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Recent researches on Allergic Bronchopulmonary Aspergillosis (ABPA) and *Aspergillus* allergens/antigens

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ABPA is an immunologically complex disease and the patients elicit Type I and Type III hypersensitivity reactions to *Aspergillus* allergens/antigens. ABPA is, often encountered in patients of bronchial asthma, cystic fibrosis, sinusitis, rhinitis etc. Many a times it poses a diagnostic dilemma to the clinicians as the clinical features mimic tuberculosis. Early detection and diagnosis is important for the successful management of the disease. Immune mechanisms operative in development of allergic broncho-pulmonary Aspergillosis are not well understood. Understanding on the immuno-pathogenesis and molecular mechanisms involved in ABPA would undoubtedly play a key role for a greater insight into bronchial asthma. This can lead to use of new predictive diagnostics and molecular medicine for patients suffering from bronchial asthma due to complex fungal allergies and infections. Current article deals with recent multifaceted researches on ABPA and *Aspergillus* allergens and antigens.

Early diagnosis of ABPA is important in view of preventing lung damage, severity of asthma, worsening the course of cystic fibrosis, misdiagnosis of patients for tuberculosis etc.

Detection of elevated IgE and IgG antibodies specific to *A. fumigatus* by various serodiagnostic techniques is one of the important diagnostic criteria for ABPA. Reference standards of *Aspergillus* antigens for immunodiagnosis are not available till today either with the World Health Organisation (WHO) or through any other agency. This is mainly due to the complex nature of antigens *of Aspergillus* species, which requires multiples purification processes. The recombinant and synthetic peptide technologies have paved way for such an approach towards preparation of standardised diagnostic reagents and international reference standards useful for immunodiagnosis of Aspergillosis.

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IMMUNOLOGICAL RESPONSES IN ABPA

The immune response of the host to *A. fumigatus* and its allergens/antigens, results in the clearance of the organism or in the induction of significant pathological or even lethal effects in the host. Continuous exposure to *Aspergillus* through either inhalation of spores or fungal colonization in the bronchii results in late-phase asthmatic reactions. The eosinophilic infiltration and mast cell degranulation in response to *Aspergillus* allergens and IgE interaction may release additional pro-inflammatory mediators that play a major role in the pathogenesis of ABPA. These degranulation products may cause chemotaxis of eosinophils and other inflammatory cells such as activated CD4+ lymphocytes. In addition, *anti-Aspergillus* antibody enables soluble antigen and mycelial fragments to penetrate and deposit in the lung parenchyma. The

immune responses of patients to *Aspergillus* include immediate skin reaction mediated mainly by IgE antibody. The late reaction (Arthus reaction) to *Aspergillus* antigens is the result of IgE-mediated mast cell activation or immune complex formation with complement activation and cell lysis. Immune complexes of specific IgG *and A. fumigatus* antigens trigger the generation of leukotriene C4 from mast cells, which in turn promote mucous production, bronchial constriction, hyperemia, and edema. However, 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. Lung biopsy specimens from patients with ABPA *show Aspergillus-specific* IgE in the germinal centers, whereas IgG antibodies could be detected in the lung parenchyma. 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. Granuloma formation in the lung is not uncommon in ABPA .

Humoral Immunity

Specific humoral responses differ considerably in patients with different clinical forms of aspergillosis. Serum samples from patients with ABPA demonstrate elevated levels of antigen-specific circulating antibodies of IgG and IgE isotypes. Antibodies of other immunoglobulin classes, particularly IgM and IgA, have also been reported. Hence, the humoral responses in ABPA may be polyclonal resulting from exposure to complex *Aspergillus* antigens. The protein antigens *of A. fumigatus* react frequently with serum IgG_1 and IgG_2 antibodies in patients with ABPA. Patients with aspergilloma have increased levels of specific IgG and IgM, mostly against carbohydrates and glycoproteins, but show only low levels of specific IgE. Patients *with Aspergillus* skin test positive asthma invariably show elevated IgE antibodies in vitro. These differences in the *A. fumigatus-speciftc* antibodies in the serum samples of patients with ABPA, aspergilloma, and skin test positive asthma suggest the differences in the fundamental immune regulatory response to *Aspergillus* antigens in these disorders.

Cell-mediated Immunity

The most significant characteristics of the disease are the enhanced IgE expression, Th2 cytokine profile, and eosinophil proliferation and activation. *Aspergillus* antigen-specific *in vitro* proliferation of peripheral blood mononuclear cells (PBMCs) and active *in vitro* production of IgE in PBMC cultures have been reported as significant features in ABPA. Similarly, B-cells of patients with ABPA and patients with ABPA and cystic fibrosis spontaneously secrete IgE . In addition, recent reports indicate that the production of B-cell-activating factors enhancing secretion of IgE and B-cell suppressive factors suppressing IgE production by antigen stimulated T-cells from patients with ABPA. Moreover, CD4+ Th2 cells in ABPA patients secrete cytokines, interleukin-4 (IL-4), and IL-5; the former enhances IgE synthesis of B-cells, while the latter activates the differentiation and recruitment of eosinophils. Activated basophils also secrete Th2 cytokines and stimulate further activation of Th2 pathway, resulting in increased IgE and eosinophil production.

Other Factors in Immunopathogenesis

In a mice model of ABPA there was a significant increase in eosinophils, T-lymphocytes, leukocyte function-associated antigen 1 (LFA-1) positive cells, and up-regulation of expression of intercellular adhesion molecule I (ICAM-1) in the lung tissue. The intensity of ICAM-1 expression correlated with the proliferation of T-lymphocytes and may have a role in the accumulation of T-lymphocytes in the lung. T-cell lines stimulated with *Asp f* 1, a major allergen *of A. fumigatus,* synthesized IL-4 along with a non-detectable amount of IL-2 and interferon. These results suggest that antigen-stimulated T-cell lines of patients with ABPA are mainly Th2 type based on their cytokine synthesis patterns. In addition, *Asp f* 1 -specific T-cell clones have been reported to be IL-4-producing CD4+ T-cells of Th2 phenotypes. Most of these T-cell clones isolated from patients with ABPA responded to two peptide fragments of *Asp f* l, representing amino acids 46-65 and 106-125 and are restricted by HLA-DR2 and HLA-DR5 alleles.

Serodiagnosis of ABPA

Serodiagnostic criteria are useful in the diagnosis of ABPA. 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. In patients with severely compromised immunity, histological and cultural confirmation of tissue invasion is very difficult to obtain during the life of the patient. Moreover, fungal elements often demonstrate a typical 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. Further, positive cultures from sputum represent inconclusive evidence of disease in that, they may constitute aerial contamination of the mucoid secretions. The isolation *of Aspergillus* from BAL fluid is positive in only 50-57% of cases of aspergillosis. On the other hand, in severely immuno-compromised patients due to the occurrence of rapid systemic disease, the occurrence of specific antibodies is very low or absent. Detection of circulating antigens in the serum and urine of patients with invasive aspergillosis is a more promising approach. However, as antigenemia and antigenuria are transient in nature, detection of antigen may not always serve the purpose. With availability of sensitive immunodiagnostic methods such as ELISA, detection of low levels of antibodies in serum samples of invasive patients is now possible.

ANTIGENS AND ALLERGENS OF *A. FUMIGATUS*

The disease states associated with the fungus, *A. fumigatus* are attributed to secretion of biologically and immunologically active complex macromolecular proteins during colonization and establishment of infection. These proteins can be categorized into metabolic products (secreted in the medium), proteins expressed on the surface (mycelial and somatic proteins released by autolysis, or rupture of the call wall of the organism). Some have been shown relevant for immunodiagnosis and virulence.

Both clinical and soil isolates *of A. fumigatus* show protein bands ranging from MW 12-80 kDa When three week culture filtrate antigens/allergens are fractionated by electrophoresis. The antigenic repertoire of *A. fumigatus* illustrates a variety of proteins. Some of them are immunogenic, allergenic and/or both and a few are pathogenic determinants. These exhibit multitude of biological activities like catalytic, elastinolytic, proteolytic, ribonucleotic, cytotoxic etc. Exhibition of the multifunctional activities is a very interesting phenomenon of *Aspergillus* allergens/antigens.

A. fumigatus antigens and allergens are subjected to post translational modifications (glycosylation, phosphorylation, methylation and acetylation) during protein folding pathway which facilitate the structure in cell-cell interaction. Many of the cell surface receptors are glycosylated. Antigen binding sites of immunoglobulins have carbohydrate residues. Structural proteins viz., collagen, laminin and fibronectin also possess carbohydrates that help in mucous secretion necessary for lubrication and subsequent protection of epithelial tissues.

CHARACTERISTICS OF ALLERGENIC PROTEINS

A prerequisite property of a protein to be an allergen is the ability to activate Th 2 response in affected individuals. The induction of allergic response in genetically predisposed individuals involves the cooperation of several functional and effector cells in recognizing allergen-induced stimulation leading to synthesis of IgE antibodies to *Aspergillus* allergens. It is still unclear why certain proteins possess allergenicity. Allergens triggering immune responses must be polyvalent with several epitopes for mediating cross linking of IgE antibodies on mast cells resulting in release of mediators. Thus, allergens are thought to be more or less constrained in their molecular conformations. A common structural or functional feature is yet to be found among the characterized allergens, which would allow the prediction of whether a certain protein has any intrinsic property to behave as an allergen or not.

MAJOR ALLERGENS/ANTIGENS OF *A. FUMIGATUS* ARE GLYCOPROTEINS

A number of immunodominant antigenic components of viruses and fungi are complex glycoproteins and lipoproteins. Majority of allergens and antigens in *A. fumigatus* are highly glycosylated. Enzymatic, chemical mapping and immunologic crossreactivity studies have shown that culture components of *A. fumigatus* (60, 40 and 30 kDa proteins) have a common peptide core. The cell sap antigens of 53 kDa and 41 kDa sizes are also believed to be of the same peptide family. The differences in molecular weight and antibody binding profiles of these proteins, appear to be due to glycosylation and phosphorylation. Differences in antibody binding profiles of various antigens may be attributed to varied glycosylation patterns. This important finding lead researchers to isolate and characterize the glycoprotein of *A. fumigatus* and characterize their carbohydrate moieties and polypeptide chain. Majority of these glycoproteins have glucose and mannose sugars and bind to lectin concanavalin A. Kurup *et al* demonstrated that *A. fumigatus* antigens resolved into 18 bands, and 12 to 14 of them were con A binding molecules. Several investigators have identified con A-binding components *of A.fumigatus* as the major antigenic fraction consistently showing reactivity with patient's sera. Four antigens (Ag 3, Ag 5, Ag 7, Ag 13) purified by Longbottom have been found useful for detection of antibodies in patients of ABPA and aspergilloma by specific ELISA. Antigens of molecular weight 20, 40, and 80 kDa exhibited significant antibody binding with patients of allergic aspergillosis. Con A binding antigens like, gp45, gp55, gp66 have been studied. They showed reactivity with both IgG and IgE antibodies present in the sera of patients of aspergillosis. A 93 kDa glycoprotein isolated by affinity chromatography using mannose-specific lectin exhibited IgE binding with sera from ABPA patients.

Thus glycoproteins are significant components of *A. fumigatus* showing both IgG and IgE binding in sera of patients with Aspergillosis. An insight into their molecular nature and interaction with the host factors is necessary to facilitate understanding of pathogenesis of *Aspergillus* induced diseases.

DIAGNOSTIC RELEVANCE OF ANTIGENS/ ALLERGENS OF *A. FUMIGATUS*

The demonstration of presence of specific IgG and IgE antibodies to *Aspergillus* antigens is diagnostically useful for patients suffering from aspergillosis.

Four major glycoprotein components Ag 5 (30 kDa), Ag 7 (150 -200 kDa), Ag 3 (24 kDa) and Ag 13 (70 kDa) have been found useful for patients of ABPA and aspergilloma. In specific ELISA, a cell sap glycoprotein with four polypeptide chains of MW 45 kDa showed reactivity with sera of patients of aspergilloma and ABPA. Antigens with MW 20, 40 and 80 kDa exhibited strong antibody reactivity with sera from allergic Aspergillosis patients. Major antigens are with MW 70, 60, 45, 34, 28 and 18 kDa in third week culture filtrate (3 WCF) preparation of *A.fumigatus.* Culture filtrate proteins of *A.fumigatus* with molecular weight of 30, 55, 45,34, 18 and 12 kDa are also observed useful in serodiagnosis. An immunodiagnostically relevant glycoprotein antigen of 55 kDa from *A.fumigatus* mycelia was purified to homogeneity.

Recombinant antigens/allergens have shown potential in serodiagnosis. Response to *Asp f* l was documented by Arruda *et al* (1992) in 85% of the patients with *A. fumigatus* induced allergic disorders *Asp f* l is the recombinant of a major 18 kDa allergen, which is excreted in urine of patients with invasive aspergillosis . Immunoreactivity of gp45 antigen with sera from ABPA and aspergilloma patients has been observed. *Asp f* 2, a glycoprotein with N-terminal sequence homology to gp55 has been observed to possess diagnostic relevance.

The array *of A. fumigatus* antigens identified for diagnosis depends on factors like culture conditions, (culture filtrate is mycelial intracellular extract), the strain used, the method of extraction etc. The carbohydrate antigens are reported to be useful in antigen-based immunodiagnosis of invasive Aspergillosis. In spite of identification of several immunodiagnostically relevant antigens, reference antigens for serodiagnosis of aspergillosis are yet to be formulated. Presence of cross-reactivity among different species of *Aspergillus* and between Aspergilli, other fungi, bacteria and human proteins

has also hampered development of sensitive and effective diagnostic assays. Hence there is a need to have a detailed study of structural, immunological and molecular aspects of diagnostic antigens for universal applications.

WHY RECOMBINANT ANTIGENS AND ALLERGENS?

The major problem hampering the immuno-diagnosis of *A.fumigatus-mediated* disease revolves around the molecular complexity of the fungus and inadequate information on the structure, function and biochemical nature of its antigens/allergens.

Extracts *of A. fumigatus* are mixtures of about 200 different proteins and glycoproteins and low molecular weight compounds. The mould generates highly variable IgE binding profile in immunoblots with sera from different allergic individuals . The immunoblot patterns depend on the sensitization of the subject, extract employed, and stage of the disease. In view of this, standardization of allergenic extract from *A.fumigatus* was attempted. However, batch to batch variation, time dependent secretion of IgE binding allergens during the fungal growth, instability of the extract due to proteases and culture conditions hampered the development of standardized extracts. Due to this, serious attempts were made to isolate fungal allergens with a view to obtain purified molecules for serodiagnosis. This required cumbersome purification techniques and often resulted in their degradation during isolation. Also, even biochemically purified allergens were observed to be contaminated with traces of IgE-binding components.

Molecular biology .offered the possibility of producing large quantities of standardized single, highly pure recombinant allergens and antigens. These recombinants can be used to generate standard diagnostic reagents. In addition, recombinant antigens and allergens are also useful in dissecting the functional domains of some of these macromolecules and understanding the structure-function relationships. Recombinant allergens also show a potential for immunotherapy of allergic diseases.

RECOMBINANT ANTIGENS AND ALLERGENS OF *A.fumigatus*

Molecular DNA procedures have resulted in cloning, characterization, production in pure form, and partial evaluation of antigens for diagnosis by ELISA and skin tests. A number of recombinant antigens/allergens from the pathogenic strains of *A. fumigatus* have been prepared. The recent listing of IUIS-WHO Allergen Nomenclature Committee includes 22 allergens from *Aspergillus and Penicillium* species. Table 1 gives some of the recombinant proteins produced and characterized from *A.fumigatus.* These can be subdivided into two classes: those with homology to described proteins and those without any homology to sequences deposited in gene banks.

It is suggested that the recombinant allergens can be used for developing standard diagnostic reagents for diagnosis of ABPA although studies involving a large number of sera from ABPA patients and *A. fumigatus-sensitized* individuals from different clinics need to be carried out before deciding on the diagnostic value of the allergens. For standardization purposes, pure recombinant allergens/antigens are superior to native molecules because of their consistency, high production and compatibility with fully automated diagnostic devices.

STRUCTURE-FUNCTION RELATIONSHIP OF IMPORTANT ALLERGENS

Many *A. fumigatus* allergens were shown to have biological activities and some have been described as multifunctional molecules. But various properties, and intrinsic factors predisposing them as allergens have not been worked out. Extensive studies using recombinant antigens/allergens for understanding molecular structure, functional domains and epitopic regions of antigens/allergens are being carried out in different laboratories.

CLINICAL APPLICATION FOR DIAGNOSTICS

Total extracts obtained from natural sources at times lack certain components due to either poor extraction or enzymatic degradation during preparation. Hence, recombinant allergens can be

Table 1. 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	Aspartic 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		
		1095	40	+	
-	Protein disulfide isomerase	1179	42	+	
-	L3 60S ribosomal	protein			

envisaged as new generation molecules for improving *in vitro and in vivo* diagnostic methods.

Recent results of immuno CAP carrying immobilized *r Asp f* 1 (Pharmacia CAP System) and allergen specific direct enzyme-linked immunosorbent assay correlated closely (r=0.997) and corroborated with skin test results. Other recombinant allergens *Asp f 2, Asp f 3 , Asp f* 4 and *Asp f* 6 have shown considerable promise as standardized allergens.

However, there may be some disadvantages with the use of recombinant antigens/allergens in diagnosis. It has been reported that some recombinant allergens show less IgE-binding capacity when compared with their natural counterparts. This is quite evident because of the existence many isoforms of allergens with less or increased IgE binding capacity. It is, therefore, necessary to use only those recombinants which possess the same immunological characteristics as their natural counterparts.

ANTIGENIC AND ALLERGENIC EPITOPES

Each antigen is recognized by the immune system by its three dimensional structure by immunoglobulins (B-cell epitopes) or, after ingestion and processing by antigen presenting cells, as small peptide fragments bound to major histocompatability complex (MHC) class II molecules by T cells (T-cell epitopes). T-cell epitopes are sequential and as such, they usually do not react with allergen specific IgE antibodies. A prerequisite for the identification of epitopes is knowledge of the primary structure (amino acid sequence) of the desired allergen. Overlapping peptides can be subsequently produced by recombinant DNA methods, *i.e.*, by cloning and expression of overlapping cDNAs, produced by either sequential deletions or random fragmentation of the cDNAs, and, after that, by the synthesis of the corresponding recombinants in *E. coli*, phage, yeast, or mammalian cells. Alternatively, peptides may be chemically synthesized. Epitopes can then be detected *in vitro* by antibody assays (direct or inhibition ELISA, RAST or by histamine release assays), by T-cell proliferation, or, by *in vivo* tests (skin tests or immunogenecity in animals). Few epitopes have been characterized from *A fumigatus* antigens/allergens.

Availability of deduced amino acid sequences of some of the major antigens/allergens *of A. fumigatus* has facilitated characterization of their epitopes by algorithms and by experiment (chemical/enzymatic cleavage). The identified epitopic sequences can be synthesized and evaluated for immunodiagnosis and their protective potential in animal models.

Three major antigens/allergens- *Asp f* 1, *Asp f2* and *Asp f* 3 have been analyzed for the presence of epitopic domains. The carboxy terminal region of *Asp f 1* (115-149 amino acids) is involved in both humoral and cell-mediated immune responses in ABPA patients. Out of five linear peptides studied for cytokine profile in mice, one showed a protective Th 1 response . The majority *of Asp f 1* specific T-cell clones isolated from PBMCs of ABPA patients responded to two peptide fragments representing amino acid residues 46-65 and 106-125 . Evaluation of synthetic peptides from N-terminal of *Asp f* 1 suggested their diagnostic relevance by ELISA, dot-blot, lymphoproliferation, cytokine analysis, and histamine release assays. One of the promising peptides for immunodiagnosis was characterized with respect to structure-function relationship.

Tryptophan residue plays an important role in the immunoreactivity of the peptide. The N-terminal region of *Asp f* 1 comprising of immunodominant domain was expressed in *E. coli* and expressed recombinant protein was observed to react with specific IgG and IgE antibodies of patients with ABPA.

A recent study showed protective nature of *Asp f 2* against crude antigenic extract *in vivo*. The N and C terminal regions *of Asp f 2* have IgE binding epitopes. Three IgE binding immunodominant epitopes in the C-terminal region of *Asp f 13* showed specific binding to *A. fumigatus-sensitive* patients. Synthetic epitope based diagnosis has several advantages over assays based on mixture of antigens/ allergens which are susceptible to batch variation. By examining more immunodominant epitopes preparing a panel of diagnostic peptides and formulation of subunit vaccines seems feasible in future.

STRATEGIES FOR PREVENTION AND THERAPY OF ABPA

The ubiquitous nature and universal distribution of *A. fumigatus* makes prevention of exposure difficult. This pathogen is associated with invasive aspergillosis and allergic disorders in humans (allergic asthma, allergic rhinitis, allergic sinusitis, ABPA and hypersensitivity pneumonitis). Due to high mortality rates observed in the patients of invasive aspergillosis and the side effects caused by corticosteroid therapy in allergic disorders, there is need to examine new therapeutic strategies for fungal

diseases. Better understanding on the mode of infection of pathogenic fungus, mechanisms of host-pathogen interactions and the pathogenesis of aspergillosis would facilitate development of safer and effective therapeutic reagents.

Development of vaccine for *Aspergillus* induced infections has been hampered by lack of knowledge about protective potential of *A. fumigatus* antigens/ allergens. Administration of *A. fumigatus* antigens results in development of disease rather than protection. Few reports are available indicating existence of protective acquired immunity against A. *fumigatus* when sublethal doses of conidia are administered to the animals before challenge. Acquired immunity could be transferred to naive animals via sensitized macrophages and lymphocytes from challenged animals. Thl mediated resistance on administering mice with *Aspergillus* crude culture filtrate antigens, suggests the protective potential of antigens and allergens of the mould.

Cytokine therapy still in primitive stage, shows promise in treatment of fungal diseases. There is observed increase in phagocytosis of *A. fumigatus* conidia (15 to 35%) on pre-exposure of neutrophils to IL-8. The oxidative response and hyphal damage by normal and cortisone treated neutrophils is enhanced by granulocyte colony stimulating factor (G-CSF) and interferon gamma (IFN-γ) . The killing ability of neutrophils and macrophages of HIV patients enhances in presence of G-CSF and IFN-γ . Eosinophilia could be abrogated by injecting IL-5 in a murine model of allergic aspergillosis . Treatment of a chronic granulomatous disease (CGD) patient (with multiple brain abcesses due to *A. fumigatus)* with IFN-γ resulted in survival of the patient.

ABPA AND PULMONARY SURFACTANT PROTEINS, SP-A AND SP-D

Surfactant protein A (SP-A) and D (SP-D) belong to a group of mammalian lectins containing collagen regions, called 'collectins'. Pathogen recognition by these proteins in the lungs is mediated by binding of terminal monosaccharide residue characteristic of bacterial and fungal cell surfaces. The broad selectivity of the monosaccharide binding site and the geometrical arranement of the multiple CRDs in the intact collectins explain the ability of these proteins to bind tightly to arrays of carbohydrate structures normally found on the surfaces of the micro-organisms and thus mediate discrimination between self and non-self.

The surfactant proteins are known to interact with carbohydrate structure on the surface of a wide range of pathogens such as viruses, bacteria and fungi, via their CRDs, and thus enhance phagocytosis and killing by neutrophils and alveolar macrophages. SP-A and SP-D are also known to interact with phagocytic cells and enhance their chemotactic, phagocytic and oxidative properties.

The experiments carried out using the transgenic mice, genetically deficient in SP-A and SP-D emphasise a key role played by SP-A and SP-D in pulmonary immune response. The SP-A gene-deficient (SP-A-/-) mice are less effective in clearing lung pathogens. Concentrations of TNF-α, IL-6, and IL-o are increased in bronchoalveolar lavage (BAL) fluid of SP-A -/- mice, which can increase further on adenoviral administration. Co-administration of adenovirus and purified human SP-A can ameliorate adenoviral-induced lung inflammation in SP-A -/- mice. Mice genetically deficient in SP-D shows chronic inflammation, foamy alveolar macrophages secreting ten-fold higher levels of hydrogen peroxide, increased activity of metalloproteinases, emphysema, and fibrosis in the lungs.

INTERACTION OF SP-A AND SP-D WITH *ASPERGILLUS* ALLERGENS

SP-A has been shown to bind a variety of allergenic pollens such as Lombardy poplar, Kentucky blue grass, cultivated rye and short ragweed, via CRDs SP-A, SP-D and a recombinant fragment of human SP-D composed of homotrimeric neck and CRD regions (rSP-D) can also bind house dust mite extract *(Dermatophagoides pteronyssinus;* Der P_1) in a carbohydrate-specific and Ca^{2+}-dependent manner, inhibit specific IgE binding to these glycoprotein allergens, and block allergen-induced histamine release from basophils isolated from *Derp*- sensitive patients. Similarly, SP-A, SP-D and rSP-D can also bind to the three-week culture filtrate (3 WCF) of A *fumigatus* as well as purified glycoprotein allergens, gp55 and gp45, inhibit the ability of *A. fumigatus*-specific IgE to bind these allergens, and block *A. fumigatus* allergen induced histamine release from

sensitized basophils isolated from ABPA patients. SP-A and SP-D have been reported to reduce the proliferation of peripheral blood mononuclear cells (PBMCs) isolated from mite-sensitive asthmatic children and SP-D, in particular, has a suppressive effects on the secretions of IL-2 by PBMCs. SP-A can attenuate production of IL-8 by eosinophils in a concentration-dependent manner. Thus, SP-A and SP-D inhibit histamine release in the early phase of allergen provocation and suppress lymphocyte proliferation in the late phase of bronchial inflammation - the two essential steps in the development of asthmatic symptoms.

The roles of SP-A and SP-D in the pathogenesis of airway inflammation and asthma have also been investigated. Abnormal levels of SP-A and SP-D in the lung lavage have been reported in the acute respiratory distress syndrome (ARDS) and pulmonary infections caused by influenza virus, mycoplasma and *Pneumocystis carinii* in AIDS patients, hypersensitivity lung diseases and cystic fibrosis . Asthmatics show increased amounts of SP-A in bronchio alveolar lavage and SP-D in alveolar lavage as compared with those in controls.

Serum SP-D levels for two allergic patients have been found elevated at diagnosis which decrease following corticosteroid therapy. The patients of birch pollen allergy and pulmonary alveolar proteinosis showed a shift towards lower oligomeric forms of SP-A in comparison to healthy volunteers. Depolymerisation of SP-A may lead to loss of binding affinity for carbohydrate-rich surfaces, with subsequent loss or alteration of biological function. SP-A has been shown to inhibit the LPS-induced TNF-α and IL-2 production by human alveolar macrophages in ARDS patients . Transtracheal administration of SP-A can downregulate T cell-dependent alveolar inflammation, thus decreasing lung injury, in a murine model of donor T cell-dependent lung dysfunction following bone-marrow transplantation.

The positive role of lung surfactant proteins A and D in strengthening innate immunity against fungal allergy and infections observed in in-vitro and in-vivo experiments has brought new dimension of thinking to the diagnosis and therapy of fungal allergies such as ABPA. The genes of surfactant protein A and D are considered as candidate genes and are being examined for SNPs and polymorphisms that may have a direct correlation with clinical outcome and presentation. Any such studies may lead towards development of molecular probes for predictive diagnosis of respirtory allergic disease in near future. Lung surfactant protein therapy and *Aspergillus* peptide based vaccines are distinct possibilities in the near future.

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Patents Related to ABPA research by the Author's Group:

S.No.	Title of the Patent	Inventors	Country	Patent appl. No. & filing Date
1	A process for the preparation of a novel synthetic peptide epitope useful for diagnosis of aspergillosis.	P.U. Sarma, T, Madan P. Priyadarsini, W. Haq and S.B. Katti.	India	Patent no. : 184440
2	A process for the preparation of novel synthetic peptide epitope (1) useful for the diagnosis of Aspergillosis.	P.U. Sarma, T. Madan, P. Priyadarsini, W. Haq and S.B. Katti.	India	Accepted 2001 to be sealed in due course)
3	A process for the preparation of novel synthetic peptide epitope (ii) useful for the diagnosis of Aspergillosis.	P.U. Sarma, T. Madan, P. Priyadarsini, W. Haq and S. B. Katti.	India	Accepted 2001 (to be sealed in due course)
4	A process for the preparation of novel synthetic peptide epitope (iii) useful for the diagnosis of Aspergillosis.	P.U. Sarma, T. Madan, P. Priyadarsini, W. Haq and S.B. Katti. due course)	India	Accepted 2001 (to be sealed in
5	A process for the preparation of novel synthetic peptide epitope (iv) useful for the diagnosis of Aspergillosis.	P.U. Sarma, T. Madan P. Priyadarsini, W. Haq and S.B. Katti.	India	754/Del/98
6	Novel polypeptides useful for diagnosis of Aspergillus fumigatus and a process for preparing the same.	P.U. Sarma, T. Madan, P. Priyadarsini, and W. Haq and S. B. Katti	USA	Granted. Patent Number: 6262231
7	Novel polypeptides useful for diagnosis of Aspergillus fumigatus and a process for preparing the same.	P.U. Sarma, T. Madan, P. Priyadarsini, and W. Haq and S.B. Katti	Europe	Appl No. 99309700.5-2204

Expressed Sequence Tags (EST's) submitted from *A. fumigatus* cDNA library by the Author's group:

Accession Nos.
BG310456
BG310457
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BG695819
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BG695821
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