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Multifunctional Antigens of *A. fumigatus* and Specific Antibodies

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Abstract

The majority of *Aspergillus*-induced infections in man are caused by the pathogenic fungus *A. fumigatus*, which secretes biologically and immunologically active glycosylated and nonglycosylated proteins. The complexity in the antigenic structure of *A. fumigatus* and the varying host immune responses lead to a wide spectrum of clinical conditions such as allergic bronchopulmonary aspergillosis (ABPA), aspergilloma, and invasive aspergillosis. It is reported that 15–20% of allergic asthmatics suffer from *Aspergillus*-induced allergies. The incidence of opportunistic infections, including *Aspergillus* infections, has risen because of the increase in the incidence of HIV and tuberculosis. Allergic bronchopulmonary aspergillosis is an immunologically significant clinical form where type I and type III hypersensitivity reactions are involved in pathogenesis. High levels of specific IgE and IgG antibodies in these patients are of diagnostic value. Molecular characterization of certain immunodominant allergens and antigens of *A. fumigatus* revealed the presence of complex carbohydrate moieties, heat-shock proteins, enzyme activities such as elastase, protease, catalase, dismutase, and cytotoxic ribonuclease. A Con A binding allergen/antigen (45 kDa) and Con A nonbinding allergen/antigen (18 kDa, Asp fI) have a multifunctional nature. The multifunctional nature of these antigens may play an important role in the pathogenesis of the disease. Significant amounts of a major allergen/antigen of molecular weight 18 kDa is excreted in large amounts through the urine of patients with invasive aspergillosis. Studies on the structure–function relationship of the 18-kDa allergen/antigen revealed the involvement of tryptophan residues in binding with monoclonal antibodies (MAbs). Also, the histidine residues and cysteine disulfide bonds of the 18-kDa allergen are involved in its catalytic activity. The high load of multifunctional antigens in the serum of patients for prolonged periods, the presence of high levels of specific antibodies, and the absence of protective

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antibodies in ABPA patients have necessitated studies on the functional properties of the antibodies. The present study shows significant immunoreactivity of antibodies in patients of ABPA to fibronectin and collagen. Analysis of IgG antibodies from the patients of ABPA showed the presence of DNA-cleaving activity. These observations offer a new line of thinking in understanding the mechanism of pathogenesis of *Aspergillus*-induced clinical manifestations, and may lead to novel approaches to intervention in the inflammation and infection caused by fungal pathogens.

Index Entries: Allergic bronchopulmonary aspergillosis (ABPA); *Aspergillus fumigatus*; ribonuclease; protease; fibronectin; collagen type IV; DNA cleaving activity.

Introduction

Aspergillus fumigatus is the principal fungal species that causes allergic and invasive infections in human beings and animals. This important fungal pathogen elicits complex immunological responses leading to varied clinical symptoms in invasive aspergillosis, aspergilloma, bronchial asthma, extrinsic allergic alveolitis, and allergic bronchopulmonary aspergillosis (ABPA). Virulence of *A. fumigatus* is attributed to the secretion of various immunologically and biologically active proteins such as toxins, elastases, proteases, and ribonucleases (*1*). Antigens with collagenase, elastase, and protease activities have been reported from different isolates of the fungi *(2–4)*. Cytotoxic metabolites of *A. fumigatus* (gliotoxin and fumagillin) are thought to facilitate fungal growth by inhibiting macrophage functions and causing immunosuppression *(5)*. Spores of *A. fumigatus* secrete low-molecular-weight diffusates, which inhibit chemotaxis and phagocytosis *(6)*. The identification, characterization and study of the role of various antigens from different sources is therefore considered essential.

Extensive tissue damage caused by granuloma formation, interstitial fibrosis, and tissue necrosis has been observed in *Aspergillus*-induced disorders, which results in irreversible lung damage *(1)*. The tissue damage observed may be attributed to the biological activity of some of these allergens/antigens and the immune responses in the host. Significant levels of specific IgG and IgE antibodies have been observed in ABPA patients compared to the normals. Animals that have been passively immunized with these antibodies do not show any protection against the disease *(7)*. When animals are immunized with a mixture of mycelial and secretory antigens, high levels of IgG and IgE antibodies along with symptoms similar to human allergic aspergillosis are observed *(8)*. We anticipate that investigations on the functional role of antibodies will lead to an understanding of clinical progression of the disease induced by *A. fumigatus*.

Microbial triggering of autoimmune reactions and molecular mimicry are observed in a number of autoimmune disorders, such as SLE, thyroiditis, and glomerular nephritis caused by streptococcal infections, and so on *(9)*. Sequence homology of a 65-kDa *Aspergillus* antigen has been

observed with human hsp 90, and the 88-kDa allergen/antigen showed homology to dipeptidyl peptidase *(10,11)*. Yet it still remains to be seen whether antibodies in *Aspergillus* patients can interact with the host's self-proteins. Analysis of antibodies in the sera of autoimmune diseases has shown the presence of catalytic activity such as protease and DNA-cleaving activity *(12,13)*. It is interesting to note that *Aspergillus* infections form a part of fungal allergies in 15–20% of total asthmatics, and malfunctioning of the lung has been reported in a number of SLE patients. These observations indicate the need for investigations on the functional role of the antibodies in *Aspergillus* patients.

In our study, the functional aspects of *A. fumigatus* antigens and the antibodies were investigated to determine their role in immunopathogenesis. A cytotoxic ribonuclease allergen/antigen (18 kDa) and an elastinolytic protease allergen/antigen (45 kDa) have been characterized with respect to immunological and biological activities. Specific antibodies to these two allergens/antigens were observed in ABPA patients. The immunoreactivity of the antibodies of patients against the host's self-proteins was also examined. The IgG antibodies isolated from sera of ABPA patients were evaluated for DNA-cleaving activity.

Materials and Methods

Human Sera and Monoclonal Antibodies

Sera of patients of ABPA were received from the VP Chest Institute, Delhi, India. Sera from healthy donors with no indication of pulmonary disease were taken as normals. MAbs raised against Asp fI were the gift of Dr. L. K. Arruda, Division of Allergy and Clinical Immunology, University of Virginia.

Purification and Characterization of Biologically Active Antigens of A. fumigatus

Asp fI was purified as reported previously *(14)*. Immunoblot and indirect ELISA analysis of HPLC-purified antigen was carried out with sera of ABPA patients and monoclonal antibody against Asp fI according to the method reported by Madan et al., 1997 *(14)*. The ribonuclease activity of purified Asp fI was examined and estimated using a qualitative and quantitative assay, as described previously *(14)*; 45 kDa antigen was purified, as reported previously *(15)*. Indirect ELISA was carried out as described by Bannerjee et al. *(16)*. The immunoblot analysis was carried out with sera of ABPA patients as per the method described by Towbin et al. *(17)*. Protease activity of 45 kDa antigen was analyzed in SDS-polyacrylamide gels containing copolymerized gelatin, as described by Heussen and Dowdle *(18)*. Assay for elastinolytic activity was carried out as described by Tomee et al. *(2)* with Congo red-elastin as the substrate.

ELISA Assay for Fibronectin and Collagen Antibodies in ABPA Patients

An indirect ELISA was carried out with fibronectin and collagen (obtained from Sigma) to assay the level of specific antibodies in the sera of ABPA patients and normals. Fibronectin/collagen was coated onto an ELISA plate with 1 μg of protein/well in 0.05 *M* carbonate–bicarbonate buffer, pH 9.6, for 3 h at 37°C. The nonspecific sites were blocked with 3% bovine serum albumin in phosphate-buffered saline (0.01 *M*, pH 7.4). The plate was then incubated with the sera of ABPA patients and normals (diluted to 1 : 100) for 3 h at 37°C followed by incubation with anti-IgG peroxidase (1 : 5000) for 2h at 37°C. The *o*-phenylene diamine (1 mg/mL) in citrate-phosphate buffer (0.05 *M*, pH 4.2) containing H_2O_2 (1μL/mL) was used as substrate, and the plate was read at 490 nm.

The presence of antifibronectin antibodies in sera of ABPA patients was also analyzed by immunoblot as per the method of Towbin et al. *(17)*. Briefly, 50 μg of fibronectin in each lane was electrophoresed in SDS-PAGE (10% gel) and electroblotted onto nitrocellulose membrane. The nonspecific sites were blocked with 3% defatted milk in 0.01 *M* Tris-HCl, 0.15 *M* NaCl, pH 7.4 (TBS). The blot was incubated with pooled sera (diluted 1 : 100 with TBS) of ABPA patients or normals for 3 h at 37°C followed by incubation with anti-IgG peroxidase/anti-IgE peroxidase (1 : 2000) for 2 h at 37°C. The blot was developed using diaminobenzidine in TBS (1 mg/mL) containing 30% H_2O_2 (1 μL/mL).

Purification of IgG Antibodies from Sera of ABPA Patients and Normals

IgG antibody fractions were purified from the sera of ABPA patients and normals using protein A Sepharose column as described in the antibody manual *(19)*.

Assay for DNA-Cleaving Activity in the Antibodies

For assaying the DNA-cleaving activity, varied amounts of the IgG antibody fractions (1–20 μg of protein in 0.01 *M* Tris-HCl pH 8.0 containing 5 m*M* $MgCl_2$) were incubated with 0.3 μg of DNA isolated from p_{vex} plasmid (plasmid DNA contained both form I, or supercoiled DNA, and form II, or relaxed DNA) for varying time intervals (1 h–overnight) at 37°C. To examine the role of Mg^{2+} ions in the DNA-cleaving activity of the antibody fraction, the activity was assayed in the presence of a chelating agent, ethylenediaminetetraacetic acid (EDTA, 1 m*M*). Heat denaturation at 100°C for 2 h was carried out with the antibody fraction to evaluate the specific DNA-cleaving activity. The reaction products were electrophoresed in a 1% agarose horizontal slab gel in 50 m*M* Tris-acetate, 1 m*M* EDTA, pH 8.0. After staining with 1 μg/mL ethidium bromide, DNA bands were observed under short-wavelength ultraviolet light and photographed.

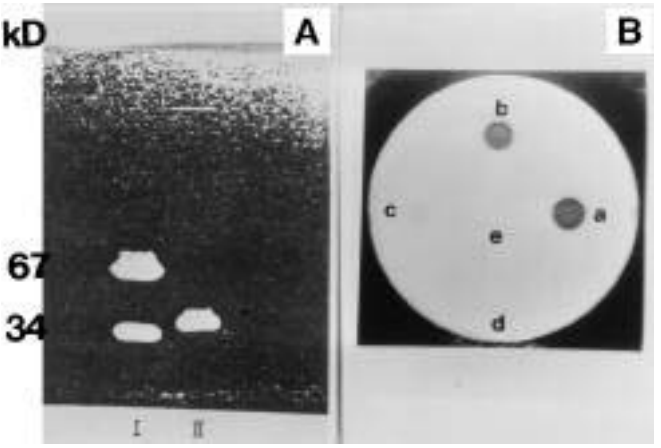

Fig. 1. **(A)** Protease activity of 45-kDa antigen in SDS-PAGE with copolymerized gelatin. Clear zones indicate the sites of protease activity. Lane I. Serum-free melanoma-cell harvest protein (MW 67 kDa) and pepsin (MW 34 kDa) as markers. Lane II. Purified 45 kDa antigen. **(B)** Ribonuclease activity on Agar gel (1.0%) with yeast RNA (0.2%): a. Protein enriched antigenic fraction, b. Purified Asp fI, c., d., and e. Controls.

Results

Multifunctional Allergens/Antigens of A. fumigatus *and Their Apecific Antibodies in ABPA Patients*

The ribonuclease activity of Asp fI was demonstrated on agar–yeast RNA gels as indicated by a clear region at the site of application (*see* Fig. 1B). The approximate ribonuclease activity of Asp fI, in comparison with the standard pancreatic ribonuclease, was found to be 70,000 U/mg. Purified 18 kDa allergen/antigen reacted with MAb raised against Asp fI by ELISA (OD at 490 nm; 18-kDa allergen/antigen + MoAb = 1.389; control = 0.067).

Protease activity of 45-kDa antigen at pH 8.3 is shown in Fig. 1A. Elastase activity of the 45-kDa allergen/antigen was 0.65 U/mg of purified protein. Inhibition of protease activity by 0.5 m*M* PMSF, and 0.5 m*M* EDTA was observed in the zymogram. Measurement of specific IgE and IgG antibodies by ELISA in sera of different patients against the multifunctional 18-kDa and 45-kDa purified allergen/antigen clearly indicated an increase from twofold to 10-fold in IgE antibodies, and a 5–12-fold increase in IgG antibodies, indicating their diagnostic significance and relevance.

Antibodies to Fibronectin and Collagen Type IV in ABPA Patients

Specific IgG antibodies to fibronectin were observed in ABPA patients by ELISA. The ELISA absorbance values with sera of ABPA patients varied from 0.200 to 0.300 against absorbance values of normals (0.010 to 0.060)

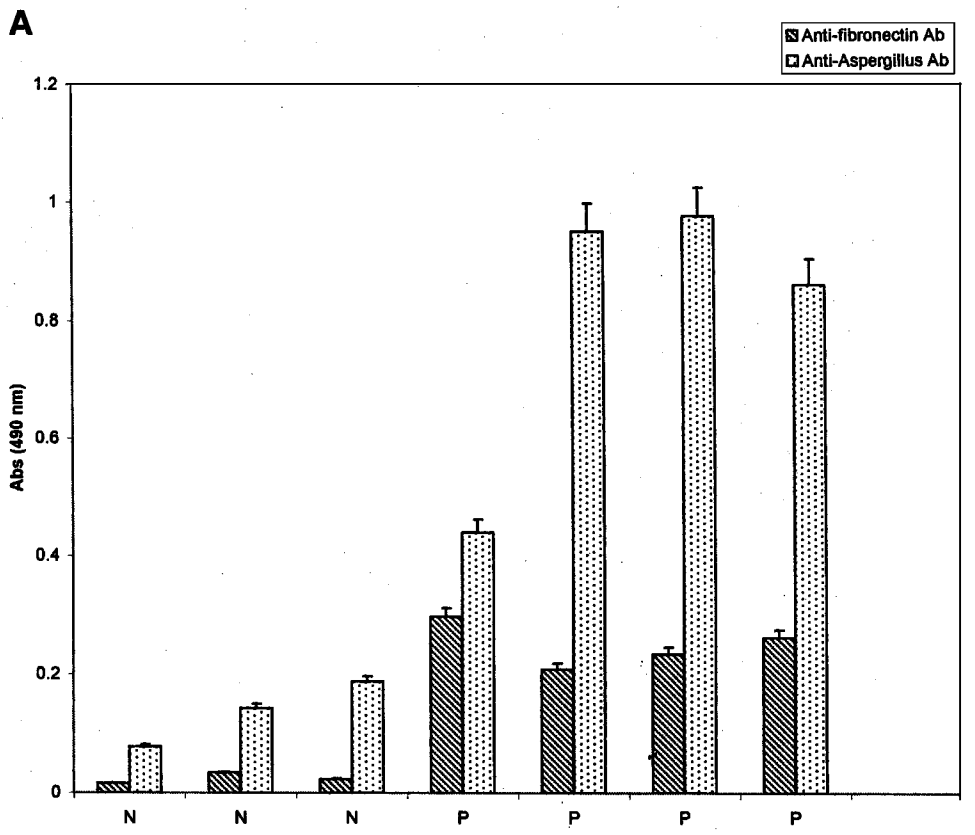

Fig. 2. **(A)** Levels of Antifibronectin IgG antibodies in sera of ABPA patients by ELISA. N: Normal P: Patient **(B)** *(opposite page)*. Levels of Anticollagen type IV antibodies in sera of ABPA patients by ELISA. N: Normal P: Patient.

(*see* Fig. 2A). ELISA absorbance values with collagen type IV varied from 0.200 to 0.250, while the value in normals was 0.010 to 0.050 (Fig. 2B). Figure 3 shows the immunoreactivity of fibronectin with sera of ABPA patients on the Western blot. Sera of ABPA patients showed the presence of a significant amount of immunoreactivity of specific IgG antibodies against fibronectin both by ELISA and immunoblot (*see* Fig.3, lane 1).

DNA-Cleaving Activity in the IgG Antibodies in the Sera of ABPA Patients

The purified IgG antibodies from the patient sera showed DNA-nicking and cleavage, while no such activity was observed in case of normals or in purified IgG from Sigma under identical conditions (*see* Fig. 4). DNA-cleaving activity was inhibited in the presence of EDTA, suggesting the positive role of Mg^{2+} in the DNA-cleaving activity (*see* Fig. 5). Incubation of plasmid DNA with increasing concentrations of antibodies showed DNA-cleaving activity even with 1 μg of antibodies (*see* Fig. 6). Heat-

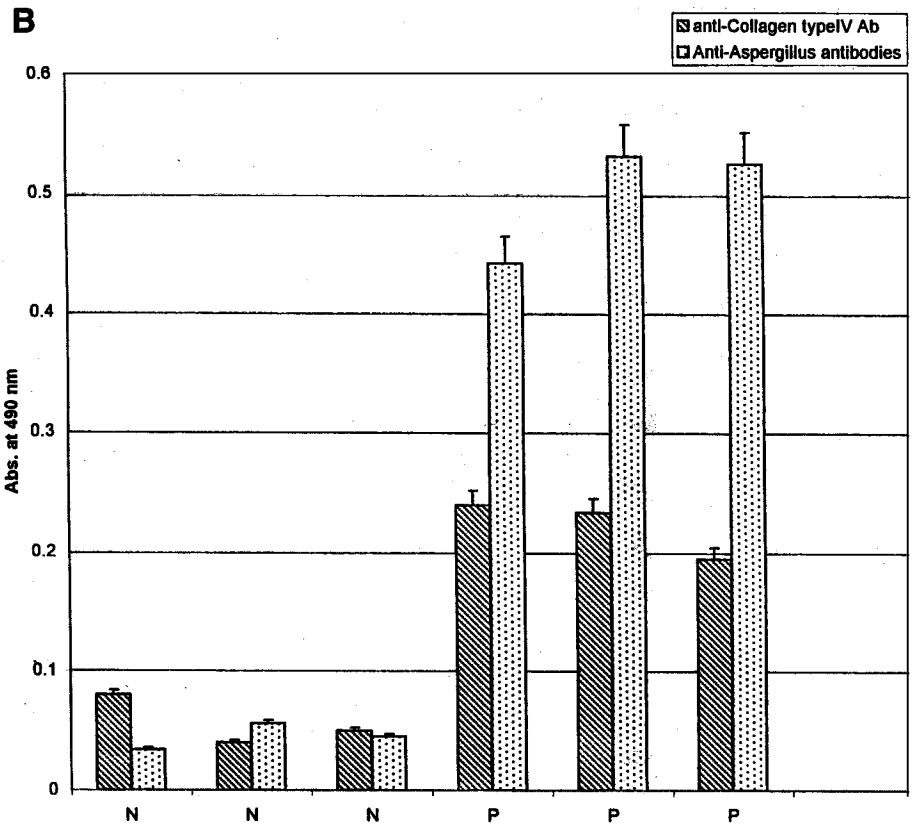

Fig. 2.

denatured antibodies (for 2 h at 100°C) showed reduced DNA-cleaving activity (*see* Fig. 7). DNA cleaving and nicking by the antibodies was observed to be time-dependent, as seen in Fig. 8 from lane 4 onward. Control DNA contained form I or supercoiled DNA (lower band in lane 1) and form II or relaxed DNA (upper band in lane 1). At first, only the supercoiled form is nicked by the antibodies to yield the relaxed form (lane 2). The trailing/smear below the band of relaxed form of DNA in each lane from lane 4 onward indicate a slow but steady cleavage of the relaxed form after an incubation period of 3–24 h. Incubation for 30 h resulted in complete cleavage of DNA (*see* Fig. 4, lane 1).

Discussion

ABPA is an immunologically significant clinical disease, yet its mechanism of immunopathogenesis is not well understood. Several groups have attempted to characterize the pathogen-related virulent factors (immunodominant multifunctional antigens of *Aspergillus)* and the host-immune factors in animal models in order to understand the immunopathogene-

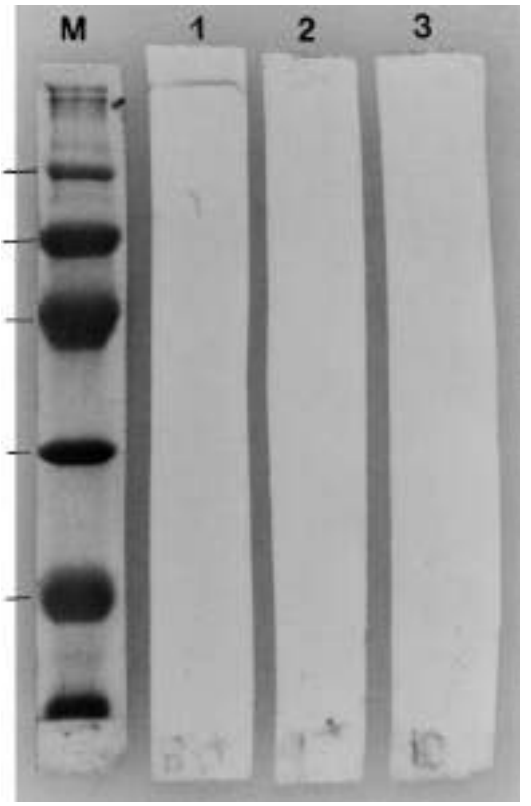

Fig. 3. Immunoblots with fibronectin. Lane M: Molecular weight markers (97 kDa, 68 kDa, 43 kDa, 29 kDa, 14 kDa); Lane 1: Fibronectin incubated with pooled sera of ABPA patients probed with anti-IgG peroxidase; Lane 2: Fibronectin incubated with pooled sera of ABPA patients probed with anti-IgE peroxidase; Lane 3: Fibronectin incubated with pooled sera of normals probed with anti-IgG peroxidase.

sis *(1)*. The high levels of IgE and IgG antibodies observed in patients during *Aspergillus* infections have not shown any protective role in these infections. Instead, the IgG antibodies have been implicated in the pathogenesis of the disease through an introductory IgE-mediated mechanism *(7)*. The presence of circulating immune complexes was reported in these patients. Characterization of the immune complexes revealed the presence of a 45-kDa allergen/antigen with elastase, protease activities, and IgG type of antibodies *(25)*. The immunological complexities involved in ABPA and the clinical outcome which includes inflammatory reactions, tissue damage, and other factors—has necessitated investigations on the functional role of specific antibodies in these patients.

Our study clearly demonstrates the presence of specific IgE and IgG antibodies to an immunodominant 18-kDa (Asp fI) allergen/antigen of *A. fumigatus* in ABPA patients. The presence of ribonuclease activity in this allergen/antigen also indicates its multifunctional nature. Asp fI is reported

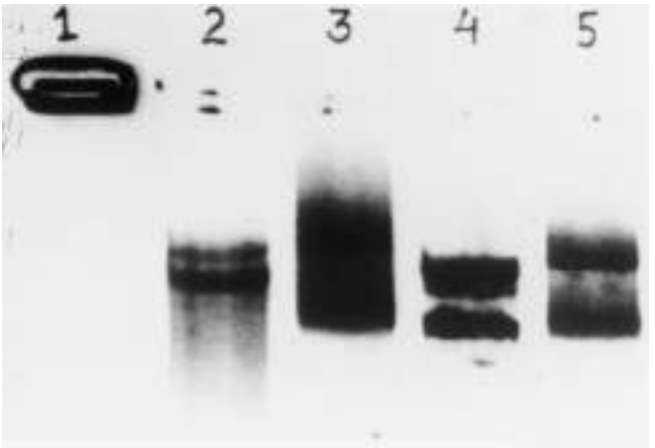

Fig. 4. DNA-cleaving activity of IgG antibodies of ABPA patients. Lane 1: DNA incubated with IgG antibodies of ABPA patients. Lane 2: DNA incubated with IgG of normals. Lane 3: DNA incubated with human IgG (Sigma) (20 μg). Lane 4: Control (DNA only) Lane 5: DNA incubated with low concentration of human IgG (10 μg).

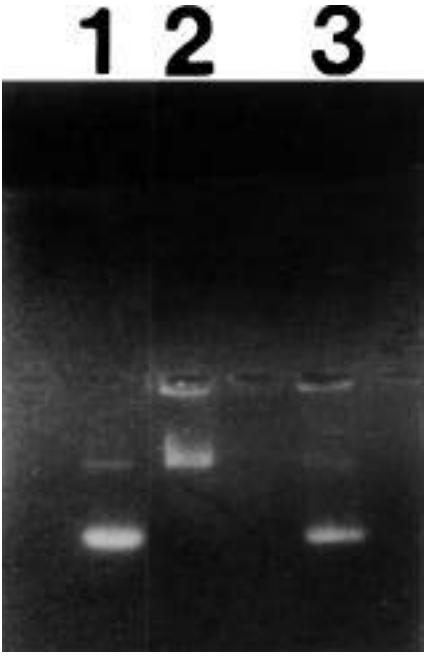

Fig. 5. Inhibition of DNA-cleaving activity with EDTA. Lane 1: Control (DNA only). Lane 2: DNA incubated with IgG antibodies of ABPA patients in the presence of 5m*M* $MgCl_2$. Lane 3: DNA incubated with IgG antibodies of ABPA patients in presence of $MgCl_2$ and EDTA.

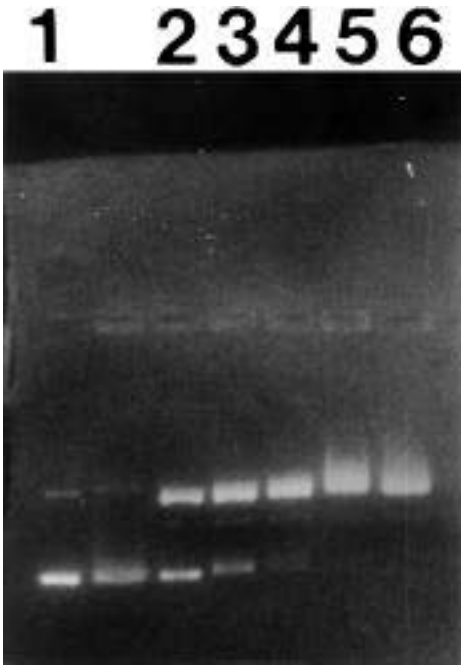

Fig. 6. Agarose gel (1%) showing DNA-cleaving activity with increasing concentration of IgG antibodies of ABPA patients. Lane 1: Control (DNA only). Lanes 2, 3, 4, 5 and 6: DNA incubated with 1, 2, 5, 10 and 15 μg of IgG antibodies respectively and contain 5 m*M* $MgCl_2$.

to be a major allergen/antigen involved in the pathogenesis of *Aspergillus*-related disorders *(20,21)*.

An IgE-mediated response to Asp fI has been reported in 85% of patients with *A. fumigatus*-induced allergic disorders *(20)*. The 18-kDa protein is also a major antigen, which is excreted in large quantities in the urine of the patients with invasive aspergillosis *(22)*. This is suggestive of prolonged presence of this antigen in the host in large quantities. Cytotoxicity of this allergen/antigen toward eukaryotic cell lines was well-established in an earlier study *(23)*. Recombinant Asp fI was also observed to be cytotoxic to EBV (Epstein-Barr virus)-transformed PBMCs (Peripheral blood mononuclear cells) of ABPA patients *(24)*. The cloning and sequencing of Asp fI showed homology to the mitogilllin family of cytotoxins (Sarma et al., unpublished data; *21*).

Another immunodominant glycoprotein allergen/antigen (45 kDa) from *A. fumigatus* reacted with the IgG and IgE antibodies in the sera of patients with ABPA and aspergilloma. Presence of proteolytic elastase activity in this antigen suggests its possible role in pathogenesis, as the lung tissue is made up of elastin. High levels of specific antibodies to this antigen were observed in patients of ABPA and aspergilloma by ELISA and Western-blot techniques. Circulating immune complexes in the ABPA

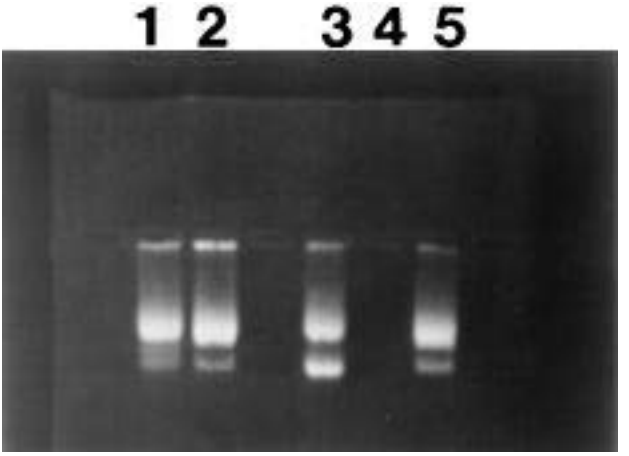

Fig. 7. Agarose gel (1%) showing DNA-cleaving activity with heat-denatured IgG antibodies of ABPA patients. Lane 1: DNA incubated with antibodies without $MgCl_2$. Lane 2: DNA incubated with heat-denatured antibodies without $MgCl_2$. Lane 3: Control (DNA only). Lane 4: DNA incubated with antibodies in the presence of $MgCl_2$. Lane 5: DNA incubated with heat-denatured antibodies in the presence of $MgCl_2$.

patients showed the presence of 45-kDa antigen as one of the predominant antigens *(25)*. These observations suggest that 45-kDa allergen/antigen may play an important role in the progression of the disease.

The presence of specific antibodies to fibronectin and collagen type IV human extracellular matrix proteins in ABPA patients has been observed for the first time in this study. Fibronectin plays an important role in host defense as an opsonin, and regulates leukocyte migration and activation. The presence of significantly high levels of antibodies to these proteins in ABPA patients suggests a possible interference in the normal immune mechanism leading to inflammation and tissue damage through the complement cascade during the progression of the disease. Fibronectin function as an opsonin supports the innate immune nature of phagocytosis of fungal conidia. The presence of antibodies to a host opsonin may result in down-regulation of innate immunity. Several autoimmune disorders in humans are triggered after microbial infections. The microbes are known to exhibit molecular mimicry and to trigger the autoimmune reactions leading to tissue damage. Several bacterial and viral proteins are reported to have sequence homologies with that of MHC molecules, although the pathologic significance of these findings is unclear *(9)*. None of the *Aspergillus* proteins sequenced thus far has shown sequence homology with fibronectin and collagen type IV. However, the advances in recombinant DNA and sequencing technology are anticipated to achieve clear indications of existence or nonexistence of such molecular homology.

The IgG antibodies isolated from ABPA patients exhibited Mg^{2+}-dependent DNA-nicking and DNA-cleavage activity. Addition of EDTA resulted in the complexing of Magnesium ions in the reaction mixture, thus

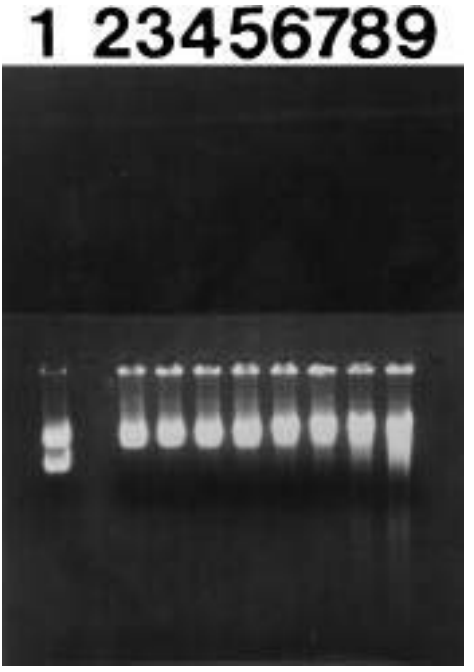

Fig. 8. Agarose gel (1%) showing DNA-cleaving activity with antibodies (IgG fraction from sera of ABPA patient) at variable time intervals. Lane 1: Control (DNA only). Lane 2, 3, 4, 5, 6, 7, 8, and 9: DNA incubated with antibodies (IgG fraction from sera of ABPA patient) for 1, 2, 3, 4, 6, 8, 14 and 24 h respectively. All reactions contain 5 m*M* $MgCl_2$.

inhibiting the cleaving activity. The Mg^{2+}-dependent cleaving activity of the IgG antibodies in the sera of patients of ABPA appears to be similar to that of Mg^{2+}-dependent DNA-cleaving activity of type II restriction endonucleases. Of interest here is the finding that the antibodies only show DNA-nicking initially, but go onto cleave DNA further upon prolonged incubation. The absence of DNA-cleaving activity in IgG from control subjects suggests that the activity is not an artifact caused by contaminants.

Catalytic activity can arise through natural means in antibodies to several naturally-occurring peptides. Prolonged exposure to antigens may also lead to generation of catalytic activity in the antibodies *(26)*. The presence of catalytic activity has been reported in antibodies isolated from patients of autoimmune disorders, such as thyroiditis and SLE *(12)*. It is yet to be shown that antibodies with DNA-cleaving activity are specific to *A. fumigatus* antigens. However, the significant levels of *A. fumigatus*-specific antibodies to antigens such as 18 kDa and 45 kDa indicate that there may be an association of catalytic activity with *A. fumigatus*-specific antibodies.

Immunoreactivity of IgG antibodies in ABPA patients with fibronectin and collagen type IV of the host and the occurrence of DNA-cleaving activ-

ity in these antibodies may eventually lead to a better understanding of the mechanism of pathogenesis of ABPA, although additional studies are needed to understand the functional role of the antibodies.

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Discussion

Christen: From your own work and that of other laboratories, is a list of the allergens of *A. fumigatus* available? Are there any common features of

these proteins that would indicate their ability to act as allergens? You mentioned, for instance, that many of them are glycoproteins, but you also showed a slide of allergens with CPK on it, which is probably not a glycoprotein.

Sarma: The few antigens that were isolated from different laboratories were sequenced and shown to have homology. The 18-kDa antigen I showed was isolated in our lab.

Christen: There are several proteins not known to be allergens. But antibodies against these proteins are found in patients. Do these have some special features that might eventually help characterize them as allergens?

Sarma: You are asking whether any specific region or motif of the protein can act as an allergen. So far, there is no such definition of allergenic epitopes.

Christen: Is it possible that the allergenic proteins are characterized by a high abundance in the cells?

Sarma: The proteins are present in reasonably good amounts. There is no doubt about that, but many other proteins present in large amounts apparently do not elicit interesting IgM, IgG, or IgE antibodies.

Zouali: The question that you just asked is not answerable for any kind of allergen. The allergenicity of a protein is very difficult to predict on a structural basis.

Christen: Could the intracellular location—the half-life or the abundance of the antigen in the cell—determine the immune response?

Sarma: There are many abundant glycoproteins in the cell. This does not distinguish an allergen.

Zouali: Some people talk about superallergens. People here would like this idea. I know there are data in the process of being submitted or published that describes some allergens behaving like superantigens.

Paul: The allergenicity of a protein is dependent to a large extent on the amount of IgE antibodies it induces. The IgE antibodies are different from some other antibodies that we heard about today—the IgG, IgMs, and so on. That is, they differ in the constant domains, as opposed to the variable domains. So the switches that lead to production of IgE by B cells are different from the stimuli involved in affinity-maturation of the variable domains. Interleukins are involved in utilization of the epsilon-constant domain, which is what defines the IgE form of antibodies. Beyond that, I am not sure if there is a predictable pattern of stimuli that would answer Dr. Christen's question.

Brahmachari: We have about 23,000 proteins circulating in blood. Peptide sequences expressed on the surface of these 23,000 circulating self-proteins

are unlikely to be allergens. So if an organism generates a new peptide, which is not in this collection of blood self-peptides, then it is likely to be an allergen. Now, if one sits down on a computer and feeds the 23,000 protein sequences, breaks them into peptides, and subtracts the collection from all known sequences from *A. fumigatus*, perhaps one could arrive at an answer.

Paul: Are you suggesting it's feasible?

Brahmachari: It's feasible! It's absolutely feasible. We just looked into *M. tuberculosis*. It has 3,923 ORFs, and we tabulated 610,000 decapeptides. If you now take this peptide library, compare with peptides made in a nonpathogenic organism like *E. coli*, and subtract, you start getting peptides which are *M. tuberculi*-specific. Nobody has done the analysis yet for humans. So one can imagine that the common peptides are very much responsible for all of the immune evasion. One has to do a lot of computational analysis, but I think it would be possible to predict which peptides are superantigens.

Paul: Certainly. Dr. Sarma, I wanted to ask you about molecular mimicry. Would you say that every time there is an immune response to the antigens you are dealing with, or to other infections for that matter, that there might be autoimmune consequences by necessity? This is one of the unifying theories in autoimmunity—molecular mimicry as the cause of autoimmune disease.

Sarma: That's right. Nobody is looking into this in detail. The microbe is reacting with the human system, but we don't even know the whole structures of functional proteins in the pathogen. Many antigens are not characterized at all. Only in the last three or four years have a few sequences been characterized, and they do have some structural homologies to host proteins.

Sushila: Prophylins from wheat have been shown to be allergic moieties, and they are present in circulation. But exactly what prophylins are from wheat? They all show a certain amount of similarity in peptide analysis, and all of them induce IgE antibody production in certain individuals. Whether the allergy is really antigen-associated or whether it is individual-associated—for example, someone with an autoimmune disorder—is not known. Each person may have a different response to the antigen.

Paul: The basic problem is that microbes use same proteins we use. If they were to use completely different structures, we would not have the autoimmune consequences. The extent to which divergence has occurred over evolution might in fact be determined by the immune system, because differences must be introduced in order to distinguish self from nonself.

Vijaylakshmi: This is a simpler question. You showed the proteolytic activity in the antibody, correct?

Sarma: Not in the antibody; in the antigen.

Vijaylakshmi: Is the antibody specific for an elastase antigen?

Sarma: We have not looked at that. We have plans to analyze the details of the specificity soon.