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IMMUNODIAGNOSIS OF ABPA

Usha P. Sarma [1], Viswanath P. Kurup[2], Taruna Madan [1]

[1] Molecular Biochemistry and Diagnostics Division, Institute of Genomics and Integrative Biology, CSIR, Mall Road, Near Jubilee Hall, Delhi 110007, India and [2] Allergy-Immunology Division, Medical College of Wisconsin, 8701 Watertown Plank Road, Milwaukee, WI, and V A Medical Center 151-I, 5000 West National Avenue, Milwaukee, WI 53295, USA

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1. ABSTRACT

For the diagnosis of allergic aspergillosis demonstration of specific immune responses to allergens has been accepted as a significant paradigm. Elevated levels of total IgE and *Aspergillus fumigatus* specific IgE and IgG antibodies are important criteria for diagnosis of ABPA. Although reference antigens or standardized methods are not available, there are a number of relevant recombinant antigens, which have been isolated in recent years. Several techniques have been employed in the demonstration of specific antibodies against antigens of *Aspergillus fumigatus* in the sera of patients. Of these methods, the widely followed ones are ELISA, radioimmunoassay, Western blot, and agar gel double diffusion. Recently, semi-automated methods have been developed using recombinant allergens to detect circulating antibody against *Aspergillus*. However, these methods have not been evaluated widely. Here we review the immunodiagnostic methods currently in use for allergic bronchopulmonary aspergillosis.

2. INTRODUCTION

The allergic form of the *Aspergillus* induced disease is named as Allergic bronchopulmonary aspergillosis (ABPA), which is an immunological disease and depicts the immune mechanisms similar to that of asthma. Variable prevalence of ABPA has been reported in patients of bronchial asthma (1-2%, 16%, 2-38%), cystic fibrosis (2%, 7.8%, 35-81%), sinusitis (13%, 23%) and rhinitis (5%) (1-11). A study with 35 patients of ABPA showed that 12 (33%) of them were misdiagnosed as patients of pulmonary tuberculosis and were treated with various antitubercular drugs (12).

3. DIAGNOSIS OF ABPA

Central bronchiectasis, fleeting pulmonary shadows, bronchial asthma, immediate skin reactivity to *Aspergillus*, elevated levels of total IgE, specific IgE and IgG antibodies, peripheral and pulmonary eosinophilia and precipitins against *Aspergillus fumigatus* (Af) antigens are characteristic features for diagnosis of ABPA patients. However, often there is difficulty to differentially diagnose ABPA from tuberculosis, pneumonia, bronchiectasis, lung abscess and bronchial asthma (12-15).

Early diagnosis of ABPA is important for the following reasons :

- Firstly, to prevent irreversible damage of the bronchi and the lungs. Bronchiectasis and bronchiolitis are known sequelae of the disease and if undiagnosed in

early stages, may lead to pulmonary fibrosis and respiratory failure. Progression of ABPA has been classified clinically into five stages viz. Acute, Remission, Exacerbation, Corticosteroid dependent asthma and Pulmonary fibrosis (16).

- ABPA may be the cause of recurrent pneumonias in children and may increase the severity of asthma in some patients.
- Association of ABPA in cystic fibrosis may worsen the course and prognosis.
- ABPA has also been found in multiple members of a family and a need has been felt for screening all the family members (10, 17-21).
- Occurrence of ABPA is also known to be associated with pulmonary tuberculosis. It has been observed that both diseases show similar clinical symptoms that cause a diagnostic dilemma.

4. SIGNIFICANCE OF SEROLOGY IN DIAGNOSIS OF ABPA

Despite the recent progress achieved in understanding the pathophysiological mechanisms, problems relating to a reliable diagnosis of aspergillosis remain to be solved. The methods used currently are based on detection of the pathogen on i) direct microscopic examination or in histopathologic sections, ii) isolation and identification of the pathogen in culture, and iii) detection of an immunological response to the pathogen or some marker of its presence, such as a metabolic product by using various serodiagnostic methods. Each approach has its advantages as well as limitations. Histological and culture confirmation of the disease is often difficult, as, fungal elements demonstrate atypical morphology depending on a number of factors including steric orientation, age of fungal lesion, effects of antifungal therapy, type of infected tissue and host responses (22-24). Further, positive cultures from sputum represent inconclusive evidence of disease in that, they may constitute aerial contamination of the mucoid secretions.

Elevated levels of total IgE and Af specific IgE and IgG antibodies detectable by various serodiagnostic techniques are important diagnostic criteria for ABPA (25-29). Reference standards of Aspergillus antigens for immunodiagnosis are not available till today either with the world health organisation (WHO) or any international agencies (30). This is mainly due to the complex nature of antigens of Aspergillus species, which require multiple purification processes. The recombinant and synthetic peptide technology have paved way for such an approach towards preparation of standardised diagnostic reagents and international reference standards useful for immunodiagnosis.

5. IMMUNODIAGNOSTIC TESTS FOR ABPA

A variety of tests with varied sensitivities have been developed to detect circulating antibodies to *Aspergillus* antigens. Some of these are more of researcher's tools such as Western blot, Immunogold assay, Immunoelectrophoresis, the Radioallergosorbent test (RAST), Biotin avidin linked immunosorbent assay (BALISA), Luminiscence Immunoassay (LIA), Lymphocyte Transformation, while some of them have been put to routine clinical application such as, Immunodiffusion test, Enzyme linked immunosorbent assay (ELISA) and Pharmacia ImmunoCAP system. These tests show diverse sensitivities depending on the quality of antigen and the type of antibodies being investigated.

5.1. Immunoelectrophoresis

Several types of immunoelectrophoretic techniques have been used in diagnosing aspergillosis. Among these counterimmunoelectrophoresis (CIE) has been widely used, mainly because of the short time required to obtain the results. By using CIE, Longbottom detected precipitins in 78% of patients with allergic aspergillosis whereas the immunodiffusion test was positive in only 64% of same cases (31). A positive correlation was reported between number of precipitation lines and the titres of complement fixing antibodies (32-35). However, the nonprecipitating nature of antigen-antibody complexes proves disadvantageous in performing these techniques.

5.2. Immunoblotting

Reactivity of the particular protein to the antibody in the sera of patients of aspergillosis can be visualized by this assay which was shown to be specific and sensitive, and often correlated with clinical status (36, 37). Immunoblotting involves i) separation of antigens in polyacrylamide gel ii) transfer of separated proteins onto nitrocellulose membranes and iii) detection of specific antigen-antibody binding. Several studies have shown that glycoprotein antigens, a major constituent of both the mycelium and the culture filtrate of Af, have high immunological reactivity (38).

5.3. Lymphocyte Transformation

The correlation between proliferative response of lymphocytes from ABPA patients to purified antigens with the disease activity in these patients is well known (39,40).

5.4. Immunogold Assay

An immunogold assay was developed by Gugnani *et al*, 1990, for detection of Af specific IgG and IgE antibodies. Slide cultures of Af, fixed in methanol, are treated with different dilutions of sera of patients, then with goat anti-human IgG or IgE antibodies and finally with rabbit anti-goat IgG conjugated with gold (41). This method is more sensitive and does not require any specialised equipment but is only semi-quantitative.

5.5. Biotin avidin linked immunosorbent assay (BALISA)

BALISA, a more sensitive version of ELISA is based on biotin-streptavidin system (biotin linked secondary antibody probed with streptavidin-conjugated enzyme), and is useful to detect relatively low levels of specific IgE and IgG antibodies in some of the ABPA patients (42).

5.6. Luminiscent Immunoassay (LIA)

Luminiscent immunoassay, a modified and sensitive ELISA for detection of antibodies, is based on the

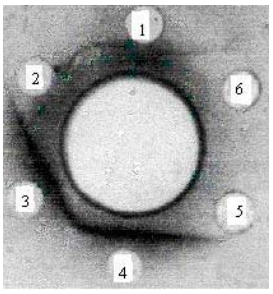

Figure 1. Immunodiffusion of culture filtrate antigens of *A. fumigatus* (central well) with sera of ABPA patients (wells 1, 2, 3 and 4) and healthy controls (wells 5 and 6).

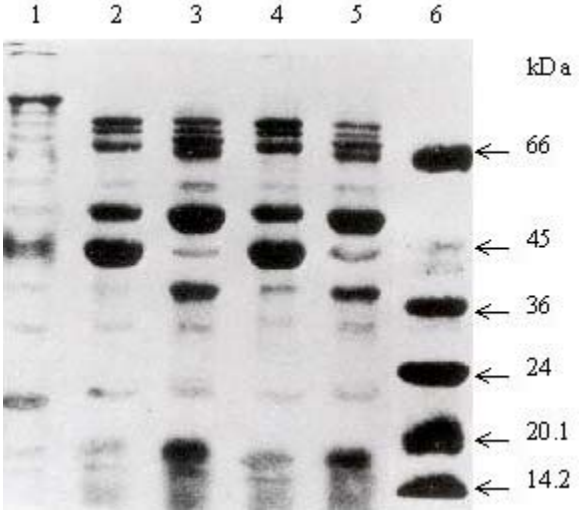

Figure 2. SDS-PAGE analysis of three week culture filtrate allergens/ antigens of various soil and clinical strains of *A. fumigatus*. Lane 1: Clinical isolate 1 (isolated from sputum of ABPA patient), Lane 2: Clinical isolate 2 (isolated from sputum of ABPA patient), Lane 3: Clinical isolate 3 (isolated from sputum of ABPA patient, strain No. 285), Lane 4: Clinical isolate 4 (isolated from sputum of Aspergilloma patient), Lane 5: Soil Strain, Lane 6: Molecular weight markers.

substrate luminol for the peroxidase conjugated secondary antibody, which produces luminiscence through emission of photons, measured in specialised microtiter plates in a luminiscence analyser (43, 44).

5.7. Radioimmunoassay (RIA)

The most commonly used RIA, for diagnosis of ABPA, is the radio allergosorbent test (RAST), used extensively for the detection of specific IgE antibodies (45). RAST has several disadvantages for use in a routine diagnostic laboratory such as radioactive hazard, disposal of wastes and need for expensive radioactive detectors.

6. IMMUNODIAGNOSTIC TESTS FOR ABPA ROUTINELY PERFORMED IN THE CLINICAL LABORATORY

6.1. Immunodiffusion test

The diagnostic utility of the immunodiffusion test (ID) has been reported by various groups. ID is useful to determine the precipitins in sera of ABPA patients and is the most common technique used for the serodiagnosis of aspergillosis in various countries where facilities are inadequate to perform an ELISA test (46, 3, 10). A precipitin reaction seen with the culture filtrate allergens/ antigens of Af and sera of ABPA patients is shown in Figure 1. Although the ID test lacks sensitivity and conveys no quantitative information about antibody concentrations, a number of clinical laboratories still use this test because of its simplicity and ease of performance.

6.2 Indirect ELISA

ELISA has been demonstrated to be a sensitive, reliable, and quantitative diagnostic tool for allergic disorders. Although the sensitivity of the conventional ELISA system depends on many variables, the nature and type of antigens used and their ability to bind to polystyrene plates are the most important decisive factors. Currently, a mixture of diagnostically relevant antigens of Af, is being used in ELISA based detection for specific IgG and IgE antibodies (47, 48) (*Aspergillus fumigatus* specific IgA/IgG/ IgM ELISA by IBL Immuno-biological Laboratories, Hamburg). Total IgE antibodies in the circulation are also estimated by ELISA using monoclonal antibodies raised against human IgE antibodies (Human IgE ELISA quantitation kit, BETHYL Laboratories, Texas, USA).

6.3. Automated antibody determination assay (Pharmacia immunoCAP system)

The Pharmacia company has developed an automated system called immunoCAP system for detection of circulating antibodies. For estimation of specific IgE antibondies to rAsp f 1, an immunoCAP assay has been standardised by Crameri *et al*, 1996 (49). For IgE antibody estimations, this immunofluoro assay was calibrated against the World Health Organization (WHO) standard for IgE and allows quantitative expression of total or allergen specific IgE. The results are expressed as kilo units of allergen specific IgE per liter (KU_A/l). The antigens are immobilised on cap like structures made of spongy matrix which capture the specific IgE antibodies from the patient sera.

7. DEVELOPMENT OF IMMUNODIAGNOSTIC REAGENTS FOR ABPA

7.1. Diagnostically relevant allergens/ antigens of *A. fumigatus*

Currently the antigens used to detect circulating antibodies to Af are based on crude extracts or partially purified extracts containing allergens/ antigens obtained from mycelial extracts or culture filtrate. A typical sodium dodecyl polyacrylamide gel elctrophoresis (SDS-PAGE) profile of three week culture filtrate allergens/ antigens of Af (both clinical and soil isolates) with molecular weights ranging from 12 to 80 kDa, is shown in Figure 2 (50). Immunoblot analysis with pooled sera of ABPA patients showed both IgG and IgE antibodies reacting predominantly with 66 kDa, 55 kDa, 45kDa, 34 kDa and 18 kDa antigens (Figure 3). An acidic glycoprotein with 12.5% neutral hexose and with an isoelectric point (pI) of

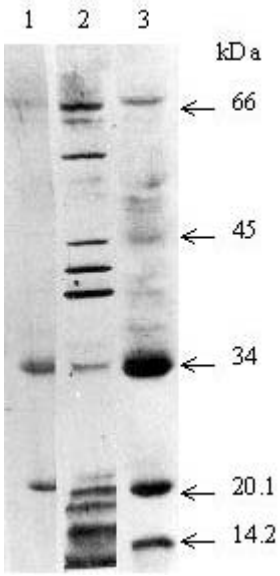

Figure 3. Western blots of three week culture filtrate allergens/ antigens of *A. fumigatus* with sera of ABPA patients probed for specific IgG and IgE binding. Lane 1: Specific IgE binding activity, Lane 2 : Specific IgG binding activity, Lane 3: Molecular weight markers.

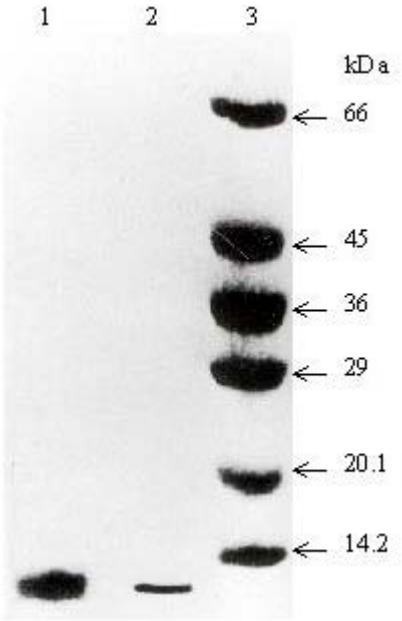

Figure 4. SDS-PAGE profile of purified Asp f 1 allergen/ antigen of *A. fumigatus.* Lane 1& 2: Different concentrations of purified Asp f 1 (18 kDa) protein loaded, Lane 3: Molecular weight markers.

5.2-5.6 and 4 polypeptide chains of molecular mass 45 kDa, was isolated from cell sap (51). This protein detected specific antibodies in 75% of sera of patients of ABPA and did not react with normal sera. Using a combination of protein purification techniques Ag3, Ag5, Ag7 and Ag 13 antigens were purified, which reacted with sera of patients with ABPA and aspergilloma (52-55). Antigens with molecular weights 20, 40 and 80 kDa showed significant antibody reactivity with sera of patients of allergic aspergillosis (56). An IgE antibody mediated response to Asp f 1, a major allergen/ antigen/ cytotoxin, has been reported in 85% of the patients with Af induced allergic disorders (57). Moreover, the Asp f 1 has also been reported to be one of the major antigens secreted *in vivo*, and excreted in the urine of patients of invasive aspergillosis (58). Figure 4 shows an SDS-PAGE profile of 18 kDa antigen purified by a combination of conA sepharose, phenyl sepharose CL-4B chromatography and reverse phase- HPLC (59). Figure 5 shows SDS-PAGE profile of immunodominant antigens such as 66 kDa, 45 kDa and 34 kDa (Figure 5 a) and 56 kDa and 27 kDa (Figure 5 b), in a purified form. Immunoreactivity of the purified 45 kDa glycoprotein antigen with the sera of ABPA and aspergilloma patients was observed by immunodiffusion, Western blot and ELISA (Table 1) (60). Teshima *et al*, reported another diagnostically relevant glycoprotein allergen/ antigen, gp 55, from Af (61). Diagnostic relevance of Asp f 2, a glycoprotein of molecular weight 37 kDa with N-terminal homology to gp 55, has been recently demonstrated (62).

Polysaccharide fractions containing galactomannans from either cell wall or cytoplasm also showed reactivity with antibodies in the sera of patients. These antigens, however, frequently demonstrate cross-reactivity with antigens from other fungi and microbes which create a major limitation in interpreting test results (63, 64). The carbohydrate antigens are reported to be useful in antigen-based immunodiagnosis of invasive aspergillosis.

The array of Af antigens identified for diagnosis depends on various factors like culture conditions (culture filtrate or mycelial intracellular extract), the strain used, the method of extraction etc. Moreover, the cross-reactivities among different species of *Aspergillus* as well as between strains *Aspergilli* from saprophytic and human origin, other fungi, bacteria etc. also affect the sensitivity of various serodiagnostic methods. Hence, characterisation of diagnostic antigens is necessary for giving a definition to antigens for universal diagnostic applications.

7.2. Recombinant allergens/ antigens

The allergenic/ antigenic proteins relevant to pathogenesis and diagnosis are highly complex macromolecules and are difficult to purify by single step purification methods. Two major difficulties encountered by immunologists in using fungal antigens for immunodiagnosis are non-availability of standardised antigens and insufficient knowledge of the molecular nature of fungal allergens. 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 allergens. Use of recombinant DNA and synthetic peptide approach has also resulted in dissection of various functional domains of some of these macromolecules and understanding of structure-function

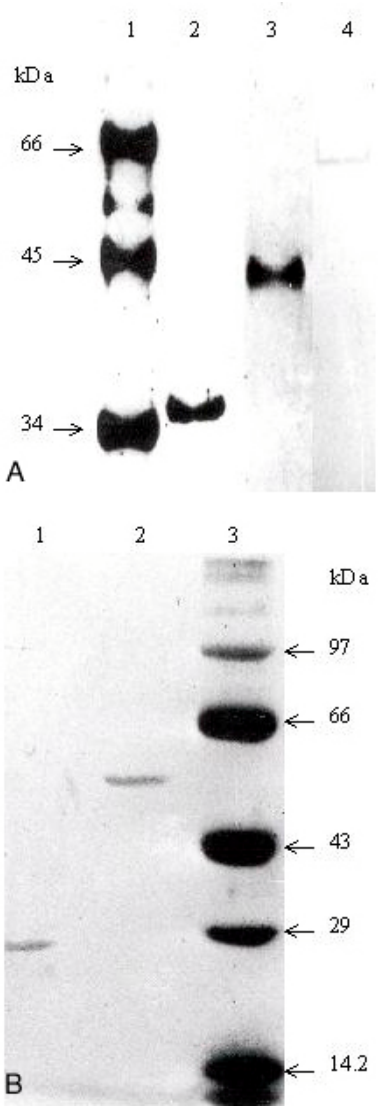

Figure 5. SDS-PAGE profile of (a) purified 66 kDa (Lane 4), 45 kDa (Lane 3) and 34 kDa (Lane 2) allergens/ antigens of *A. fumigatus*, molecular weight markers (Lane 1), (b) purified 56 kDa (Lane 2) and 27 kDa (Lane 1) allergens/ antigens of *A. fumigatus*, molecular weight markers (Lane 3).

relationship. Several of these proteins have been individually cloned and expressed in prokaryotic and eukaryotic vectors (Table-2). One of the major allergens and a proposed virulent factor, Asp f 1, was cloned and studied in greater detail with respect to its structure and function (65-67).

Some of the recombinant proteins showed specific binding to IgE antibodies from asthmatic and allergic bronchopulmonary aspergillosis (ABPA) patients (Table-2b). Some of these allergens are highly specific for ABPA and allow serologic discrimination between ABPA and Af allergy with a specificity of 100%. However, none of these recombinant allergens from Af showed a sensitivity of 100% suggesting that a combination of well characterised recombinant allergens and synthetic epitopes needs to be identified to facilitate diagnosis of ABPA with 100% sensitivity and specificity.

Asp f 2, a 268 amino acid protein, exhibited N-terminal sequence homology to a 37 kDa *A. fumigatus* culture filtrate protein and gp 55, a 55 kDa glycoprotein allergen/ antigen secreted by Af (62). The immunochemical characterisation of various Af allergens/ antigens and the amino acid sequence data suggest that various allergens/ antigens may have some common epitopes. Differential glycosylation and proteolytic processing of the antigens and the strain variability in antigen preparations may contribute to the variations in the performance of antigens in immunodiagnosis.

In an enzyme-linked immunosorbent assay (ELISA), serum IgE antibody reactivity to rAsp f 3 could be detected in 72% of 89 individuals sensitized to Af, demonstrating that the protein represents a major allergen of the mold (68). A secreted ribotoxin (rAsp f 1) and a peroxisomal protein (rAsp f 3) were recognized by sera from –Af sensitized CF-patients with or without ABPA, while an intracellular manganese superoxide dismutase (rAsp f 6) and rAsp f 4, a protein with unknown function, were recognized exclusively by IgE antibodies from sera of CF patients with ABPA. Therefore, rAsp f 4 and rAsp f 6 represent specific markers for ABPA and allow a sensitive, fully specific diagnosis of the disease (69-70). Analysis of IgE-antibody mediated immune responses to single recombinant Af allergens in asthmatic patients facilitates discrimination between ABPA and Af sensitization with high specificity (100%) and sensitivity (90%) (70). Recombinant *Aspergillus* allergens Asp f 1, f 2, f 3, f 4, and f 6 were studied for their specific binding to IgE antibodies in the sera of ABPA patients, Af skin prick test positive asthmatics, and normal controls from USA and Switzerland. The results demonstrate that Asp f 2, f 4, and f 6 can be used in the serodiagnosis of ABPA, while IgE antibody binding to Asp f 1 and f 3 was not specific (71). On the other hand, Asp f 1 was found to be an important allergen with respect to immunoreactivity and skin testing with patients of allergic asthma sensitised to Af (72).

Few of these Af allergens also exhibited high sequence homologies with the known proteins and enzymes derived from other sources. Alkaline serine proteinases from *A. fumigatus, A. flavus,* and *A. oryzae* (Asp f 13, Asp f l 13, and Asp o 13 respectively) have been reported to have allergenic properties (73). Another group of homologous vacuolar serine proteinases, Asp f 18, with conserved sequence have been reported from *Aspergillus* and *Penicillium*. Another allergenic protein of Af, Asp f 12 (65 kDa fusion protein) belongs to heat shock protein (Hsp 90) family. A novel enolase allergen from Af (Asp f 22) was identified, cloned and sequenced which showed significant homology and IgE cross-reactivity with enolase of P. citrinum (Pen c 22) (74). A novel histidine kinase gene AFHK1, a probable virulence factor, has been cloned and sequenced from AF which has homology to histidine kinases of *Candida albicans* and tesA gene of *A. nidulans* (75).

Table 1. ELISA absorbance values for specific IgE and IgG antibodies against various allergens/antigens in sera of patients with aspergillosis

Allergens	Subjects	Numbers	ELISA		Western blot	
			IgG	IgE	IgG	IgE
70 kD	ABPA	5	5/5 (100%)	4/5(80%)	+	+
	Aspergilloma	2	2/2 (100%)	0	+	-
	Non-atopic, normals	5	0	0	-	-
34 kD	ABPA	5	5/5 (100%)	4/5(80%)	+	+
	Aspergilloma	2	2/2 (100%)	0	+	-
	Non-atopic, normals	3	0	0	-	-
gp 45	ABPA	5	5/5 (100%)	4/5(80%)	+	+
	Aspergilloma	2	2/2 (100%)	0	+	-
	Non-atopic, normals	3	0	0	-	-
Asp f 1	ABPA	9	9/9(100%)	9/9(100%)	+	+
	Non-atopic, normals	10	0	0	-	-
	Allergic asthma	2	0	0	-	-

Table 2. Recombinant *A. fumigatus* Allergens

Aspergillus Allergens	Properties	Size (bp)	Mol.Wt. kDa	IgE Reactivity	Gen Bank Accession No.
rAsp f 1	Ribonuclease	678	18	++++	M83781
rAsp f 2	-	1572	37	++++	U56938
rAsp f 3	Peroxisomal protein	630	21	++++	U58050
rAsp f 4	-	1080	40	++++	AJ001732
rAsp f 5	Metalloprotease	1270	42	++	Z30424
rAsp f 6	Manganese superoxide dismutase (MnSOD)	666	26.7	++++	U53561
rAsp f 7	-	860	12	++	AJ223315
rAsp f 8	-	870	11	++	AJ223333
rAsp f 9	-	920	43	++++	AJ223327
rAsp f 10	Aspertic protease	1000	34	++	X85092
rAsp f 11	Peptidyl-prolyl isomerase	684	20	++	AJ006689
rAsp f 12	Heat shock protein	1649	47	++	U92465
rAsp f 13	Alkaline protease	-	34	++	
rAsp f 15	Serine protease	-	16	++	AJ002026
rAsp f 16	-	-	43	++++	G3643813
rAsp f 17	-	-	-	++	AJ224865
rAsp f 18	Vacuolar serine proteinase	-	34	++	
Asp f X*		758	19	++++	AJ006688
-	Protein disulfide isomerase	1095	40	++	AF363787
-	L3 60S ribosomal protein	1179	42	++	BG695822

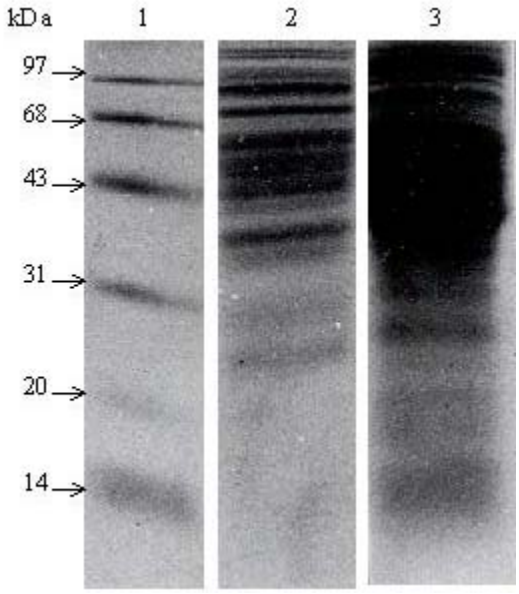

Figure 6. Overexpression of a novel PDI-family gene of *A. fumigatus* in *E. coli*. Lane 1: Molecular weight markers, Lane 2: SDS-PAGE profile of supernatant of uninduced *E. coli* cells, Lane 3: SDS-PAGE profile of supernatant of induced *E.coli* cells overexpressing the PDI-family gene of *A. fumigatus* (40.2 kDa).

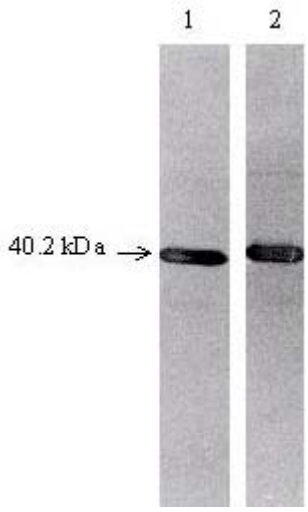

Figure 7. Western blots for specific IgG and IgE binding of purified recombinant protein of the PDI-family gene of *A. fumigatus*.

Recently, one of the cDNA's sequenced from Af showed sequence homology with protein disulphide isomerase (PDI) gene family (76) (GenBank Acc. No. Nigam BF 112419). A protein from PDI family is being reported for the first time from Af, which may play an important role in protein folding and conformation. The gene has been cloned and expressed in *E. coli* (Figure 6). Immunological characterisation of the expressed protein showed reactivity to specific IgG and IgE antibodies in the sera of ABPA patients (Figure 7).

High levels of serum IgE antibodies to Asp f16, a 43 kDa protein were observed in 70% of patients with ABPA, whereas patients with allergic asthma, Aspergillus skin test positive asthmatics without clinical evidence of ABPA and normal controls failed to show Asp f16 specific IgE binding by ELISA (77).

The immunologic evaluation of recently purified and characterised recombinant allergens of *A. fumigatus,* using various diagnostic methods mentioned above, is shown in Table 3.

7.3. Epitopes of allergens/ antigens

Application of recombinant DNA technology to fungal allergens provided scope for better understanding on molecular nature of the these allergens and in the identification of immunodominant epitopes. The knowledge of IgE- binding epitopes of the major Af allergens may be of importance for increasing the specificity and sensitivity of diagnostic tests for designing model allergens representing such epitopes for *in vitro* tests. Advances made in epitope mapping of major antigens of pathogenic microbes resulted in the rapid development of diagnostic technologies and products. Availability of the deduced amino acid sequences of a few major Af allergens/ antigens and partial sequencing of several other allergens/ antigens have now provided opportunity to analyse regions both by algorithms and by experiment (by chemical/ enzymatic cleavage of allergenic/ antigenic proteins). Identified peptide fragments can be synthesised and evaluated for their potential in immunodiagnosis. Synthesis of overlapping peptides spanning the whole protein is one of the approaches for development of peptide based diagnostics for infectious diseases. Another approach is based on the recombinant expression of the epitopic sequences.

Few antigens of Af, Asp f1, gp55, Asp f2, Asp f 3, have been studied with respect to immune responses to their peptides. Evaluation of synthetic peptides from the N-terminal of Asp f 1 suggested their diagnostic relevance by ELISA, dot-blot, lymphoproliferation, cytokine analysis and histamine release assays (78,79). One of the promising peptides for immunodiagnosis was characterised with respect to structure-function relationship. Tryptophan residue plays an important role in the immunoreactivity of the peptide (Figure 8). The N-terminal region of Asp f 1 comprising the immunodominant domain was expressed in *E. coli* and expressed recombinant protein was observed to react with specific IgE and IgG antibodies of patients of ABPA (80).

Various epitopes of Asp f 1 were observed to be differentially induce Th1 and Th2 responses (81). Of the five peptides studied for their cytokine profile, one showed a clear Th1, whereas another showed a Th2 response. Hence, for the first time an epitope of Asp f 1 was reported to induce protective Th1 response in mice. While the protective nature of *Aspergillus* allergens has not been observed so far, the Th2 responses due to mixture of allergens have been implicated in the pathogenesis of ABPA. Further, synthetic peptide epitopes of Asp f 1,

Table 3. The Reactivity of Various Recombinant Allergens to IgE Antibodies in Sera of Patients with Allergic Aspergillosis (%)

Allergens	Subjects	Numbers	ELISA	Western blot	Immediate skin test reactivity	Reference
	ABPA	15	10/15 (67%)	+	8/15(53%)	
	Allergic asthma	10	5/10 (50%)		5/10(50%)	
	Atopics non A. fumigatus	6	0		0/6	85
	Non-atopic, normals	7	0	-	0/7	
	ABPA	9	+	-	5/9(56%)	
	Allergic asthma	5	2 (40%)		2/5(40%)	
	Atopics non A. fumigatus	6	0		0/6	65
	Non-atopic, normals	7	-		0/7	
	ABPA	30	24(80%)		24/30(80%)	
	Allergic asthma	5	2(40%)		2/5(40%)	
Asp f 1	Atopics non A. fumigatus	9	0		0/9	49
	Non-atopic, normals	10	0		0/10	
	ABPA	24	20(83%)			
	Allergic asthma	16	13(81%)			
	Non-atopic, normals	10	1(10%)			71
	ABPA	24	16(67%)			
	Allergic asthma	16	0			
	Non-atopic, normals	10	0			
	ABPA	60	50(83%)			
	Allergic asthma	40	18(45%)			70
	Non-atopic, normals	20	0			
Total		339	161		46	
	ABPA	20	15(75%)			
	Allergic asthma	8	1(13%)			
	Non-atopic, normals	5	0			
	ABPA	24	21(88%)	+		
Asp f 2	Allergic asthma	16	0	-		62
	Non-atopic, normals	10	0	-		
	ABPA	24	21(88%)	+		
	Allergic asthma	16	1(6%)	-		
	Non-atopic, normals	18	0	-		
Total		141	59			
	ABPA	11	11(100%)		11/11(100%)	
						69
	Allergic asthma	8	5(63%)		5/8(63%)	
	Non-atopic, normals	4	0		0/4	
	ABPA	24	20(83%)			
Asp f 3	Allergic asthma	16	6(38%)			
	Non-atopic, normals	10	1(10%)			71
	ABPA	24	19(79%)			
	Allergic asthma	10	5(50%)			
	Non-atopic, normals	10	1(10%)			
	ABPA	60	58(97%)			
	Allergic asthma	40	21(53%)			70
	Non-atopic, normals	20	0			
Total		237	147		16	
	ABPA	60	48(80%)			
	Allergic asthma + Ab	40	0			70
	Non-atopic, normals	20	0			
	ABPA	24	22(92%)			
	Allergic asthma + Ab	16	0			
Asp f 4	Non-atopic, normals	10	0			71
	ABPA	24	15(63%)			
	Allergic asthma + Ab	16	3(19%)			
	Non-atopic, normals	10	0			
	ABPA	12	10(83%)		10/12(83%)	
	Allergic asthma	12	0		0/12	86
	Non-atopic, normals	5	0		0/5	
Total		249	98		10	
	ABPA	60	33			
	Allergic asthma + Ab	40	0			70
	Non-atopic, normals	20	0			
	ABPA	24	14			
	Allergic asthma	16	0			71
	Non-atopic, normals	10	0			
Asp f 6	ABPA	24	16			
	Allergic asthma + Ab	16	4			71
	Non-atopic, normals	10	0			
	ABPA	12	5		5/12	
	Allergic asthma	12	0		0/12	
	Non-atopic, normals	5	0		0/5	86
Total		249	76		5	
PDI-Af (40kD)	ABPA	5	5/5(100%)	+	n.d	76
	Non-atopic, normals	2	0	0	n.d	
L3 60S ribosomal protein	ABPA	3	n.d	+	n.d	Unpublished data
	Non-atopic normals	3	n.d	-	n.d	

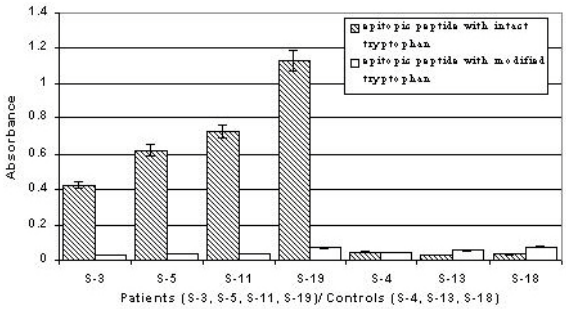

Figure 8. Absorbance values of the eleven amino acid synthetic epitope of Asp f 1with intact and modified tryptophan binding the specific IgG antibodies in the sera of ABPA patients by ELISA.

based on their possible T-cell stimulatory properties were observed to stimulate the peripheral blood mononuclear cells from ABPA patients and normal controls (81). Several peptide epitopes of Asp f 1 also demonstrated distinct IgE antibody binding with sera of ABPA patients. The carboxy-terminal region of Asp f 1, representing amino acid residues 115-149, as an epitope was involved in both humoral and cell mediated immune responses in ABPA patients (83).

Evaluation of synthetic decamer peptides of Asp f 2 on derivatized cellulose membranes showed that either the N- or C-terminal region of Asp f 2 is essential for its correct folding and conformation, needed for binding to specific IgE antibodies (63). A recent study showed protective nature of some of the epitopes of Asp f 2 against crude antigenic extract *in vivo* (83). Several epitopes of Asp f 13 were found to react with rabbit anti-Asp f 13 antiserum. Three of these immunodominant epitopes, near the C-terminal region of the protein, bound to IgE antibodies from Af-sensitive patients (73). The C-terminal amino acids 143-150 of Asp f 3 was found to be significant in the IgE binding and deletion of this sequence resulted in no or lesser reactivity with patient sera (79).

8. CONCLUDING REMARKS

Diagnostic relevance of specific IgE and IgG antibodies in patients of ABPA is well established. *Aspergillus* researches have lead to characterisation of diagnostically relevant allergens and antigens of Af. Expression of recombinant fungal allergens from different isolates opened up opportunities to prepare international reference antigens for immunodiagnosis. Further, some of the peptides from allergens/ antigens of Af, relevant for immunodiagnosis, have been identified. These advances are anticipated to lead to development of immunodiagnostic kits with quality antigens enhancing the sensitivity and specificity of test systems for laboratory diagnosis of ABPA. This undoubtedly strengthens the clinical diagnosis of ABPA.

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Send Correspondence to: Usha Sarma, Ph.D., Centre for Biochemical Technology, Mall Road, Near Jubilee Hall, Delhi – 110007, India, Tel: 91-11-766-6158, Fax: 91-11-766-7471, E-mail: u_sarma@hotmail.com