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# IMMUNOPATHOGENESIS OF ALLERGIC BRONCHOPULMONARY ASPERGILLOSIS AND AIRWAY REMODELING

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## 1. ABSTRACT

Allergic bronchopulmonary aspergillosis (ABPA) is a hypersensitivity lung disease clinically characterized by manifestation of wheezing, pulmonary infiltrates and bronchiectasis and fibrosis which afflicts asthmatic and cystic fibrosis (CF) patients. The pathophysiologic mechanisms are mediated by a first and direct interaction of Aspergillus fumigatus (Af) antigens with airway epithelial cells, followed by an immunologic/allergic inflammatory response to Af antigens. In the first phase, Af proteases directly activate epithelial cells by damaging the epithelial integrity and by a direct interaction with epithelial cells, resulting in a rapid wave of pro-inflammatory cytokines and chemokines that initiates an inflammatory response. During the second phase, this fungus induces a strong Th2-type immunological response with markedly elevated Aspergillus-specific and total serum IgE levels and a strong eosinophilic inflammatory response. It is proposed that strong repair reactions, similar to that described for asthmatic patients, are induced by fungal proteinases followed by concurrent release of epithelial derived growth factors. Furthermore, Th2-type cytokines e.g. IL-4 and IL-13 and proteases from inflammatory cells and mast cells, will also promote the release of growth factors from airway epithelial cells driving a strong and irreversible remodeling reactions in airways of patient with ABPA, resulting in bronchiectatic lesions and fibrosis.

## 2. INTRODUCTION

Allergic bronchopulmonary aspergillosis (ABPA) is a hypersensitivity lung disease mediated by allergic early and late-phase inflammatory response to Aspergillus fumigatus (Af) antigens that occurs in a minority (less than 1%) of asthmatic and more often (7-10%) in cystic fibrosis patients (1-6). ABPA is characterized by markedly elevated Aspergillus-specific IgE and total IgE levels and eosinophilia, and manifested by wheezing, pulmonary infiltrates, bronchiectasis and fibrosis. The immune response to Aspergillus antigens in ABPA as well as in allergic asthmatic and cystic fibrosis patients is characterized by a Th2 CD4+ T-cell response (7-9).

The allergic inflammatory response in ABPA appears to be more intense greater than in Aspergillus-sensitive atopic asthma and cystic fibrosis patients and may result in severe changes in airway structure (remodeling), characterized as proximal and/or central bronchiectasis in bronchography or high resolution computerized tomography (HRCT) (10). Immunopathogenesis of ABPA starts with Af spores that are trapped in the bronchial airway by the luminal mucus, bind to epithelial cells, germinate and form local patches of mycelia (7,11). Antigens released by Aspergillus mycelium interacts directly with airway epithelial cells resulting in the release of pro-inflammatory cytokines and growth factors that mediate both inflammatory responses and induce airway remodeling (11-16). Furthermore, Af mycelia release antigens/allergens that are processed by antigen-presenting cells (APC) and presented to T-cells within the bronchoalveolar lymphoid tissue (BALT). The T-cell response to Aspergillus allergens in allergic asthmatic patients is skewed toward a Th2 CD4+T-cell response with IL-4, IL-5, IL-13 cytokine synthesis and secretion. This cytokine pattern will favor IgE antibody formation and mast cell degranulation and promote a strong eosinophilic inflammatory response. Proteases released from mast cells and eosinophilic leukocytes may interact with airway tissue

cells e.g. epithelial cells and fibroblasts, additionally promoting the release of mediators involved in remodeling of the airway tissues.

## 3. AIRWAY REMODELING IN ALLERGIC ASTHMA

Remodeling of the airways of asthmatic patients is a general feature that has been described for a long time. The first characteristic changes in airway wall structure was described in patients at necropsy after death from asthma. Later these changes were observed in biopsies of patients with asthma as a thickening of the epithelial reticular basement membrane and may contribute to the severity of asthma (5,17-22). More recently it has been shown that thickening of the basement membrane in biopsies parallels a more general thickening of the whole airway wall, including features observed in HRCT in patients with asthma (23). Therefore, remodeling of the airway wall appears to be a general feature in patients with asthma. The mechanism by which this airway wall thickening may occur is less clear. It is generally assumed that damage of the epithelial cell layer by environmental allergens is the main cause of epithelial repair mechanisms. Observations on increased numbers of epithelial cells in sputum (Creola bodies) and in bronchoalveolar lavage fluid (BALF) support this view. However, it is not yet fully clear if this injury of the epithelial cell layer is due to an intrinsic property of the asthmatic epithelial cells itself or is due to an increased susceptibility of the epithelial cells to environmental insult. This increased susceptibility of the airways in patients with asthma was shown to be present early in life and was unrelated to the duration of chronic inflammation and severity of the disease (20,21,24). In the mechanism of remodeling, the epithelial cells of the airways appear to play a key role. It is generally proposed that allergen-induced injury of the airway epithelium is the first step that may result in a repair process. It was suggested that this repair process is abnormally regulated in asthma (25). Furthermore, it was proposed that chronic injury and/or defective repair will result in persistent activation of epithelial cells and continuous release of pro-inflammatory epithelial all derived cytokines and growth factors that will drive the chronic inflammatory and remodeling responses (25,26). These growth factors may alter the function of both fibroblasts and airway smooth muscle cells into myofibroblast and fibromyocyte secretory phenotypes, finally resulting in the thickening of the RBM as well as in the increase of smooth muscle mass (20,24). In addition to these direct effects of environmental stimuli, the release of mediators from the skewed Th2-type immunological response e.g. IL-4 and IL-13 may also influence the release of epithelial-derived growth factors. Recently it has been shown that both IL-4 and IL-13 can induce the release of TGF-beta from primary airway epithelial cells (27). Additionally, it was demonstrated that this TGF-beta 2 was able to transform bronchial fibroblasts into myofibroblasts that actively secrete endothelin-1 (ET-1) and vascular endothelial growth factor (VEGF), that are directly involved in the remodeling of the airway wall. Finally, factors secreted by mast cells (28) and eosinophils (29-32) may enhance the remodeling response by promoting epithelial, endothelial, smooth muscle and fibroblast proliferation.

## 4. AIRWAY REMODELING IN PATIENTS WITH ABPA

One of the most characteristic features of patients with ABPA are the bronchiectatic lesions as have been demonstrated in most of the patients using HRCT. Generally these lesions are both centrally and more often proximally found in the airways. These damaged sites may be found in different degrees ranging from mild and hardly detectable forms to more severe and extended lesions. In the latter situation there was also more extensive damage detectable as atelectasis and cavitation. The mild form of bronchiectasis in ABPA as detected by HRCT is quiet similar to that recently shown in patients with asthma and is mainly characterized by airway wall thickening (23). This suggests that the more extensive lesions as seen in patients with more severe and/or chronic ABPA, may be due to similar but quantitatively different mechanisms. It has been suggested that fungal micro-invasion may contribute to airway destruction. Several studies suggested that in patients with ABPA there is evidence for “limited fungal invasion” of the parenchyma (33-35). A lung biopsy specimen from a child with cystic fibrosis showed marked disruption of elastin layers in the bronchioles and Aspergillus fumigatus in the parenchyma (36). As was described above the strong release of antigens and concurrent fungal proteases by Aspergillus that grow between the epithelial cells of the airway wall, shape the conditions for a continuous and strong activation of airway tissue cells involved in remodeling of the airways. Although this remodeling of the airways is interpreted as a defense reaction to environmental insult, the observation that in active ABPA the continuous damage of the airways and occurrence of haemoptysis, suggest that damage by the inflammatory events may exceed the protective mechanism.

## 5. INTERACTION OF AIRWAY EPITHELIUM WITH ASPERGILLUS

One of the characteristic features in patients with ABPA is the observations that *Aspergillus fumigatus* (Af) is bound to the surface of epithelium growing on and in between the epithelial cells without being efficiently killed by mononuclear cells and eosinophils (36). It has also been shown that spores of Aspergillus are attached to epithelial surfaces cultured in vitro (37). The physical presence of Af is of importance for the modulation of the immunological response towards a Th2-type (7,38-40). In order to evade the very effective natural defense system of the human airways against fungi, various virulence factors of Af have been detected over the past decades that have been shown to interfere with or even block normal functions of the humoral and cellular defense of the airways (16,41-47). Some of these virulence factors are the proteolytic enzymes of Af, which facilitate the immune response to the fungal antigens and play a role in the inflammatory response.

## 6. ASPERGILLUS PROTEOLYTIC ENZYMES AND INTERACTION WITH EPITHELIAL CELLS

It has been found that certain strains of Af release proteolytic enzymes with elastolytic and collagenolytic activities. The possible role of these proteolytic enzymes as a pathogenic factor in fatal invasive aspergillosis is still uncertain. However, findings in patients with ABPA or aspergilloma indicate that these proteases may be involved in the pathogenesis of these diseases. The culture filtrate extracts from Af showed marked elastase and collagenase activity and the 32 kD elastase protein bound to IgG from sera of patients with ABPA and aspergilloma (44). The pronounced binding of IgG antibodies to the 32 kD fungal elastase suggest that these proteases are produced in vivo in patients with ABPA and aspergilloma. Furthermore, during exacerbations of aspergilloma, antibody concentrations against different antigens including the 32 kD and 40 kD proteases were markedly increased (48), indicating that these fungal proteases may play a role in the pathogenesis of the disease. A direct destructive activity to the epithelial cell layer and structural components of the airways is suggested by the observations in a patient with CF, showing Af in the parenchyma and disruption of elastin layers in the bronchioles (49).

An important feature of pathogenic microorganisms is their capacity to interact with epithelial cells of the mucosal surface. Previously it has been shown that products released in vitro by Af are able to cause epithelial cell detachment (15,50). This capacity to induce epithelial cell detachment is also characteristic for other proteases released by different fungi, e.g. Alternaria and Cladosporium, but Aspergillus proteases are more active at much lower concentration (12). Recent studies performed with proteases from various sources, e.g. Der p1 from house dust mite (51,52) have shown that degradation of epithelial cell structures results in facilitated transport of antigens and allergens across the epithelium that will result in enhanced exposure to antigen presenting cells and concurrent immune responses.

In addition to damaging the epithelial cell layer integrity, we have shown that human bronchial and alveolar epithelial cell lines produce pro-inflammatory cytokines such as IL-8, IL-6 and MCP-1, after incubation with protease containing culture filtrates of Af. This cytokine releasing activity could be ascribed to the proteolytic activities of these extracts (12,47,50,53). These observations suggest that proteolytic enzymes released by Aspergillus growing on and between epithelial cells may be responsible for the induction of chemoattractive cytokines by epithelial cells and corresponding inflammatory responses. It has been proposed that induction of a severe inflammatory response by the direct activation of epithelial cells, may induce additional epithelial injury (12). Destruction of the epithelial cell barrier by proteases from either the fungus or from the eosinophilic and neutrophilic inflammation, is followed by repair mechanisms, resulting in the influx of serum proteins and extracellular matrix proteins to the luminal site of the epithelium (24,54). Since spores and mycelia of Af have surface structures that are able to interact with extracellular matrix molecules, damage and concurrent repair of the airway mucosa with concurrent release of repair molecules, may facilitate the binding of Aspergillus to the damaged sites of the airways. The interaction of enhanced release of proteolytic enzymes and allergens on the epithelial surface will induce a continuous inflammatory response and a strong Th2-type immunological response.

In addition to the induction of cytokine responses of epithelial cells, it has been shown that proteases from Af at higher concentrations also inhibit epithelial cell cytokine production even below the spontaneous cytokine production levels (12). This silencing mechanism which was specific for the elastase/collagenase containing extracts of Af may represent an additional virulence factor by preventing effective targeting by infiltrative phagocytic cells and persistence of Aspergillus in the airways .

## 7. HOST IMMUNE RESPONSE TO ASPERGILLUS; ROLE OF TH2-TYPE CYTOKINES AND EOSINOPHILS IN REMODELING

The persistent presence of Af in the airways and the continuous release of antigens and allergens will lead to a strong activation of the Th2-type immunological response with very high productions of total and specific IgE antibody. Furthermore, an additional Th1 response with formation of IgG and IgA antibodies to antigens of Af, as is observed in patients with ABPA (55-60).

T-cell lines that were generated from ABPA patients with an Aspergillus allergen, Asp f1 showed a T-cell phenotype that was exclusively CD4+CD25+HLA-DR+ T cells (39). Subsequent studies performed by this group (8,9,61) demonstrated that T-cell clones obtained from asthmatic ABPA patients were either Th2 (IL-4+, IFN-gamma−) or Th0 (IL-4+, IFN-gamma+) T cells. Furthermore, it was shown that IL-13 is also enhanced in ABPA mouse model (62). The production of both IL-4 and IL-13 in airways may directly induce the release of TGF-beta2 from epithelial cells (27). TGF-beta2 is able to transform bronchial fibroblasts into myofibroblasts that actively secrete endothelin-1 (ET-1) and vascular endothelial growth factor (VEGF), that are directly involved in the remodeling of the airway wall. In addition to affecting the remodeling of the airways, IL-4 and IL-13 will also facilitate the infiltration of eosinophilic leukocytes by inducing the expressing of ICAM-1 and VCAM-1 on all tissue cells (endothelium, epithelium and fibroblasts). The continuous release of allergens followed by production of Th2-type cytokines and chemokines from epithelial cells during exacerbations of ABPA, induces a massive infiltration of eosinophils, as has been described in BAL and biopsies from these patients (36,63). Infiltration of eosinophils in atopic asthmatic patients are the cause of additional damage to the epithelial cell layer by release of their toxic granular proteins e.g. major basic protein (MBP), eosinophilic cationic protein (ECP), eosinophil-derived neurotoxin (EDN) and eosinophil peroxidase (EPO). Eosinophils in biopsies of asthmatic patients were shown to be in a state of degranulation with deposition of

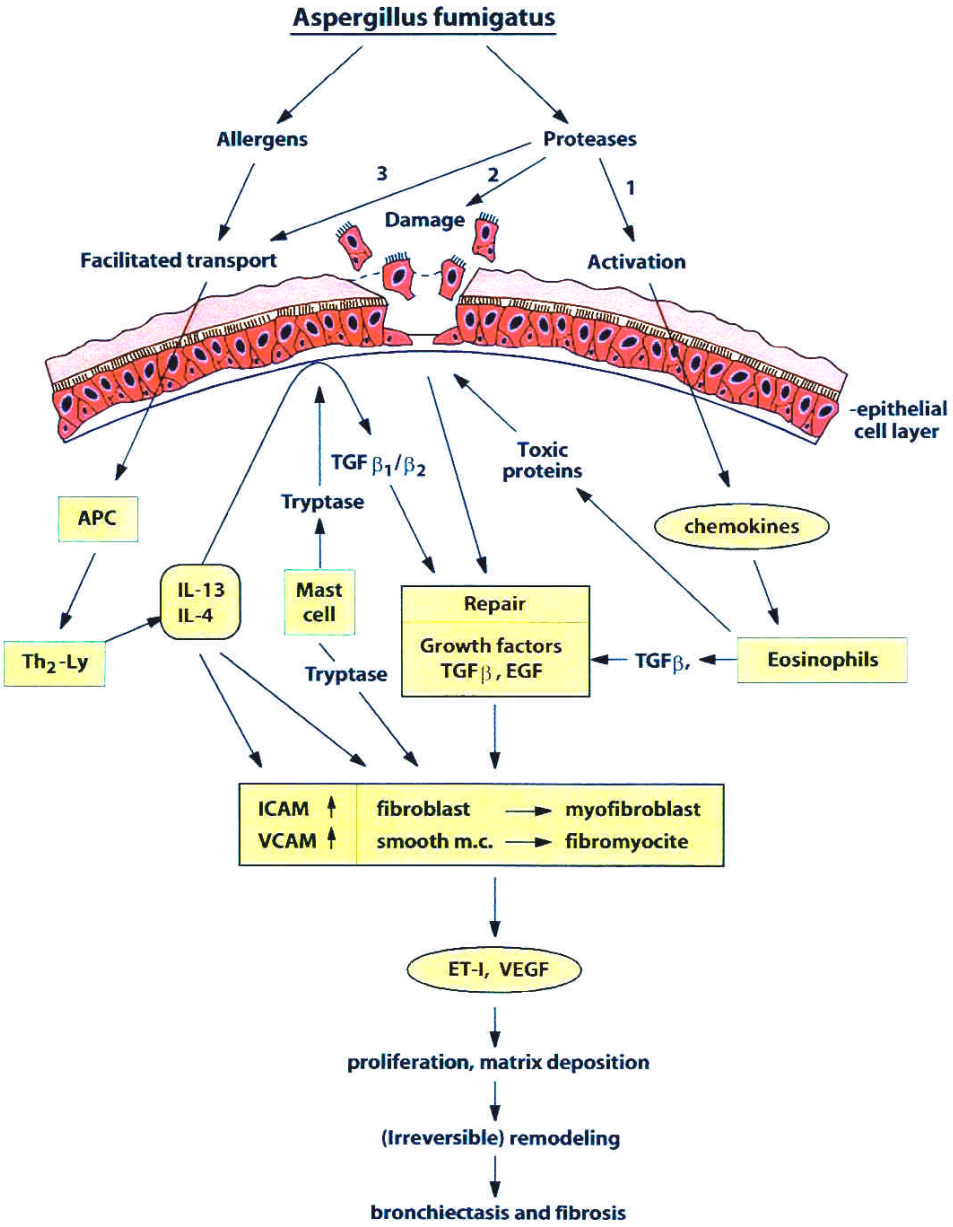


**Figure 1.** This figure reviews the mechanisms described in the text. Interactions of Aspergillus fumigatus allergens and proteases with the airway epithelial surface is shown in the upper part of the figure (corresponding text see 5 and 6). Proteases and allergens interact with epithelial cells inducing facilitated antigen and allergen transport to the immune system by both activation of epithelial cells as well as by disruption (damage) of epithelial adhesion contacts (left and middle side of the figure 1). The immune response in ABPA is a Th2 type response resulting in production of IL-4 and IL-13 that are responsible for the formation of an IgE antibody response against Af allergens and corresponding sensitization of mast cells. In addition IL-13, IL-4 and tryptase will generate the release of TGF-beta subtypes that will drive a remodeling response by changing the phenotypes of both fibroblasts and smooth muscle cells (middle part of figure 1). IL-4 and IL-13 will also cause and increased expression of both ICAM and VCAM on endothelial cells, fibroblasts and epithelial cells, facilitating the Th2-type inflammatory response characterized by infiltration of Th2 lymphocytes, monocytes and eosinophils. The direct and strong activation of epithelial cells by proteases from Af will induce the release of chemokines (eotaxin, IL-8, TARC) that together with IL-5 from Th2 lymphocytes will generate a strong infiltration of eosinophils (right side of figure 1). The strong eosinophilic infiltrate will be an additional cause of damage of the epithelial surface, while the release of TGF-beta1 will enhance the remodeling of the airways (left side of figure 1). Proteolytic damage together with uncontrolled proliferation and matrix deposition by myofibroblasts and fibromyocites will finally result in irreversible remodeling (scarring) of the airways known as bronchiectasis and fibrosis (lower part of figure 1).

their toxic granular proteins in the bronchial tissue (EG2 positive cells), after allergen induced asthmatic responses (54,64,65). This degranulation of eosinophils is associated with damage of the airway surface structures such as detachment of epithelial cells (66). Activated eosinophils (EG2 positive cells) have also been shown in bronchial biopsies of patients with ABPA (67). As has been argued above , damage of epithelial cells will be followed by repair reactions, thereby inducing both TGF-beta1 and TGF-beta2. While TGF-beta2 will promote the formation of myofibroblasts and fibromyosites (20), the release of TGF-beta1 will act both as an anti-inflammatory factor and as a pro-fibrotic substance in wound repair (68,69). In addition to the damaging effect of eosinophils it is important to note that eosinophils may be an important source of TGF-beta (29-32), and in this way may enhance the remodeling response by promoting epithelial, endothelial, smooth muscle and fibroblast proliferation. Finally, factors secreted by mast cells e.g. tryptase are also able to activate epithelial cells and promote the proliferation of epithelial, endothelial smooth muscle cells and fibroblasts (28,70).

## 8. FUTURE PERSPECTIVE

As discussed above, it can be seen that the remodeling in ABPA follows similar pathways as in allergic asthma (Figure 1). Damage of epithelial cells by proteolytic enzymes from Af will induce repair reactions with the release of growth factors that promote proliferation of different tissue cells and deposition of matrix proteins. The physical presence of Af in the airway surface in ABPA, induces more damage and an exaggerated activation of the innate (epithelial cells) and cognate Th2-type immunological response. The burden of proteolytic enzymes from fungal origin and from mast cells and inflammatory cells (eosinophils) is followed by a dysregulated repair response, resulting in extensive and irreversible remodeling of the airway tissue finally characterized by bronchiectatic lesions and fibrosis.

Based on this model it is obvious that therapeutic regimens will include anti-inflammatory agents such as systemic and locally applied corticosteroids, while additional anti-fungal treatment may also be helpful. New therapeutic treatments may come from specific anti-proteases, that are currently developed in Pharmaceutical Industries, and that will inhibit the proteases from both fungal origin and those derived from the inflammatory response (eosinophils, mast cells).

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