

# THE ASSOCIATION OF ALLERGIC BRONCHOPULMONARY ASPERGILLOSIS AND CYSTIC FIBROSIS

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As has been discussed in other articles in this issue, allergic bronchopulmonary aspergillosis (ABPA) is a complication of asthma that may be more common than initially expected; however, it is a syndrome that occurs with variable frequency throughout the United States. In fact, some clinicians believe they have never seen a case, whereas in other parts of the United States a fairly large number of such cases are diagnosed and treated.

Cystic fibrosis (CF) is the most common lethal genetic disease in the white population, with a prevalence of one in 2500 births. This disease is autosomal recessive, with the CF gene localized on the long arm of chromosome 7. CF has been associated with a deletion at position 508. The CF gene gives rise to a protein product called the *cystic fibrosis transmembrane regulator protein* (CFTR), which is related to a class of proteins that is involved in the transport of substances across cell membranes. An abnormal protein may interfere with chloride transport or may interfere with activation of transport mechanisms by protein kinases. The basic abnormality in CF is abnormal exocrine gland function, which is due to defective transport mechanisms that lead to the production of hyperviscous secretions.<sup>2</sup> These hyperviscous secretions in the bronchial tree can become inspissated, and bacterial infections can

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occur with subsequent development of bronchiectasis and progressive pulmonary destruction from chronic and recurrent inflammation.

In 1976, Mitchell-Heggs et al reported an association between CF and ABPA for the first time.<sup>13</sup> Since that time, other investigators have reported that ABPA can occur in as many as 11% of patients with CF. This statistic may be an under-estimation because the lung disease in patients with CF may obscure some of the pulmonary findings considered typical of ABPA. However, distinguishing ABPA from other infectious complications in CF is crucial for proper treatment because untreated ABPA causes long-standing pulmonary inflammation, bronchiectasis, and fibrosis itself.<sup>1, 6, 12, 13, 17</sup> On the other hand, when one small CF population was systemically examined for ABPA, only 1 of 53 patients was found to have ABPA.<sup>1</sup>

*Aspergillus* is a species of fungi that are ubiquitous throughout the environment and are tolerant to varied environmental conditions. These fungi are commonly cultured in surveys of homes as well as outdoors. Although several species of *Aspergillus* have been found to cause disease in humans, *Aspergillus fumigatus* is the most common. Studies determining the atmospheric spread of *A. fumigatus* spores indicate a worldwide distribution. Of interest is that there are similar levels reported in both North America and the United Kingdom. *A. fumigatus* grows at temperatures between 15°C and 53° C. Major antigenic glycoproteins that appear under different growth conditions have been identified.<sup>2, 8</sup>

The classical diagnostic criteria for the diagnosis of ABPA have already been reviewed by Patterson and Varkey (see their articles elsewhere in this issue). The applicability of these criteria to all cases of patients who may have both CF and ABPA has been put in question. Hutcheson et al have described a cohort of 79 patients where variable responses to *A. fumigatus* were seen over a period of time.<sup>7</sup> This data shows the pitfall of basing the diagnosis of ABPA in CF patients solely on the basis of serology and skin test results.

## IMMUNOLOGIC STUDIES

Further studies have been carried out that look at various parameters of the immune response to *A. fumigatus* antigens in several ways in an attempt to examine the relationship between CF and bronchopulmonary aspergillosis. In 1989, Knutsen and Slavin reported that patients with ABPA and CF patients with ABPA have *A. fumigatus*-specific T cells that respond with proliferation and enhanced IgE synthesis in an in vitro system.<sup>10</sup> In vivo activated T cells spontaneously synthesized increased amounts of immunoglobulin E (IgE). Peripheral blood mononuclear cells of patients with positive skin tests proliferated in vitro in response to stimulation with *A. fumigatus* antigen. Such patients, however, when compared with patients with ABPA, did not have the same magnitude of increased serum IgE concentration, and their B cells did not spontaneously synthesize increased amounts of IgE. Their T-cell supernatants did

not enhance B-cell IgE synthesis. Furthermore, patients with ABPA alone had phenotypically normal percentages and numbers of T suppressor cells that showed increased function.<sup>11</sup> Knutsen et al reported the serial measurements of serum anti-*A. fumigatus* IgE and IgG antibodies in patients with CF who were suspected of developing ABPA. Significant increases in both IgE and IgG antibody levels occurred when compared with other groups of patients who had positive skin tests and serum precipitins to *A. fumigatus*. Therefore, some overlap with other patients is seen, and serologic data cannot totally be relied upon. In extending observations on the production of immunoglobulins in patients with these diseases, Knutsen et al looked at T- and B-cell function in several groups of patients, including 11 CF patients with ABPA, 37 patients with positive skin tests or IgG precipitating antibodies but without ABPA, and 35 patients without skin test reaction to *A. fumigatus* or evidence of specific IgE or IgG antibody to *A. fumigatus*.<sup>9</sup> Supernatant cultures of *A. fumigatus*-stimulated T cells from ABPA patients significantly induced B-cell IgE synthesis compared with the other two groups. Thus T cells stimulated with *A. fumigatus* antigens secrete cytokines that induce B-cell IgE synthesis. It would appear that B-cell IgE hyperactivity is manifested both in vivo and in vitro by increased IgE concentration. As a result, perhaps analysis of T-cell regulation and B-cell IgE synthesis will distinguish patients with CF and ABPA from *Aspergillus*-sensitive but non-ABPA patients with CF.<sup>10</sup>

## MICROBIAL COLONIZATION IN CF

Because patients with CF are very susceptible to recurrent and chronic colonization of the lower respiratory tract, perhaps because of the unusual hyperviscous mucus they produce, it is very important to distinguish true infection from simple colonization. It is clear from other literature that there is a high prevalence of infection with *Pseudomonas aeruginosa* in these patients; it is found in the airways of all CF patients who die from their lung disease.<sup>5</sup> Colonization of the airways with *A. fumigatus* can lead to sensitization of the host. Sensitization can be manifested by an immediate wheal and flare skin test reactivity or development of *A. fumigatus*-specific antibody classes.

Lymphocyte transformation responses to *A. fumigatus* culture filtrates and mycelial extract antigen were examined in patients with CF and in CF patients with ABPA.<sup>14</sup> The responses in these groups were not significantly different when crude antigens were used. However, using purified fractions of ConA nonbinding *A. fumigatus* antigens, the lymphocyte stimulation results were more discriminatory between the two groups. It is of great interest that a semi-purified protein fraction is more specific to ABPA as a disease itself and as a complication of CF than simply as a reflection of sensitization. It appeared that a positive test was sufficient for diagnosis of ABPA in a patient with CF, but a negative test was not sufficient to eliminate this diagnosis.<sup>14</sup>

## A. FUMIGATUS ANTIGENS IN DIAGNOSIS

Much of the research in ABPA, with or without the complication of CF, has been hampered by difficulties in obtaining quality allergen preparations. Recombinant allergen produced with resultant defined allergenic activity has recently been used and recombinant *A. fumigatus* antigen I/a, a major allergenic protein of *A. fumigatus* has been cloned and developed in enough quantity to be purified.<sup>15</sup> This defined allergen has been used for clinical and serologic diagnosis of *A. fumigatus*-associated diseases and has recently been evaluated in patients with CF and ABPA, *A. fumigatus* allergic patients, and CF control patients without ABPA.<sup>15</sup> Serologic assays in this small group of patients allowed accurate identification of all patients with coexistent ABPA and CF, which suggests that use of purified recombinant allergen in serologic investigations may be a definite advancement in the diagnosis of CF ABPA patients.

## GENETICS AND ASSOCIATION OF CF AND ABPA

Finally, some of the recent studies on the cause of ABPA have shown fascinating results. Reports of familial occurrence of ABPA along with the increased incidence of ABPA in CF patients suggested the possible value of genetic testing for diagnosis.<sup>16</sup> Eleven individuals who met strict criteria for the diagnosis of ABPA and who had normal sweat electrolytes were studied for the entire coding region of the cystic fibrosis transmembrane regulator gene.<sup>16</sup> In this group, one patient carried two CF mutations and five patients were found to carry one CF mutation. The one patient had a mutation involving F508/R347H; of the other five, four had a mutation involving F508 and the remaining patient had a mutation involving R117H. The frequency of the F508 mutation in patients with ABPA was significantly higher than in 53 white patients with chronic bronchitis and in the general population. This set of findings in an admittedly small group suggests that the CF transmembrane conductor regulator gene can play an etiologic role in a subset of patients with ABPA. The fact that it is a CF gene is of specific interest with regard to patients carrying both diseases.<sup>16</sup> In a separate study, a highly significant association between the histocompatibility gene antigen (HLA)-DR2/5 serotypes and ABPA have been described.<sup>4</sup> In addition, it was shown that the DR2/5 genotypes identified in 16 ABPA patients match, without exception, two previously described HLA-DRB1 class II molecules that functioned to activate Asp f1-specific T helper 2 (Th2) cells derived from ABPA patients. The high correlation between HLA genotypes, T-cell reactivity, and disease suggest that several HLA-DR2/5 genotypes potentially convey a greater-than-average risk for ABPA in patients with CF and in patients with asthma. These findings suggest the need for further genetic studies, which may lead to new approaches in therapeutic intervention.<sup>3</sup>

## **PATHOPHYSIOLOGY**

Presently, it may be fair to hypothesize that the pathophysiology of ABPA is similar to any chronic late-phase allergic reaction to persistent allergen exposure in the bronchial tree. T-cell responses that lead to the production of cytokines that influence IgE synthesis and eosinophilia would be prominent in this reaction. In addition to the radiologic findings of transient pulmonary infiltrates, ongoing inflammation can lead to the development of bronchiectasis. These mechanisms certainly explain the utility of diagnostic criteria for ABPA that are described elsewhere in this publication by Knutsen et al.

## **DIFFERENTIAL DIAGNOSIS AND THERAPY**

The coexistence of ABPA and CF may lead to less defined diagnostic criteria for ABPA. Such criteria must distinguish colonization from sensitization and from infection. The simple presence of skin test reactivity, asthma, serum precipitins to *A. fumigatus*, infiltrates, and bronchiectasis are not sufficient to reach a definitive diagnosis of ABPA in CF. It may be that HLA testing or CFTR testing will be necessary to establish the diagnosis of ABPA in CF patients. If these tests cannot be obtained, a diagnostic and therapeutic trial of prednisone may be considered. It is important to consider ABPA in all CF patients because ABPA could further compromise the respiratory status of patients. Additionally, as new treatments for ABPA become available, diagnosing ABPA accurately in CF patients becomes even more important.

## **CONCLUSIONS**

CF is a disease of exocrine glands in which many patients secrete hyperviscous respiratory tract secretions. This abnormally tenacious sputum can lead to colonization of the airway with many microorganisms. In the case of colonization with *A. fumigatus*, the result can be one of several. The first would be a potentially immunologic sensitization of the patient with resultant production of various types of antibodies, which would not necessarily lead to any disease. On the other hand, colonization in some patients can lead to full-fledged development of a hypersensitivity airways disease known as allergic bronchopulmonary aspergillosis. ABPA is a disease featuring asthma, recurrent pulmonary infiltrates, severe airways inflammation, with the potential of developing bronchiectasis and pulmonary fibrosis. Several immunologic assays involving both immunoglobulin production of several types and B- and T-cell function have been carried out in an effort to distinguish these types of cases. In addition, there are problems with unreliable test results due to problems of allergen purification. Cloning genes of *Aspergillus* antigenic molecules may lead to more reliable results. Finally, several

approaches involving genetic testing suggest that there are particular HLA types and several specific genetic mutations in mucous transport protein production that are peculiar to ABPA and that may be shared with some patients with CF. The implications of these findings will be important for future approaches to the disease. Currently, early detection is critical so that appropriate therapy of ABPA in CF patients can be carried out.

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