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Immunology of Allergic Bronchopulmonary Aspergillosis

Viswanath P. Kurup*Allergy-Immunology Division, Department of Medicine, Medical College of Wisconsin, and
Research Service, Veterans Affairs Medical Center, Milwaukee, Wisconsin, USA*

Abstract

Allergic bronchopulmonary aspergillosis (ABPA) is a hypersensitivity lung disease caused predominantly by the ubiquitous fungus *Aspergillus fumigatus*. ABPA is characterized by eosinophilia, fleeting pulmonary infiltrates, central bronchiectasis, elevated serum IgE and *Aspergillus* specific IgG and IgE. The pathogenetic mechanism implicated in ABPA is not completely understood. The cytokine response detected in ABPA patients is of a CD4+ Th2 type as evidenced by the production of IL-4, IL-5, and very little or no IFN- γ on stimulation of T-lymphocytes with *Aspergillus* antigens. Animal model studies using wild type and gene knockout mice indicate a more precise mechanism of lung injury in antigen exposed animals. IL-4 knockout mice invariably showed a predominant Th1 response. B-cell deficient and IgE knockout mice exposed to *A. fumigatus* antigens showed airway response similar to wild type mice indicating a lesser role for IgE and other antibodies in the pathogenesis of murine experimental ABPA. RAG negative mice failed to show airway hyperreactivity response, although airway hyperreactivity was induced in naïve RAG negative animals when T-cells from wild type *Aspergillus* immunized mice were transferred. The results of these studies indicate a multifactorial immunopathogenesis in ABPA, which include T-cells, IgE, eosinophils, mast cells, and various cytokines and chemokines.

Key words : *Allergic bronchopulmonary aspergillosis, Aspergillosis, Immunology, Cytokine response, Animal model, Multifactorial immunopathogenesis.*

Introduction

Allergic bronchopulmonary aspergillosis (ABPA) is a disease primarily occurring in patients with asthma resulting from inhalation of *Aspergillus* antigens particularly *A. fumigatus*. *A. fumigatus* (*Af*), a ubiquitous fungus, has been implicated in a number of airway diseases ranging from saprophytic colonization to extensive life threatening invasion with a multitude of intermediary conditions resulting from host and environmental factors (1,2). The development of allergy to *Aspergillus* may depend on the mode and frequency of exposure and genetic and other predisposing factors of the host. Frequently, sensitization of *Af* allergens also occurs along with one or more of other aeroallergens. Among the various members of the genus, *Af* is associated with over 80% of all human syndromes caused by aspergilli. Other species that have been associated with aspergillosis include *A. flavus*, *A. nidulans*, *A. niger*, *A. terreus*, *A. oryzae*, and *A. ochraceus* (3-5). The predominance of *A. fumigatus* over other aspergilli in human disease may be due to its adaptability to human body temperature, comparative resistance to oxidative killing, and small spore size capable of reaching the small airways.

Aspergillus fumigatus is a thermotolerant fungus that grows at a wide range of temperatures from 15 °C to 53 °C. *Aspergillus* spores have been demonstrated from all geographical and climatic conditions. The spores have been recovered from potting soil, mulches, grass, wood chips, decaying vegetables, compost piles, sewage treatment facilities, bird droppings, moldy hay, and outdoor as well as indoor air. There is no consensus about the extent to which exposure to larger quantities of *Af* may contribute to ABPA (1,6). It is also not clear whether the size of the spores is a factor in the causation of ABPA. However, exposure to high concentrations of *Af* spores from dumpsites, bird droppings, and smoking moldy marijuana have been reported to cause ABPA (7-9). Increasing mold counts in the patients' environment have been correlated with increasing number of exacerbations of ABPA. Similarly it has been reported

that worsening in airway obstruction is correlated with the increase in *Af* spores in the immediate environment of ABPA patients (10,11). However, other investigators have not found such a correlation (12). There is agreement among experts that the host immune status and response to *Af* are more important than environmental factors in the development of ABPA. Asthma and cystic fibrosis predispose to the development of ABPA and about 1-2% of all asthmatic patients and at least 10% of cystic fibrosis patients present with ABPA.

ABPA has been described as an immunological disease with diagnostic characteristics of immediate and late cutaneous reactivity to *Af* antigens, elevated total serum IgE concentration and precipitating antibodies to *Af*. Elevated quantities of *Af* specific serum IgE and IgG when compared with sera from *Af* skin test positive asthmatics, central bronchiectasis with normal distal tapering, eosinophils in peripheral blood and pulmonary infiltrates are classical findings. Upto one third of ABPA patients show dual response of immediate hypersensitivity followed by a late phase reaction of erythema and induration to *Af* antigens if intradermal skin tests are carried out.

Immunology of ABPA

ABPA patients demonstrate elevated total serum IgE concentrations and elevated anti-*Af* IgE antibodies. The available evidences indicate a major difference in the IgE response in ABPA compared to *Af* sensitized non-ABPA atopic patients (13-18). ABPA patients also have increased amounts of anti-*Af* antibodies belonging to IgG and IgA isotypes (18-22). The response in ABPA patients is predominantly of a Th2 type as evidenced by elevated IgE (17,23-29). The B-cells from ABPA patients spontaneously synthesize increased amounts of IgE *in vitro* compared to *Af* sensitized non-ABPA patient groups indicating increased activation of IgE secreting B-cells in the peripheral blood (23).

ABPA occurs in approximately 1-2% of asthmatic and 7-10% of cystic fibrosis patients (24-27). In ABPA, *Af* spores are inhaled into the bronchial airway, where they are trapped by the luminal mucus and germinate. The hyphae of the germinating spores of *Af* release antigens that are processed by antigen-presenting cells such as residential macrophages, B-cells, etc; and present to T cells. The T-cell response to *Af* antigens is initially skewed towards a Th2 response, manifested by production of interleukin-4 (IL-4), IL-5, IL-10, and IL-13 (30-37). IL-4 and IL-13 induce IgE isotype switching of B-cells (31,36). Though IL-4 is necessary for IgE isotype switching, IL-4 alone is not sufficient. In order for IgE secretion to occur, a second signal mediated by cognate T- and B-cell interactions *via* CD23-CD21 and CD40-CD40L ligand interactions is essential (38-40). In addition, IgE binding factors, such as CD23 and soluble CD23 (sCD23), augment B-cell IgE synthesis. Furthermore, cognate interaction between CD23 and CD21 enhance the synthesis of IgE by B-cells (41-43). It has been shown that the important source of CD23 in augmentation of IgE synthesis is natural killer (NK) cells (41-43). Recently, Diaz-Sanchez *et al* (44) identified another B-cell signaling pathway where CD58 (LFA-3) also promotes IgE isotype switching and B-cell IgE synthesis. Consistent with a predominant Th2 response, IgG and IgA specific antibodies are also produced in addition to anti-*Af* IgE antibodies. As outlined above the interaction of T-cells, B-cells and antigen presenting cells and the resulting immune response takes place initially in the bronchoalveolar lymphoid tissue (BALT). As is typical of the mucosal immune system, some of the T- and B-cell also traffic to the systemic lymphoid tissues. Thus, the current evidences, although incomplete, definitely point to a complex immunopathogenesis in ABPA.

The *in vivo* activation of IgE bearing B-cells observed are probably due to T-cell dysregulation. A slight, but significant increase of CD4+ T-cells in patients with ABPA has been reported (19). Examination of suppresser phenotypes representing CD8+ CD11b+ T-cells revealed normal ratios of these cells in ABPA patients. It has been reported that the T-cells of ABPA patients suppressed the *in vitro* IgG synthesis by B-cells from normal subject's (19). Thus, decreased T suppresser cell number and/or function is not responsible for the increased Th2 CD4+ T-cell immune response in ABPA. On the contrary, this observed increase in T-suppresser function probably is an attempt to down-regulate the inflammatory reaction. Activated T-cells are present in the blood of patients with remission and flare stages of ABPA. The increased T-cell activity also was found to increase plasma levels of soluble IL-2R (sCD25) (45).

T-cell lymphoproliferative responses to crude *Af* extracts have been reported earlier (19,46,47). In the past few years, several groups have reported T and B-cell responses to purified *Af* antigens, which has yielded significant understanding of T-cell, antigen-presenting cell (APC) and B-cell interactions (48-54). Interestingly, lymphoproliferative responses to a control antigen and tetanus toxoid were decreased in ABPA patients, suggestive of decreased or suppressed Th1 T-cell responses (29). Since T-cells from ABPA patients suppressed IgG synthesis by B-

cells from normal subjects, this was interpreted as a probable secondary increased T-suppressor activity. These results explain the earlier observations of decreased delayed hypersensitivity in skin response to *Af* antigen.

Subsequently, *Aspergillus*-specific T-cell and B-cell interactions were examined. In these studies, T-cells were stimulated with a crude *Af* extract for 48 hours, and the *Aspergillus* stimulated T-cell supernatant was then co-cultured with atopic or control B-cells for 10 days. In these studies, supernatants obtained from *Af* stimulated T-cells from ABPA patients enhanced B-cell IgE synthesis (18). Later, T-cell lines were generated from ABPA patients with a major allergen of *Af* Asp f 1, a ribotoxin with immunostimulatory properties (29). This study shows T-cell phenotype was exclusively of a CD4⁺ CD25⁺ T-cells with only 1.3% showing a CD4⁺ CD23⁺ phenotypes. The cytokine profile was of the Th2 type T-cells as indicated by IL-4 production and no IFN- γ or IL-2 production. The lymphoproliferative stimulus for the Asp f 1 T-cell lines was of an autocrine pattern mediated predominantly by IL-4. Further studies demonstrated that T-cell clones obtained from ABPA patients were either Th2 (IL-4⁺, IFN- γ ⁻, IL-2⁻) or Th0 (IL-4⁺, IFN- γ ⁺) cells (55,56). Similarly atopic cystic fibrosis patients also produced a Th2 type cytokine pattern to Asp f 1. Thus, the Th2 T-cell response in ABPA is specific to *Af* antigens and not a universal Th2 cell response to all antigens/allergens. Although not known in ABPA patients, the chemokine receptor specificity may be similar to those shown in animal model studies, where expression of more CCR4 and CCR3 receptors have been reported (57).

Cytokines in ABPA

The effector cells responsible for the allergic inflammatory responses in ABPA are predominantly mast cells, eosinophils, and CD4⁺ Th2 cells. When *Aspergillus* antigens cross-link IgE bound to mast cells, the release of a variety of mediators occurs such as histamine, leukotrienes, and platelet activating factor (PAF), which induce bronchial smooth muscle contraction and vascular permeability (38). A number of mast cell factors, 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 endothelial cells and mononuclear phagocytic cells induce eosinophil chemotaxis and activation (59). Basophil hyperreactivity with increased histamine release, probably due to IL-4 stimulation, has also been reported in ABPA (60). Stimulated mast cells and basophils also secrete IL-4. It is well recognized that antigen exposure *via* inhalation triggers a Th2 cytokine release in ABPA patients resulting in the activation of allergic cascade. CD4⁺ Th2 cells secrete the cytokines IL-3 and IL-5 that promote bone marrow maturation of eosinophils and activation of eosinophils (61-66). Furthermore, IL-4 induces expression of VCAM-1 on vascular endothelial cells and its ligand VLA-4 on T-cells and eosinophils. Recent studies have shown that IL-4 can induce eotaxin and recruit eosinophils to the pulmonary airways (57). In addition, selective expression of the CC chemokine receptor 3 (CCR3) and eotaxin receptor on eosinophils, basophils, and CD4⁺ Th2 cells have also been reported. Furthermore, CCR3 expression is upregulated by IL-4 polarizing conditions (57,64,66). Thus, both chemotactic and cell surface adhesion molecules promote recruitment of Th2 cells and eosinophils within the allergic inflammatory site (67). In addition, IL-4 and IL-5 also induce Fc receptors for IgE68. In ABPA, significant amounts of *Af* specific IgE antibodies are produced within BALT and are present within the bronchial mucus. Thus, both IgE and IgA anti-*Af* 7, antibodies bound to their Fc receptors on eosinophils trigger mediator release when they engage allergens (68,69). When eosinophil-bound IgE, IgA, and IgG are cross-linked by *Af* antigens, the eosinophils are triggered to secrete inflammatory mediators, such as major basic protein and eosinophil derived neurotoxin (62,63,69). *A. fumigatus* growing within the airways releases high levels of allergens in the airway and lung parenchyma that produces a heightened and prolonged late-phase allergic inflammatory response mediated by eosinophils.

Immunoregulation in ABPA

Allergic diseases are thought to be the result of an overproduction of Th2 cytokines (IL-3, IL-4, IL-5, IL-10, and IL-13) by allergen-specific CD4⁺ Th2 cells (30,35,65,70-74). IL-4 is critical for IgE synthesis and IL-5 for eosinophil maturation, survival, and activation. Th2 cells amplify and prolong allergic inflammation and late phase reactions, which are the hallmark of asthma and allergic rhinitis. The cascade is self-perpetuating when the IgE molecules produced in response to T-cell help facilitate allergen capture which in turn further activates cytokine secreting T-cells. The specific T-cell response (Th2 or Th1 or mixed) that develops in allergic and non-allergic disease is thought to be influenced by the antigen type, dose, type of antigen presenting cell (APC), cytokine micro-environment, co-stimulatory signals received by T-cells and genetic (host) factors (30,71).

The presence of IL-4 during the priming of naive T-cells has been shown to be critical for the

development of Th2 responses (71,75,76). IL-12, a heterodimeric cytokine, has been shown to be instrumental for the development of Th1 cell differentiation. IL-12 supposedly induces Th1 reactions through the upregulation of IFN-g secretion by T-cells and NK cells, however, IFN-g independent effects have also been reported (77-79). IL-18 augments Th1 responses by increasing IFN-g production from Th1 cells (40,80).

There is considerable evidence for cross-regulation between Th1 and Th2 responses. Human IgE synthesis is reciprocally affected by IL-4 and IFN-g producing CD4⁺ T helper cells. A significant positive correlation was found between helper activity for IgE production and IL-4 concentration in culture supernatants of a large number of human T-cell clones (71). In contrast, there was a significant negative correlation between helper activity for IgE production and IFN-g concentration (71,75). Subsequently, it has been shown that IgE synthesis is dependent on two main signals delivered by T-cells to B-cells. The first signal, IL-4 or IL-13, induces a sterile germline IgE transcript in B-cells (81). The second signal, CD40-CD40L interaction, is mediated by T-cell-B-cell contact and leads to a productive transcript (81). The opposite regulatory roles on IgE synthesis by cytokines associated with Th2 or Th1 responses was confirmed by the observation that recombinant IL-4 induced and recombinant IFN-g or IL-12 inhibited IgE synthesis by peripheral blood lymphocytes *in vitro* (71,75,76). Yoshimoto *et al* (80) have shown that a combination of IL-12 and IL-18 induces anti-CD40-activated B-cells to enhance IgG2a production in mice. In these experiments, the proliferative response of the B-cells was unaffected. However, IL-4 dependent IgE and IgG1 production was inhibited.

Conversion of Th2 responses to Th1 responses appears to be difficult to achieve. Current data suggest that polarized Th2 or Th1 cells represent final, irreversible stages of differentiation⁸². However, within a population of T cells polarized *in vivo*, a pool of undifferentiated CD4⁺ T-cells is thought to exist. These undifferentiated cells can be primed to give rise to the opposite T helper phenotype when cultured under appropriate conditions⁸³. If applicable to humans, treatment with IL-12 or IL-18 may present a novel therapeutic approach for allergic diseases.

Animal Models of ABPA

Murine models of ABPA were originally developed by exposing mice to soluble antigens either intranasally (in) or intraperitoneally (ip). These mice developed elevated levels of IgE and eosinophils in the peripheral blood, bone marrow, and lung (84). High IgE production to *Af* antigen challenge is consequent to the expression of IL-4 by activated CD4⁺ Th2 cells (85).

Consistently enhanced production of IgE and IgG1 has been reported in mice exposed to *Af* antigens (86). The IgE response was arrested or retarded by the administration of anti-IL-4 antibody. However, no significant difference was detected in the levels of IgG1. A marked increase in the IgG2a antibody levels in these animals were also observed, indicating that anti-IL-4 antibody induced a more pronounced Th1 response, as IgG2a levels are IFN-g dependent. Thus, anti-IL-4 antibody treatment resulted in a suppressed Th2 response and an enhanced Th1 response. Similarly, a Th1 type of response was also detected in IL-4 knockout mice when challenged with *Af*. In these mice, no IgE was demonstrable after *Af* challenge, but considerable levels of IgG2a were detected (87,88).

The nature of the antigen and the route of its exposure to mice play a significant role in the type of immunological responses (33,85,86). Particulate antigens of *Af* were prepared by coupling *Af* to polystyrene microbeads with glutaraldehyde. Lung lymphocytes from mice sensitized with these beads produced IL-4, IL-5, and IL-10 in response to *Af* stimulation *in vitro*. The spleen cells, however, produced only negligible levels of IL-4 *in vitro* when stimulated with *Af* soluble antigen. There was a marked increase in eosinophils and IgE in blood and lungs of mice exposed to antigen coupled beads (85). This suggested a localized Th2 response in the organ directly exposed to particulate *Af* antigen. In a different study, mice were primed intraperitoneally with soluble *Af* antigen. This was followed by an injection of antibodies to IL-4, IL-5 or IFN-g and challenge with *Af* antigen coupled beads (88). Using anti-IL-5 antibody the eosinophilia resulting from exposure to particulate antigens was completely abrogated. IgE levels decreased in mice treated with anti-IL-4 antibody, but showed no effect on peripheral blood or lung eosinophilia. Mice treated with anti-IFN-g antibody produced elevated eosinophils in peripheral blood. These mice also showed a marked increase in the eosinophil infiltration into the lung. These results suggest that the IgE and eosinophil responses noted after *Af* exposure are due to IL-4 and IL-5 respectively and that these two cytokines are independently regulated.

Our observations with IL-4 knockout mice suggest that the lung injury observed in this instance may be due to eosinophil mediators and IFN-g, but not due to IL-4 and IgE⁸⁸. In this model we have observed eosinophil infiltration in the lung tissue, while surprisingly no mRNA for IL-5 was

detected. These mice expressed mRNA for RANTES, suggesting a role for this chemokine in the recruitment of eosinophils. It is possible that eotaxin and GM-CSF also may be involved in the recruitment of eosinophils as IL-4 induced eotaxin production by fibroblasts has been reported (89).

As predicted from the predominant IgE and IgG1 responses induced by sensitization with *Af*, the predominant T-cell response is of the Th2 type (85,86). The T-cells from the lungs, draining lymph nodes or spleens have been re-stimulated *in vitro* with immobilized anti-CD3 antibodies or with crude antigen extract (33). Cell suspensions from sensitized, but not from control mice, produced significant amounts of Th2 cytokines such as IL-4, IL-5, and IL-10-33. The Th1 cytokine IFN- γ on the other hand was made in equivalent amounts by cells from controls and sensitized mice. Similar results have been reported from the analysis of lung T-cells for the presence of intracellular cytokines, or from the examination of bronchoalveolar lavage fluids for secreted cytokines (33). Immunohistochemical analysis has shown that exposure to *Af* antigens induced peribronchial and perivascular infiltration of T-cells (90).

Using immunohistochemical staining, GM-CSF, IL-4, and IL-5 was found to be limited to T-cells and eosinophils (90). The increase in GM-CSF, IL-4, and IL-5 expressing cells in lungs was noted on day 14 after final dose of antigen. By day 21, only GM-CSF expressing cells were significantly increased. A strong correlation between a number of cells expressing GM-CSF and IL-5 *in situ* and the number of eosinophils in the lung was noted. Both tissue IL-4 and IL-5 expressing cells correlated with total serum IgE levels. This supports that IL-4, along with IL-5, could be a major modulator of IgE production.

Genetic predisposition towards a predominant Th2 cytokine profile in response to allergen/antigen may be due to immunologic modulation at several levels. Studies comparing BALB/c mice (Th2 predisposed) and C57BL/6 or DBA/2 mice (Th1 predisposed) revealed two mechanisms (91). These include a rapid loss by Th2 cells of the second chain of the IL-12 receptor thus rendering the cells unresponsive to IL-12. This will preclude the possibility of switching to a Th1 phenotype. Alternatively, substantially reduced production of IL-12 (or IL-18) by antigen presenting cells that diminishes the changes of priming naive cells towards a Th1 phenotype.

As has been shown using a number of other immunogens, IL-4 is absolutely critical for the production of IgE in experimental ABPA. IL-4 deficient mice (IL-4 $-/-$) or mice treated with neutralizing anti-IL-4 antibodies throughout the sensitization period do not have an increased serum IgE response (87,88). However, these mice make similar levels of IgG1 antibodies and increased levels of IgG2a antibodies when compared to sensitized wild type mice. The role of antibodies in the pathogenesis of ABPA was addressed using mice with a targeted deletion of the e or m chain (92,93). IgE deficient mice (129 SvEv e-chain deficient) are not capable of producing IgE. The C57BL/6 m-chain-deficient mice do not only lack antibodies of all isotype but also lack mature B-cells. Sensitization with *Af* antigens induced histological lesions, which were similar in quality, and extent in both types of gene targeted mice relative to wild type mice. These lesions typically consisted of airway inflammation and perivascular and peribronchial infiltrates. Furthermore, prominent alveolar lesions, including granulomas, were induced in response to *Af* antigens in the absence of mature B-cells and antibodies. Both IgE deficient mice and B-cell deficient mice sensitized with *Af* antigens developed airway hyperreactivity to the same extent as did wild type mice (93,94). Rag negative mice (T- and B-cells negative) when exposed to *Af* failed to demonstrate airway response whereas these animals on reconstitution with CD4 $^{+}$ T-cells from normal mice exposed to *Af* regained the airway hyperreactivity. However, when only B-cells were transplanted no response was noticed. These findings clearly indicate a major role for T-cells in the development of ABPA in mice.

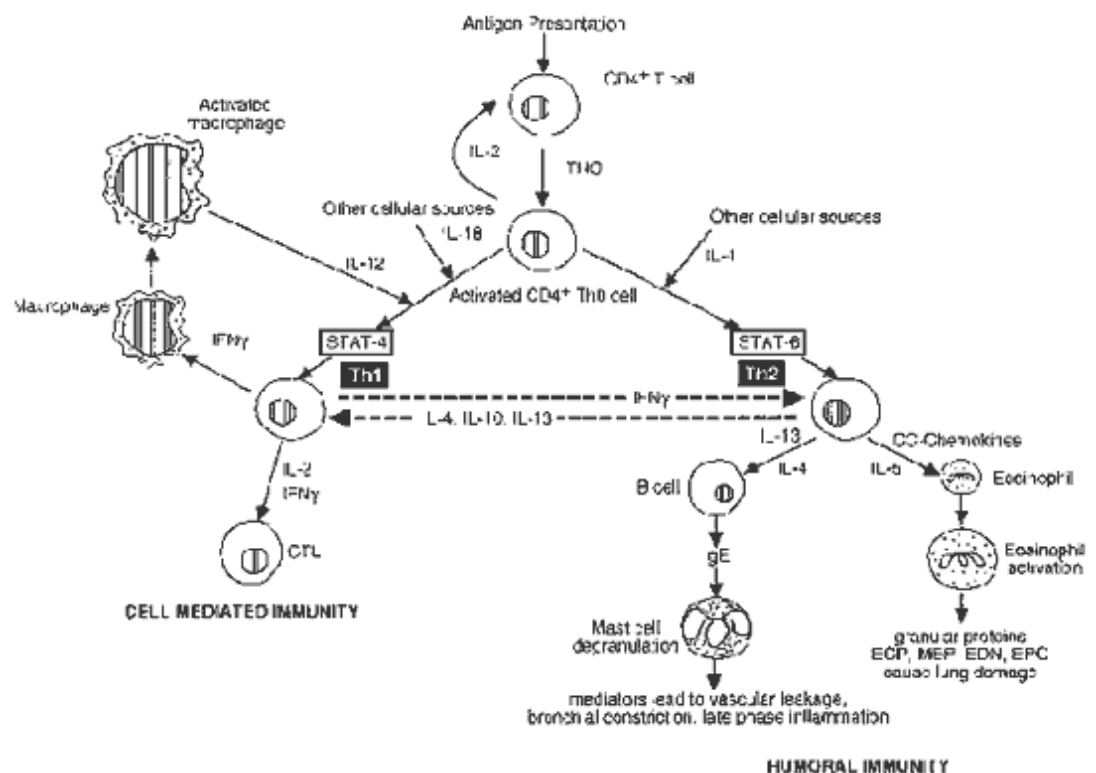
Eosinophils in ABPA

The emigration of airways and interstitium of the lungs by eosinophils is a prominent feature of human and experimental ABPA. A number of studies have addressed the requirements for eosinophil accumulation and function. Wild type mice exposed to *Af* antigens develop not only lung eosinophilia, but also blood and bone marrow eosinophilia (95-97). If these animals are treated with neutralizing antibodies to IL-5, the bone marrow, blood, and lung eosinophilia is almost completely abrogated (86,88). The essential requirement for IL-5 has also been demonstrated using many other immunogens (e.g. parasites, ovalbumin) (98,99). GM-CSF is another cytokine that can activate and maintain eosinophils in the tissues. It has been shown that GM-CSF producing T-cells accumulate in the lungs of animals exposed to *Af* antigens (90). However, GM-CSF must be ordered in hierarchy behind IL-5, because endogenous GM-CSF production cannot support eosinophilia in animals treated with neutralizing anti-IL-5 antibodies or

in IL-5 gene knockout mice (99).

The role of chemokines and adhesion molecules in mediating lung eosinophilia has not been investigated thoroughly in experimental ABPA. A recent report showed that mRNA for RANTES is upregulated following sensitization of mice with *Af* antigens (87). It will be of great interest to determine the role of eotaxin as it has been shown to be a major chemoattractant for eosinophils in the lungs (100). MIP-1a and MCP-4 are other chemokines, which may be instrumental in eosinophil recruitment to the lungs (66,101).

Eosinophils have been shown *in vitro* to secrete mediators that are proinflammatory or cytotoxic (102). In experimental ABPA, eosinophils do not appear to be critical to mediate tissue injury, since IL-5 deficient animals, and animals treated with anti-IL-5 antibodies still develop the full spectrum of microscopic lesions (92). The infiltrates consisting of mononuclear cells instead of eosinophils appear to be just as substantial as in wild type mice. The changes in the airway epithelium (goblet cell hyperplasia) are still present to the full extent. In models using ovalbumin as immunogen, the requirement for eosinophils and IL-5 in mediating airway hyperreactivity are very controversial and appear to depend on the mouse strain and the sensitization protocol (92,99,103-106). It may be that for immunogens which induce relatively poor immune responses in certain mouse strains the proinflammatory and toxic mediators released by eosinophils are necessary substitutes for the proteases and toxins which are constituents of many natural allergens such as *Af* antigens. Thus, in experimental ABPA the consequences of lung eosinophilia are still unclear. In ABPA, eosinophils may be responsible for lung injury through inflammation resulting from repeated exposures to antigens over periods of several months.



Conclusion

It is evident from the available information that ABPA is a Th2 mediated disease. A definite increase of Th2 response to *Aspergillus* was detected in both BALT and systemic immune tissues. The response also may be dependent on the route, nature, and dose of antigen exposure. In some ABPA mouse models as well as in some patients, occasionally a strict Th1-Th2 dichotomy may not be detectable. It may be a mixture of responses with a preponderance of one over the other. This may be attributable to the antigens (nature or dose), presentation of antigens, and host factors. With the advent of newer information on the role of cytokines and chemokines, our understanding on the pathogenesis of ABPA and related diseases also will expand. The current understanding of the immune mechanism is summarized in figure 1. In conclusion, additional information is needed from human ABPA and animal models to elucidate the precise mechanism of ABPA. Understanding of the mechanism will have important consequences in patient care including treatment, immunotherapy, and prevention of ABPA and related diseases.

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