

CELL-MEDIATED IMMUNITY IN ALLERGIC BRONCHOPULMONARY ASPERGILLOSIS

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Allergic bronchopulmonary aspergillosis (ABPA) is a hypersensitivity lung disease mediated by an allergic late-phase inflammatory response to *Aspergillus fumigatus* antigens characterized by markedly elevated allergen specific and total immunoglobulin E (IgE) levels and eosinophilia, and manifested by wheezing, pulmonary infiltrates, and pulmonary bronchiectasis and fibrosis. ABPA afflicts only a small percentage of susceptible atopic individuals with either asthma or cystic fibrosis (CF). ABPA is unique in that it is caused by *A. fumigatus*, a living fungus growing within the mucosal lumen of the bronchi that releases a multitude of proteins whose biologic function alters the host immune responses in addition to being immunogenic. Elucidation of the immunopathogenesis of ABPA has provided valuable information related to allergen-induced chronic asthma. More recently, immunogenetic studies have further contributed to the understanding of the immunopathogenesis of why certain individuals are susceptible to develop ABPA. Immunologic responses to *Aspergillus*, as well as to other microorganisms, may be categorized as either T helper (Th)1 or Th2 CD4+ T-cell responses.^{61, 110, 128} Th1 CD4+ T-cell cytokines, such as interferon gamma (IFN- γ), interleukin 2 (IL-2), and tumor necrosis factor (TNF)- β , enhance cytotoxic and macrophage activity (e.g., cellular immunity), whereas Th2 CD4+

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Table 1. Th1 AND Th2 CD4+ T CELLS

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From Romagnani S: Biology of human Th1 and Th2 cells. J Clin Immunol 15:121–129, 1995; with permission.

T-cell cytokines, such as IL-4, IL-5, IL-10, and IL-13, promote antibody synthesis (e.g., humoral antibody immunity) (Table 1). It is hypothesized that protective host immunity to *A. fumigatus* is a Th1 CD4+ T-cell response, whereas a Th2 CD4+ T-cell humoral response found in ABPA is nonprotective and leads to a hypersensitivity pulmonary lung disease.

TYPES OF ASPERGILLUS LUNG DISEASES

Aspergillus may cause five different lung diseases, depending on the host's immune response to *Aspergillus* antigens. Understanding the immune responses in these *Aspergillus*-induced pulmonary diseases gives us insight into the normal protective host defense to *A. fumigatus* and subsequently into the immunopathogenesis of ABPA.

1. Invasive *Aspergillus* pneumonitis. *Aspergillus* pneumonitis and septicemia occur primarily in those individuals who have either a T-cell or phagocytic immunodeficiency. The immunodeficiencies may be

congenital (e.g., severe combined immunodeficiency, leukocyte adhesion defect, and chronic granulomatous disease), acquired (e.g., AIDS or lymphoma), or iatrogenic (e.g., chemotherapy and immunosuppressive drugs for transplantation). Chronic granulomatous disease (CGD) is especially informative of the protective immune response to *Aspergillus*. In CGD, neutrophil and monocyte intracellular killing of bacteria and fungi within phagolysosomes is impaired because of a metabolic defect of the cytochrome b558 pathway.^{104, 105} The patients make appropriate anti-*Aspergillus* IgG antibodies and T-cell responses. The lung pathology demonstrates predominantly a neutrophil infiltrate with a slight increase of eosinophils. Granulomata and giant-cell formation is prominent, but this may be part of the underlying CGD condition. Thus neutrophil and macrophage phagocytic function is critical to the host's immune defense of *Aspergillus*.^{104, 105, 107-109, 121, 128} Patients with T-cell immunodeficiency are also susceptible to invasive *Aspergillus* infections, which may be related to defective cytolytic function. In CGD, administration of the Th1 cytokine IFN- γ augments phagocytic function and decreases the incidence of *Aspergillus* infection. In a murine model, Cenci et al²⁰ demonstrated that Th1 CD4+ cytokine pattern was protective of invasive aspergillosis, whereas mortality was increased when a Th2 cytokine pattern was predominant. Thus, the protective immune response to *Aspergillus* is a neutrophil and macrophage phagocytic and a Th1 CD4+ T-lymphocyte cellular response, which gives us insight that the predominant Th2 CD4+ T-cell response present in ABPA is ineffective in eliminating *Aspergillus* from airways.

2. Aspergilloma. In aspergilloma, *A. fumigatus* grows as a mycelial mat within a preexisting lung cavity, a fungus ball. There are markedly elevated anti-*Aspergillus* IgG antibody and T-cell responses. Furthermore, Platts-Mills' group has demonstrated that the T-cell response is a Th1 cytokine pattern (personal communication).

3. Hypersensitivity lung disease. This disease is a classical hypersensitivity alveolitis, characterized by fever, pulmonary infiltrates, hypoxia, and restrictive lung disease. The immune response to *Aspergillus* antigens, typically *Aspergillus flavus*, are T-cell and IgG antibody responses.

4. Asthma. Approximately 25% patients with asthma and 60% of CF patients develop IgE antibodies to *Aspergillus*. When *Aspergillus* spores are inhaled, they trigger an IgE-mediated allergic inflammatory response in the bronchial airways leading to bronchial obstruction (e.g., asthma). The immune response to *Aspergillus* antigens in both asthma and ABPA is characterized by a Th2 CD4+ T-cell response. This suggests that other host factors are important in predisposing atopic asthmatic patients to develop ABPA.

5. ABPA. ABPA is characterized by wheezing (asthma); "allergic" pulmonary infiltrates; markedly elevated serum IgE levels; positive *Aspergillus* IgE skin-prick test; positive *Aspergillus* precipitins; elevated IgE, IgA, and IgG anti-*Aspergillus* antibodies; and elevated pulmonary and

blood eosinophilia. If the allergic eosinophil or T-cell pulmonary inflammation persists, bronchiectasis and pulmonary fibrosis may develop.

IMMUNOPATHOGENESIS OF ABPA

Model of ABPA

ABPA occurs in approximately 1% to 2% of asthmatic^{39, 59, 98, 114} and 7% to 10% of CF patients.* The immune response to *Aspergillus* antigens in allergic asthmatic and CF patients as well as in patients with ABPA is a CD4+ Th2 T-cell response. The response in ABPA, however, appears to be quantitatively greater than in allergic asthma or patients with CF. In the proposed model of the immunopathogenesis of ABPA (Fig. 1), *A. fumigatus* spores are inhaled into the bronchial airway, where they are trapped by the luminal mucus and germinate, forming mycelia. *A. fumigatus* mycelia release antigens that are processed by antigen-presenting cells (APC) and presented to T cells. The T-cell response to *Aspergillus* antigens is skewed toward a Th2 CD4+ T-cell response,

*References 10, 31, 47, 48, 56, 57, 77, 92, 127, 135, and 140.

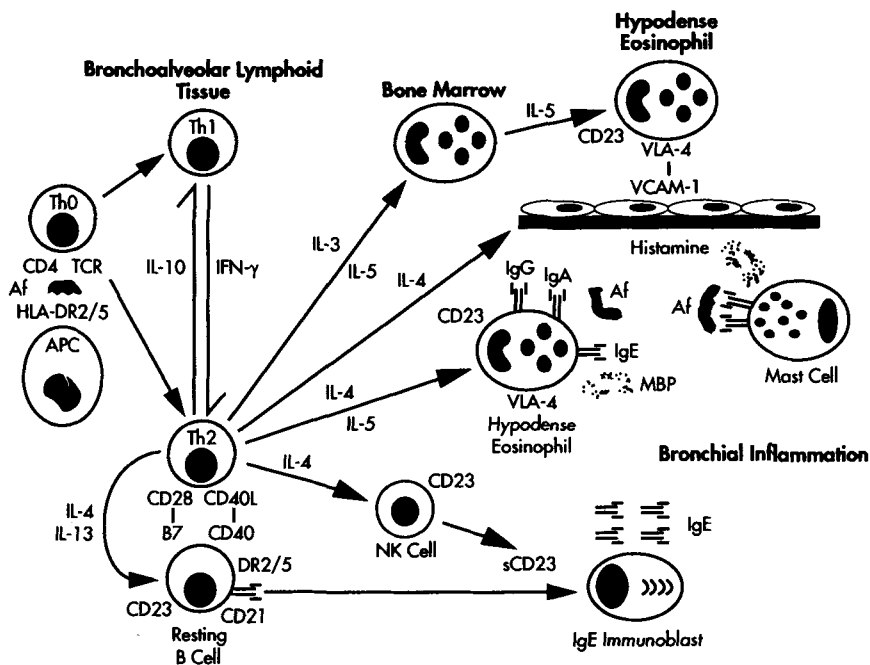


Figure 1. Hypothesized model of the immunopathogenesis of ABPA.

manifested by IL-4, IL-5, IL-10, and IL-13 cytokine synthesis and secretion. In addition, IL-10 down regulates Th1 CD4+ T cells, further skewing the responses to a Th2 pattern. IL-4 and IL-13 induce IgE isotype switching of B cells.^{14, 33, 34, 80, 115, 116, 129, 141} Although IL-4 is necessary for IgE isotype switching, it is not sufficient. In order for IgE secretion to occur, a second signal mediated by cognate T- and B-cell interactions via CD40L-CD40 and CD28-B7 ligand interactions occurs.^{14, 80, 116, 141} IgE binding factors, such as CD23 and soluble CD23 (sCD23), augment B-cell IgE synthesis.* In addition, T cell-CD23 and B cell-CD21 cognate ligation also augments B-cell IgE synthesis.^{5, 13, 103} Perhaps an important source of CD23 in augmentation of IgE synthesis is natural killer (NK) cells.^{113, 139} Recently, Diaz-Sanchez et al²⁸ identified another B-cell signaling pathway, CD58 (LFA-3), that also promotes IgE isotype switching and B-cell IgE synthesis. Consistent with a predominant Th2 CD4+ T-cell humoral response, IgG and IgA specific antibodies also are produced in addition to anti-*Aspergillus* IgE antibodies. As outlined, this T- and B-cell and APC immune response initially occurs within the bronchial lymphoid tissue (BALT) compartment. As is typical of the mucosal immune system, some of the T and B cells traffic to the systemic lymphoid system.

The effector cells responsible for the allergic inflammatory responses in ABPA are predominantly mast cells and eosinophils. In this model, when *Aspergillus* antigens cross-link IgE bound to mast cells, mast cells release a variety of mediators, such as histamine, leukotrienes, and PAF, which induce bronchial smooth muscle contraction and vascular permeability.²³ A number of mast cell cytokines, such as leukotriene B4 and PAF, are chemoattractant for eosinophils. In addition, chemokines, such as eotaxin, RANTES and MCP-3, derived from a variety of cell types such as epithelial and phagocytic cells, induce eosinophil chemotaxis and activation.³ Basophil hyperreactivity with increased histamine release also has been reported in ABPA, probably caused by IL-4 stimulation.¹⁰⁶ Th2 CD4+ T cells secrete the cytokines IL-3 and IL-5 that promote bone marrow maturation of eosinophils and activation of eosinophils.^{9, 35, 36, 51, 136} Furthermore, IL-4 induces expression of VCAM-1 on vascular endothelial cells and its ligand VLA-4 on T cells and eosinophils. Recently, studies have demonstrated selective expression of the eotaxin receptor, chemokine receptor 3 (CCR3), on eosinophils, basophils, and Th2 T cells, and CCR3 expression is up regulated by IL-4 polarizing conditions.^{44, 112} Thus, both chemotactic and cell surface adhesion molecules promote recruitment of Th2 T cells and eosinophils within the allergic inflammatory site.^{122, 137} Eosinophils possess Fc receptors for IgE, IgG, and IgA, and IL-4 also induces increased expression of the low affinity Fc IgE receptor, CD23, in eosinophils.³⁸ In addition, IL-4 and IL-5 induce Fc receptors for IgA.¹⁶ In ABPA, significant amounts of *Aspergillus*-specific IgA antibodies are produced within BALT and are present within the bronchial mucus. Thus, both IgE and IgA anti-*Asper-*

*References 4, 13, 27, 29, 37, 100, 103, 113, 123, 138, and 139.

gillus antibodies bound to their Fc receptors on eosinophils trigger mediator release when they engage allergen.^{1, 2, 26} When eosinophil-bound IgE, IgA, and IgG are cross-linked by *Aspergillus* antigens, the eosinophils are triggered to secrete inflammatory mediators, such as major basic protein and eosinophil-derived neurotoxin.^{2, 35, 36} The model proposes that *A. fumigatus* growing within the airways releases persistent high levels of allergens in the airway and lung parenchyma that produce a heightened and prolonged late-phase allergic inflammatory response.

Lung Specimens

Examination of lung specimens and bronchoalveolar lavage (BAL) fluid and cells has yielded invaluable information concerning the pathogenesis of ABPA.

IgE

Greenberger et al⁴¹ demonstrated that specific anti-*A. fumigatus* IgE and IgA antibodies are produced within BALT. In contrast, IgG anti-*Aspergillus* antibodies obtained from BAL fluid were predominantly exudative, derived from the peripheral systemic lymphoid system. Our group^{117, 118} demonstrated lymphoid follicles that stained with anti-IgE in a lung biopsy of patient with ABPA, indicating in vivo IgE-bearing B cells and immunoblasts. Thus, BALT B cells have been driven to IgE immunoblasts. Greenberger et al⁴¹ further demonstrated that the total IgE in the systemic lymphoid system was composed of only a fraction of the specific anti-*Aspergillus* IgE antibodies. This implies that the CD4+ Th2 T cells have trafficked to the systemic immune system and have activated other clones of B cells, in addition to those with *Aspergillus* specificity.

Cells in BALT

The analysis of the cells obtained from BAL in ABPA reveals an admixture of alveolar macrophages, eosinophils, and lymphocytes,⁴¹ similar to those found in asthma.¹³¹ Eosinophil infiltration predominates both in BAL and lung tissue, as is evident on lung biopsy.¹¹⁷ In addition, the eosinophils are activated and have released their mediators, such as major basic protein. Thus, eosinophils are the major effector cell causing inflammation. Lymphocytes found in BAL fluid are composed of T, B, and NK cells. The T cells are an admixture of CD4+ and CD8+ T cells, approximately in a 2:1 ratio. Interestingly, we have observed increased CD23+ NK cells and CD23+ CD4+ T cells obtained from BAL of ABPA patients. This increased CD23 expression is probably mediated by IL-4. The significance of CD23+ T cells is probably T cell CD23 and B cell CD21 T-B ligand-counterligand interaction and augmentation of IgE synthesis. Similarly, CD23+ NK cells are an important source of sCD23

or NK CD23-B cell CD21 interaction, increasing immunoblast IgE secretion. Within the lung parenchyma, our group demonstrated the presence of CD4+ and CD8+ T cells in an approximate 2:1 ratio.

Immunosuppressive Properties of *Aspergillus* Antigens

In the bronchial mucus, *Aspergillus* mycelia are quite prominent. Our group also demonstrated mycelial fragments within the lung tissue of patients with ABPA, suggesting that *Aspergillus* antigen load is probably extremely elevated in these patients.¹¹⁷ Furthermore, many of these *Aspergillus* proteins, such as Asp f1, proteases, or heat-shock protein, have biologic function that alters the host immune responses in addition to being immunogenic. *Aspergillus* antigens have demonstrated suppression of macrophages via inhibition of nuclear activation proteins NF- κ B and AP-1.^{91, 131} Our group observed that Asp f1 and mitogillin, cytotoxins that inhibit mRNA, suppressed T-cell lymphoproliferative responses to tetanus toxoid stimulation (Fig. 2). In this experiment, mitogillin, which differs from Asp f1 by only one amino acid, was nearly tenfold more immunosuppressive than Asp f1. There are also species differences of the *Aspergillus* proteins that are immunosuppressive. Thus, the host-environment interaction must be considered in the understanding of the immunopathogenesis and immunogenetics of ABPA.

Systemic T- and B-Cell Responses

Examination of peripheral blood T- and B-cell function has also revealed a predominant Th2 CD4+ T-cell activation to *Aspergillus* allergens in ABPA. Our group has focused mainly on cystic fibrosis patients, who have been especially informative in elucidating the B- and T-cell responses to *Aspergillus*. Colonization with *A. fumigatus* occurs with increasing age in patients with CF, being as high as 60% in older patients. In addition, approximately 25% of patients with CF are atopic. Therefore, there is an increased frequency of *Aspergillus* sensitization and development of IgE and IgG anti-*Aspergillus* antibodies in patients with CF; however, only approximately 7% of these patients develop ABPA. In contrast, approximately 25% of asthmatic patients have IgE anti-*Aspergillus* antibodies and 10% have precipitating antibody, but the incidence of ABPA is approximately 1% to 2%. Study within the population with CF affords the opportunity to examine and compare the immune responses in ABPA versus *Aspergillus*-sensitized non-ABPA groups.

B-Cell Antibody Synthesis

Extremely elevated total serum IgE concentrations and elevated IgE anti-*Aspergillus* antibodies manifest ABPA. There appears to be a

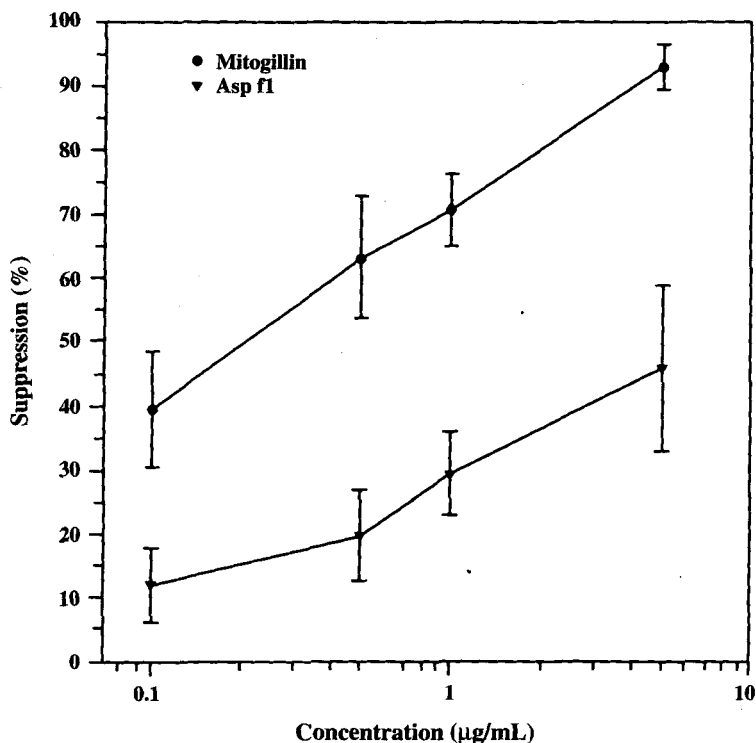


Figure 2. Immunosuppressive properties of Asp f 1 and mitogillin. Asp f 1 and mitogillin-suppressed normal control tetanus toxoid stimulated lymphoproliferation in a dose-response fashion. Mitogillin caused 50% inhibition of tetanus-stimulated proliferation at nearly one tenth the concentration compared with Asp f 1 inhibition.

quantitative, though not qualitative, difference of the B-cell IgE response in ABPA compared with *Aspergillus*-sensitized non-ABPA atopic patients. The heightened total and specific anti-*Aspergillus* IgE antibody responses have been described by our group^{47, 48, 52} and others (Fig. 3).^{18, 30, 31, 40, 60, 124} In ABPA, there are also increased amounts of IgG and IgA anti-*Aspergillus* antibodies, reflective of the Th2 humoral versus Th1 cellular response to *Aspergillus* antigens in these patients.^{11, 43, 53, 68, 78, 81, 99, 125} In Figure 6, ABPA patients develop extremely elevated amounts of IgE, IgG, and IgA antibodies to a variety of *Aspergillus* proteins demonstrated by immunoblot studies.⁵³ Although other *Aspergillus*-exposed groups develop IgE, IgG, and IgA anti-*Aspergillus* antibodies, there is a quantitative increase of IgE anti-*Aspergillus* antibodies in ABPA patients (Table 2). This in vivo activation of IgE immunoblasts is demonstrated by the observation that B cells obtained from patients with ABPA spontaneously synthesize increased amounts of IgE in vitro compared with *Aspergillus*-sensitized non-ABPA patient groups (see Table 2).⁵⁴



Figure 3. IgE (solid bar), IgG (open bar), and IgA (striped bar) immunoblot scores of *Aspergillus fumigatus* proteins in patients with ABPA and CF. Immunoblot score represents the sum of all the densities of the immunoblot bands for each isotype. Data is presented as mean $\pm$ SD. * $P < 0.01$ by ANOVA; post hoc analysis ABPA versus ST+/PPT+ not significant but significant ($P < 0.05$) compared with other groups. (From Knutsen AP, Hutcheson PS, Mueller KR, et al: Serum IgE and IgG anti-*Aspergillus fumigatus* antibody in cystic fibrosis patients with allergic bronchopulmonary aspergillosis. J Lab Clin Med 116:724-727, 1990; with permission.)

Table 2. IN VITRO IgE SYNTHESIS BY B CELLS OF PATIENTS WITH CF AND ABPA

Rights were not granted to include this data in electronic media. Please refer to the printed journal.

From Knutsen AP, Mueller KR, Hutcheson PS, et al: T- and B-cell dysregulation in cystic fibrosis patients with allergic bronchopulmonary aspergillosis. Clin Immunol Immunopath 55:129-138, 1990; with permission.

T-Cell Immunity

Because the observed *in vivo* IgE B-cell activation probably represents T-cell dysregulation, we began to examine T-cell immunity in ABPA. Our group reported that there was a significant, though only slight increase of CD4+ T cells in patients with ABPA (Table 3).⁵⁸ Examination of CD8+CD11b+ T cells, suppresser phenotype, revealed normal percentages of these cells in ABPA. Indeed, previous groups had demonstrated that Con-A-induced T-suppressor function was normal in patients with ABPA. Furthermore, our group demonstrated that ABPA patients' T cells suppressed normal control B-cell IgG synthesis *in vitro* (Fig. 4). Decreased T-suppressor cell number or function is not responsible for the increased Th2 CD4+ T-cell immune response in ABPA. On the contrary, this observed increased T-suppressor function probably is an attempt to down regulate the inflammatory reaction. During flares of ABPA, Brown et al¹⁷ reported increased T-cell activity manifested by increased plasma levels of soluble IL-2R, sCD25.

We^{58, 119} and others^{73, 89} have observed T-cell lymphoproliferative responses to crude *Aspergillus* extracts (Table 4). In the past few years, several groups have reported T- and B-cell responses to purified *Aspergillus* antigens, which has yielded significant understanding of T-cell, APC- and B-cell interactions.* Interestingly, lymphoproliferative responses to a control antigen, tetanus toxoid, were decreased in patients with ABPA, suggestive of decreased or suppressed Th1 T-cell responses. Because ABPA T cells suppressed control B-cell IgG synthesis, we interpreted

*References 6-8, 19, 24, 25, 62-64, 68, 71, 74, 79, 88, 94, 95, and 132.

Table 3. T-CELL SUBPOPULATIONS IN PATIENTS WITH CF AND ABPA*

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Figure 4. In vitro IgG synthesis by pokeweed mitogen-stimulated control B cells cocultured either with control T cells (Tc) or with control plus CF T cells (Tp + Tc). Addition of T cells from subjects with CF and ABPA significantly suppressed control B-cell IgG synthesis compared with that in subjects with CF who had *A. fumigatus* ST/PPT-positive and *A. fumigatus*-negative results ($P < 0.025$ by chi-square analysis). (From Knutsen AP, Slavin RG: In vitro T-cell responses in cystic fibrosis patients with allergic bronchopulmonary aspergillosis. *J Lab Clin Med* 113:428–435, 1989; with permission.)

Table 4. IN VITRO LYMPHOPROLIFERATIVE RESPONSES TO *A. FUMIGATUS* AND TETANUS TOXOID IN PATIENTS WITH CF AND ABPA*

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From Knutsen AP, Slavin RG: In vitro T-cell responses in cystic fibrosis patients with allergic bronchopulmonary aspergillosis. *J Lab Clin Med* 113:428–435, 1989; with permission.

this as probably secondary to increased T-suppressor activity. These results explain the earlier observations of anergy to *Aspergillus* antigens by delayed hypersensitivity skin tests.

Subsequently, *Aspergillus*-specific T-cell responses on B-cell IgE synthesis were examined. In these studies, T cells were stimulated with a crude *Aspergillus* extract for 48 hours, and the *Aspergillus*-stimulated T-cell supernatant was then cocultured with atopic control B cells for 10 days. In these studies, supernatants obtained from *Aspergillus*-stimulated T cells from patients with ABPA enhanced B-cell IgE synthesis (Table 5).⁵⁴ Subsequently, T-cell lines were generated from patients with ABPA with a single immunodominant antigen, Asp f1.⁵⁵ In these studies, the T-cell phenotype was exclusively CD4+CD25+HLA-DR+ T cells. CD23+CD4+ T cells were only 1.3% (Table 6). The cytokine profile of these T cells was IL-4+, IFN- γ -, or IL-2- (e.g., Th2 CD4+ T cells). Indeed, the lymphoproliferative stimulus for the Asp f1 T-cell lines was predominantly IL-4 mediated (e.g., an autocrine pattern) (Fig. 5). Subsequent studies performed by Chauhan in our group demonstrated that T-cell clones obtained from asthmatic patients with ABPA were either Th2 (IL-4+, IFN- γ -, IL-2-) or Th0 (IL-4+, IFN- γ +) T cells.^{21, 22} Furthermore, tetanus toxoid generated T-cell clones that were the expected Th1 phenotype, namely IL-4-, IFN- γ +. Atopic CF patients, however, also generate a IL-4 Th2 T-cell cytokine pattern to Asp f1. Thus the Th2 T-cell response in ABPA is specific to *Aspergillus* antigens and not a global Th2 cell response to all antigens. Because the Th2 T-cell cytokine pattern of ABPA and *Aspergillus* IgE sensitive patients is similar,

Table 5. ENHANCEMENT OF IN VITRO ALLOGENEIC B-CELL IgE SYNTHESIS BY *ASPERGILLUS*-STIMULATED T-CELL CULTURE SUPERNATANTS FROM PATIENTS WITH CF AND ABPA*

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Table 6. ASP F1 T-CELL LINES FROM PATIENTS WITH ABPA

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From Knutsen AP, Mueller KR, Levine AD, et al: Characterization of Asp f1 CD4+ T-cell lines in allergic bronchopulmonary aspergillosis. *J Allergy Clin Immunol* 94:215–221, 1994; with permission.

we hypothesize that there is either increased frequency or activity of *Aspergillus*-specific Th2 T cells or increased sensitivity to Th2 cytokines.

TCR

Our group and others demonstrated that there is a quantitative increase of the Th2 T-cell responses in ABPA compared with *Aspergillus*-sensitized non-ABPA patients.^{20, 55} To determine why this is so, our group investigated whether there is unique TCR recognition (T-cell epitopes), TCR-V β restriction, or HLA-class II restriction that would promote enhanced Th2 responses. Analysis of T-cell epitope mapping has revealed that there are three immunodominant regions of the Asp f1 protein in patients with ABPA that is recognized by TCR.²¹ Our findings are similar to those found in other allergen models. O'Hehir et al has evaluated T-cell responses to purified house dust mite allergens. In their model, T-cell clones were generated from atopic and nonatopic individuals.⁹⁶ Significantly, the T-cell clones from nonatopic individuals proliferated in response to allergen stimulation but did not support IgE synthesis, whereas the T-cells clone from atopic patients did. Furthermore, TCR

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Figure 5. Asp f1-specific T-cell line was rested for 2 to 17 days after IL-2 and Asp f1 stimulation (A). Supernatant IL-4 concentration was measured serially. Significant amounts of IL-4 were synthesized for up to 10 to 14 days of rest. Monoclonal anti-IL-4 antibody inhibited Asp f1-stimulated lymphoproliferation of antigen-specific T-cell lines in a dose-response manner (B). Maximum inhibition was 84% at 5% anti-IL-4 antibody concentration (v/v) in this experiment and 78% in a second experiment. Anti-IL-2 had little inhibition of proliferation indicating an IL-4-dependent autocrine lymphoproliferative response. (From Knutsen AP, Mueller KR, Levine AD, et al: Characterization of Aspf1 CD4+ T-cell lines in allergic bronchopulmonary aspergillosis. *J Allerg Clin Immunol* 94:215–221, 1994; with permission.)

epitope mapping studies revealed a limited number of epitopes reacting with TCR,^{46, 97} TCR-V β restriction or usage,^{49, 133} and HLA class II restriction.¹³³ Four major V β chains, V β 3, 6, 13, and 14, react to Asp f1. We have not yet evaluated T-cell epitopes or V β usage in non-ABPA patients, but they will probably be similar to those found in patients with ABPA. This, however, does allow us to evaluate whether mutations of the epitope might alter the T-cell cytokine or lymphoproliferative responses for potential immunotherapy of ABPA.

HLA-DR

Chauhan et al^{21, 22} have indeed identified that there is HLA-DR2 and HLA-DR5 restriction in patients with ABPA (Table 7). Furthermore, within HLA-DR2 and HLA-DR5 there are restricted genotypes. On the other hand, 40% to 44% of non-ABPA atopic *Aspergillus*-sensitive individuals have the HLA-DR2 or DR5. These results are similar to that found with purified house dust mite allergens.^{76, 126, 130} Thus, certain genotypes of HLA-DR2 and DR5 are necessary but not sufficient to cause ABPA.

Table 7. HLA-DR RESTRICTION OF APCs OF Asp f1 TO T-CELL CLONES IN PATIENTS WITH ABPA*

Rights were not granted to include this data in electronic media. Please refer to the printed journal.

*From Chauhan BA, Knutsen AP, Hutcheson PS, et al: T-cell subsets; epitope mapping, and HLA-restriction in patients with allergic bronchopulmonary aspergillosis. Reproduced from *The Journal of Clinical Investigation*, 1996, 97, 2324–2331, by copyright permission of The American Society for Clinical Investigation.

Furthermore, Chauhan et al²² demonstrated that Asp f1 allergen has a low affinity of binding to the HLA-DR, which is consistent with a Th2 T-cell response previously reported by others; strong antigen HLA-DR-Ag-TCR affinity binding favors a Th1 cellular response, and low-affinity binding favors a Th2 humoral response.^{32, 76, 101, 102, 126}

IL-4R

IL-4 receptor alpha chain (IL-4R α) mutations were evaluated in collaboration with Dr. Talal Chatila. Mutations of IL-4R α have been identified in atopic individuals with elevated IgE levels.^{45, 111} These mutations increase IL-4 and IL-4R interactions, promoting B-cell IgE isotype switching. In subjects with ABPA, there were increased mutations of IL-4R α , similar to that found in other atopic conditions, such as atopic dermatitis and asthma. There were, however, increased homozygous mutations of IL-4R, perhaps partially accounting for the increased IgE response in ABPA. IL-4 stimulation also induces increased expression of a number of receptors, including CD23 on B cells, eosinophils and NK cells, VLA-4 and CCR-3 on eosinophils and T cells, and VCAM on endothelial cells (see Fig. 1). In addition to promoting IgE synthesis, increased IL-4R activity would then also favor increased allergen triggering of eosinophil degranulation and increased eosinophils and T cells into the allergic inflammatory site.

IL-4 Promoter

Several groups have reported mutations of IL-4 promoter either in upstream or downstream regions in atopic individuals^{15, 120, 134}; however, Blumenthal et al¹² have not identified any mutations. In preliminary studies, our group has not been able to identify mutations of the IL-4 promoter in patients with ABPA.

CFTR

Because ABPA is found in highest incidence in atopic CF patients, our group examined CFTR mutations in patients with ABPA and asthma in collaboration with Patrice Miller. Clearly, there was increased frequency of heterozygous mutations of the CFTR gene in these patients with asthma (Table 8).⁸⁴ We hypothesize that in CF, the abnormal mucus promotes the trapping of *Aspergillus* spores within the bronchial airway, permitting and perhaps promoting *Aspergillus* mycelia growth. The significance of the heterozygous CFTR mutation in patients with asthma is unclear as to their mucus properties but is being investigated. Thus the abnormal mucus may allow increased *Aspergillus* colonization within the bronchial airway of patients with CF and asthma, and in a genetically susceptible individual, stimulate a Th2 T-cell response and subsequent ABPA.

Table 8. MUTATIONS OF CFTR GENE IN ASTHMATIC PATIENTS WITH ABPA

Rights were not granted to include this data in electronic media. Please refer to the printed journal.

From Miller PW, Hamosh A, Macej M Jr, et al: Cystic fibrosis transmembrane conductance regulator (CFTR) gene mutations in allergic bronchopulmonary aspergillosis. *Am J Hum Genet* 59:45–51, 1996; with permission.

Animal Models of ABPA

Murine animal models of ABPA have elucidated information concerning the T-cell responses to *A. fumigatus* antigens.^{50, 65–67, 70, 72, 75, 82, 83, 85–87, 90} Foremost, Kurup's group has demonstrated that there are murine strain differences in the response to *A. fumigatus* allergen challenges.⁶⁷ This response is analogous to the human condition in which most individuals infected with *Aspergillus* develop a brisk Th1 CD4+ T-cell and IgG antibody response; only a small percentage of atopic asthmatic individuals who develop an exaggerated Th2 CD4+ T-cell response develop ABPA. After *Aspergillus* allergen challenge in their murine model of ABPA, there are increased BAL lymphocytes and eosinophils. They have demonstrated the importance of IL-5 in the development of eosinophilia in the peripheral blood and of IL-4 in the development of IgE anti-*Aspergillus* antibodies, which correlates with bronchial eosinophilia and bronchial hyperreactivity. Recently, Kurup et al⁶⁹ demonstrated that the TCR-Asp f1 epitope–HLA-DR recognition was also important in the Th2 versus Th1 cytokine synthesis T-cell response. In this study, Asp f1 peptide 5 (amino acids 53–68) stimulated T-cell secretion of IL-4 in four strains of mice, whereas four other peptides stimulated secretion of IFN- γ and IL-2. Interestingly, stimulation of IL-5 secretion was induced by all five peptides in two strains of mice. Kurup's group has demonstrated the genetic susceptibility is composed of HLA-DR Asp f1 epitope–TCR interaction in development of a Th2 CD4+ T-cell immune response to *Aspergillus* antigens in a murine model of ABPA.

CONCLUSION

A quantitative increased Th2 CD4+ T-cell response to *Aspergillus* in both the BALT and systemic immune systems (Display box 1) characterizes ABPA. What is key in the immunopathogenesis is that the BALT is exposed to high levels of *Aspergillus* allergens, perhaps because of abnormal mucous properties due to CFTR mutations. Antigen presentation to T cells is characterized by HLA-DR 2 and DR5 restriction of low-affinity antigen binding. In addition, there is restricted TCR-V β usage. Thus

there is an immunogenetic susceptibility to develop ABPA that resides within the HLA-DR-Ag-TCR signaling of the T cells toward a Th2 CD4 + T-cell response. In addition, there may be increased sensitivity of B cells and eosinophils to IL-4 stimulation because of mutations of IL-4R α accounting for the allergic inflammatory response to *Aspergillus*.

Summary of Immunopathogenesis of ABPA

- $\uparrow$ Th2 CD4 + T-cell response to *A. fumigatus*
 - BALT mucosal immune response
 - $\uparrow$ IL-4, IL-13, IL-10, IL-5
 - $\uparrow$ IgE total and anti-*Aspergillus* antibodies
 - $\uparrow$ IgA & IgG anti-*Aspergillus* antibodies
 - Eosinophilia
 - $\uparrow$ VLA-4 and VCAM integrins
 - migration of eosinophils and lymphocytes into inflammatory lesions
- HLA-DR 2/5 restriction
 - genotype restriction
 - low-affinity binding
- TCR-V β restriction (3, 6, 13, 14)
- $\uparrow$ IL-4R homozygous β chain mutations (preliminary data)
 - $\uparrow$ IL-4 activity
- Mast-cell degranulation
 - basophil hyperreactivity
- $\uparrow$ sCD25 during ABPA flares
- $\uparrow$ T suppressor function
- Anergy to *A. fumigatus* by DTH
- CFTR heterozygous mutations
 - ? effect on mucous properties

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