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IMMUNOTHERAPY OF ALLERGIC BRONCHOPULMONARY ASPERGILLOSIS: A CLINICAL AND EXPERIMENTAL APPROACH

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1. ABSTRACT

Allergic bronchopulmonary aspergillosis (ABPA) is a severe allergic pulmonary complication caused by the saprophytic fungus *Aspergillus fumigatus.* The present review examines the pathogenesis of this disease describing in detail the role of innate and acquired immunity in the induction of sensitivity to *A.fumigatus*. Different approaches in developing specific immunotherapeutic treatments such as induction of anergy, regulatory cells, a switch from Th2 to Th1 type of immune response, CpG and genetic immunization and the usage of altered peptides or modified allergens are critically examined.

2. INTRODUCTION

Allergic bronchopulmonary aspergillosis (ABPA) is a severe allergic pulmonary complication caused by the saprophytic fungus *Aspergillus fumigatus* (Af). The present review examines the pathogenesis of this disease and critically examines the perspectives in developing specific immunotherapeutic treatments.

A.fumigatus is one of the most ubiquitous fungi disseminated through airborne conidia (1). The conidia usually measure 1-3 μm in diameter and can even reach small airways on inhalation (2,3). It has been estimated that an average human will inhale at least several hundred Af conidia per day. Most healthy individuals invariably possess low levels of IgG antibody to Af proteins (4-7). Inhalation of conidia by immunocompetent individuals rarely has any adverse effects, since they are eliminated efficiently by innate immune mechanisms, mainly by phagocytic cells, macrophages and neutrophils. Until recent years Af was considered a weak pathogen responsible for either allergic forms of the disease, such as farmer's lung, or producing cavitary diseases such as aspergilloma (8,9). Over the past 10 years Af has become the most prevalent airborne fungal pathogen, causing severe invasive infections in immunocompromised patients with cancer, HIV, bone marrow or solid-organ transplants (10-13). For most patients suffering from Af induced diseases, the main site of infection or allergic inflammation is the respiratory tract. Although almost all individuals have IgG to Af and possibly to other ubiquitous fungi only a small proportion develop IgE antibodies, a hallmark for immediate hyperreactivity. Early forms of hyperreactivity to Af include asthma, allergic sinusitis and alveolitis. They occur following repeated exposure to Af antigens even without mycelial colonization. Germination of the spore and limited mycelial growth also can occur in the respiratory track of patients with ABPA (14,15). ABPA is found mainly in patients suffering from atopic asthma or cystic fibrosis. However some cases of ABPA possibly escape diagnosis because ABPA is a very difficult syndrome to diagnose. Around 15% of asthmatic patients sensitized to Af and 6 to 35 % patients with cystic fibrosis develop ABPA (16-19). ABPA is characterized by an intense lung inflammation with eosinophils, presence of mucus plugs in the airways, immediate skin reactivity to Af antigenic extracts, elevated level of serum total IgE and Af specific IgE and IgG in the sera. Clinically, there are periods of exacerbation and remission that may lead to proximal bronchiectasis and fibrosis in the lungs. Removal of the

patients from source of sensitization results in clinical improvement. In contrast, ABPA usually require therapeutic intervention. Corticosteroids, in high doses, are the main treatment for ABPA, although, adverse side effects are rampant and severe (20,21). A group of antifungal compounds, the azoles, have activity against Af and have been proposed as an alternative treatment for ABPA. However, at present, there are no conclusive case control data available to evaluate the efficacy of the antifungal therapy for treatment of ABPA (22,23).

3. INNATE IMMUNITY

Nonspecific or natural immunity plays a major role in the defense against Af by recognition and clearance of the organism in immunocompetent hosts (24). The majority of the conidia of Af, like most airborne particles, are probably excluded from the lungs through the ciliary action of the mucous epithelium. However, the clearance of Af at this level may be less efficient than with other airborne saprophytic microorganisms, since proteases, ribotoxins, and toxic molecules produced by Af inhibit ciliary movement of the airway epithelium. In addition, proteases present in Af antigens can damage the airway epithelial cells (25,26). This finding is supported by the observation that vacuolar serine protease, a 34-kDa protein, which reacts with IgE antibodies in over 80% of serum samples from patients and may be considered a major allergen of Af, inhibits the mucociliary function of the epithelial cells (26). The proteinase secretion by Af was increased in the presence of blood serum in the growth medium (27,28). This effect was abrogated, when serum was inactivated, by heating at 56^{o}C. These data demonstrate that protein rich environment in the lungs of patients with asthma or cystic fibrosis, favors protease release by Af and other fungi, thereby acting as a contributing predisposing factor.

Macrophages and neutrophils can recognize, bind, internalize and ingest conidia of Af while neutrophils attack and kill hyphae by oxidative mechanism, as they are too large to be engulfed (29). Although conidial and hyphal structures recognized by phagocytic cells are not known precisely, it was suggested that galactomannan (GM), a major Af cell wall constituent may be a responsible factor (30-32).

When stimulated with Af conidia and hyphae, phagocytic and epithelial cells produce TNF alpha, IL-1, IL-6, IL-8 and a set of chemokines (33-36). This cocktail serves as an attractant for neutrophils and stimulates phagocytosis. The extracts from Af grown on collagen medium induce a strong dose-dependent decline in cytokine production at higher concentrations. This indicates that the enhanced presence of proteases increase the damage of epithelial cells (34,36). Specificity of cytokine gene activation and inhibition is shown at the level of the transcription factors NF-kappa B and AP-1 (34,37).

Lung surfactant proteins SP-A and SP-D play an essential role in the early antifungal defense responses in the lung through inhibiting infectivity of conidia by agglutination and by enhancing uptake and killing by phagocytic cells (30). SP-A and SP-D recognize polysaccharide structures and SP-D recognizes only certain polysaccharide configurations, likely through differential binding to nonterminal glucosyl residues (32). Lung surfactant proteins participate in the clearance of Af conidia and hyphae by phagocytic cells through a mechanism described for bacterial lipopolysaccharide (LPS) involving toll-like receptors.

Innate immunity initiates protection of the host against invasion and subsequent multiplication of microbes through specific recognition. Germ line-encoded receptors have been identified for microbial products such as mannan, lipopeptide, peptidoglycan, lipoteichoic acid, LPS, and immunostimulatory sequence of oligodeoxynucleotides (ISS-ODN). The Drosophila Toll protein has been shown to be involved in innate immune response of the adult fruitfly. Ten members of the family of Toll-like receptors (TLRs) are known (38). Six of them have been demonstrated to mediate cellular activation by distinct microbial products. Structural similarities between TLRs and receptor molecules involved in immune responses (such as CD14 and the IL-1 receptors) and their functional characteristics identified as TLR2 and TLR4 as candidate receptors for LPS and other microbial products. The complex formed from the interaction of TLR4, CD14 and MD-2 mediates LPS signaling (39). TLR4 and CD14 but not TLR2 also are involved in Af hyphae induced activation of human monocytes (40).

4. ACQUIRED IMMUNITY

A.fumigatus induced allergic diseases are believed to be due to T helper (Th) 2-like immunity to allergens in affected tissues (41-44). Immune responses to allergens are characterized by a cross-regulation between Th1 and Th2 cells (45-49). The concentration of IL-5, IL-9, IL-10 and IL-18 are significantly higher in allergic asthmatic patients than in normal control subjects, while the data available on the production of IFN-gamma, IL-4, IL-12 and IL-13 are controversial since they are elevated in some, while remain normal in others (45-49). *A.fumigatus* induced IgE production is mediated by Th2 cells by producing IL-4 and/or IL-13. New evidences not only confirm the role of eosinophil induced airway inflammation, but also suggest a role for interleukin (IL)-8/neutrophil-mediated inflammation in ABPA (20). Taken together it can be concluded that Af induced hyperreactivity is a chronic process characterized by Th2 biased specific immune response with eosinophilic inflammation, IgE production and neutrophil activation.

5. SPECIFIC IMMUNOTHERAPY (SIT) OF ATOPIC DISEASES

It is generally accepted that immunotherapy is efficient when applied to the treatment of patients sensitized to one or two allergens and with a mild form of hyperreactivity (50-58). In some trials, immunotherapy was successful when 3 to 5 allergic extracts were used, however multiple-allergen immunotherapy in allergic children with moderate-to-severe, perennial asthma was ineffective

(54,55). The best described effects observed after immunotherapy with natural allergen extracts or recombinant full length proteins are the improvement in the universal symptom-medication scores in the actively treated group. These include increase in allergen specific serum IgG, little or no change in the serum total and specific IgE levels, decrease in eosinophil counts and Th2 associated lymphokine production (51,52,56-59). Traditional immunotherapy includes subcutaneous (s.c.) injections or sublingual drops or pills of allergen in increasing doses (51,52,56-58). Allergen immunotherapy has never been used for the treatment of ABPA or asthma induced by Af, however it has been used to treat other fungal induced asthma such as those caused by *Cladosporium herbarum* and *Alternaria alternata* (60,61). Immunotherapy for allergic fungal diseases is not widely used because it is ineffective due to unstandardized mold extracts and possible danger of anaphylaxis and sensitization to new allergens. Unfortunately, the composition of fungal antigen extracts can vary between batches and from laboratory to laboratory. Characterization and production of standardized recombinant allergens greatly improve the diagnosis of ABPA and other forms of Af induced hypersensitivity diseases. This may also help in developing specific immunotherapy for fungal allergens including ABPA. It has been shown that about 100 proteins or glycoproteins from Af can bind human Ig (62). About 20 of them have been well characterized and produced in a recombinant form (63-67). However, IgE from sera of asthmatic or cystic fibrosis patients sensitized to Af, but without ABPA, binds only to a limited number of proteins, such as Asp f 1 and Asp f 3, while IgE from patients with active ABPA binds to Asp f 2, Asp f 4, Asp f 6 and Asp f 16 also in addition to Asp f 1 and Asp f 3 (66,67).

5.1. Mechanisms involved in specific immunotherapy

The generation of an effective immune response involves antigen-specific T cell expansion and differentiation of effector function. T cell activation requires at least two distinct signals, including signaling via the antigen-specific T cell receptor (TCR) and a costimulatory pathway (68). Antigen stimulation of T cells can lead either to a positive immune response, characterized by proliferation, differentiation, clonal expansion and effector function, or, in the absence of an appropriate costimulation, to a state of long-lasting unresponsiveness, termed anergy (69). Anergic T cells fail to proliferate and secrete cytokines in response to secondary stimulation. The interaction between the costimulatory molecule CD28 on T cells and members of the B7 family on antigen-presenting cell (APC) results in upregulation of T cell proliferation and cytokine production and induces the expression of the anti-apoptotic protein Bcl-xl (70). The negative regulatory mechanisms during T cell activation are not well understood, but they are crucial for the maintenance of lymphocyte homeostasis, which limits any ongoing immune responses such as normal immune response, allergic reaction or autoimmune process. Mechanisms involved in the decrease of allergic symptoms during classical immunotherapy, are also obscure. There are three concepts, which are proposed to explain the effect of modulation or down regulation of allergic immune responses in experimental systems or in disease. They include: i) induction of anergy; ii) formation of regulatory cells with suppressive function; and iii) a switch from Th2 to Th1 phenotype. Because beneficial effects of immunotherapy are observed after treatment with relatively high doses of allergen, the availability of the antigen, including the dose and half-life time of the antigen, the number of antigenic peptide-MHC complexes on the antigen presenting cells (APC), and the affinity of the MHC and TCR have been suggested to play an important role in the efficacy of immunotherapy (68,71). These concepts will be treated in more details below.

5.2. Anergy

The induction of specific anergy in peripheral T cells through SIT involves a number of steps and factors associated with immunological mechanism of disease. This phenomenon is based on lack of costimulatory signals, although APC displaying specific antigenic peptides on their surface to interact with $CD4^+$ T cell exist (70,71). The main costimulatory molecules involved are CD28/CD152 (CTLA-4)-CD80/CD86 (B7 family) and CD40-CD154 (CD40 ligand) (68-70,72,73). However, for several years the functional role of the CD28 homologue -cytotoxic T lymphocyte antigen-4 (CTLA-4), in T cell activation has been obscure and controversial (70,74). CTLA-4 was initially thought to provide a costimulatory signal in conjunction with TCR/CD3 signaling. Today it is more clear that CD28 and CTLA-4 molecules possess diametrically opposite functions: signaling via CD28, in conjunction with TCR, is required for T cell activation, while signaling via CTLA-4 is a negative signal that inhibits T cell proliferation and can not be restored by IL-2. On the other hand, CD28 costimulation is not sufficient for anergy as activated T cells progress through the cell cycle in order to escape anergy (75,76). Induction of this "division-arrest" form of anergy requires CTLA-4 signaling during the primary response. Anergy may be induced either through a combination of CTLA-4 signaling and the failure of cell cycle progression, or through a proliferation-independent mechanism in which TCR ligation occurs in the absence of CD28 (75,76). In the later case IL-2 can replace CD28 engagement.

When allergenic proteins and peptides at low concentrations are injected s.c. in a soluble form they induce anergy in mature allergen specific T cells. It was shown that the proliferative and cytokine responses in specific T cells were significantly suppressed, although in some instances IL-2 was able to restore these functions (77-79). The encounter of $CD4^+$ T cell and APC expressing specific antigenic peptide without costimulatory molecules induces partial phosporylation of ZAP70 kinases in T cells, which is not sufficient for maximum cell activation but strong enough to prime cells and induce anergy (80). The antigen able to bind MHC class II molecules without inducing costimulatory molecule expression on APC is determined by peptide housing T cell dominant epitopes (81). This approach was widely studied in experimental models (82,83). Some allergic peptides were used in clinical applications (71,84-91). Bee venom peptide immunotherapy was shown to be effective (71). Results

with Fel d 1 peptides showed considerable variations from no effects to maximum effectiveness (87,89). Based on these data it can be concluded that anergy induction down regulates both cellular and humoral immune responses. However, IgG production usually increases during the immunotherapy, and this phenomenon cannot be explained by the proposed mechanism of passive anergy induction (51-53). Bee venom peptide immunotherapy induced late-phase reaction demonstrating active immunization and not anergy (86,88,91,92). Our unpublished experiments demonstrated that repetitive injections of similar or low doses of Asp f 2 and Asp f 3 in PBS were able to mount high antibody production and not anergy. Besides, over the last decade the concept of T cell co-stimulation has emerged to take a central role in the process of T cell activation. Although co-stimulation is important, there is little evidence to link co-stimulation with T cell anergy (76).

5.3. Induction of regulatory cell

In recent years it has been demonstrated that anergy induction is associated simultaneously with an increase in IL-10 production [93-94]. Neutralization of IL-10 with a specific antibody restored the original proliferative and cytokine responses of the PBMCs. It has been shown that IL-10-producing B cells and monocytes were involved at a later stage of SIT, particularly in the maintenance of the anergy. The addition of IL-10 to stimulated PBMC or to purified B cells inhibited IgE synthesis and enhanced the IgG4 antibody formation. Finally accumulating data have led to the conclusion that SIT generates IL-10, which in turn induces specific anergy by autocrine interaction with T cells and counter-regulating IgE and IgG4 production. Further investigations demonstrated that suppression of T cells by IL-10 is an active process, which depends on the expression and participation of CD28. T cells with specific phenotypes were responsible for increased IL-10 production. Another line of evidence on the existence of regulatory cells came from the works of several groups (95-98). It was shown that $CD4^+$ $CD25^+$ T cells are naturally occurring regulatory T cells. They do not proliferate or produce IL-2 and have suppressive properties. They can be isolated from the spleens of normal mice and can be activated and expanded in vivo (98). Repetitive stimulation of naive T cells in the presence of IL-10 induces the differentiation of T regulatory cells. Costimulation of T cells via CD2 in the absence of costimulation through CD28- or LFA-1, induces T cell anergy in an IL-10-independent pathway along with the differentiation of Ag-specific regulatory T cells (97).

Although the existence of regulatory cells is an established fact their role in normal or pathological immune response is not clear. On the other hand there have been studies demonstrating significantly high levels of IL-10 before and after immunotherapy compared to controls (46,47,59). According to these data, after immunotherapy IL-10 levels in patients with allergic rhinitis or venom allergy remained unchanged or showed a slight decrease (59,92). IL-10 mRNA was over expressed in gut mucosa of patients with allergic asthma (47). The discrepancy in these results on the role of IL-10 during immunotherapy suggests that the effect of immunotherapy probably depends on cell-cell cooperation and is more complicated.

6. ALTERING TH2 TO A TH1 IMMUNE RESPONSE TO ALLERGENS

The central effector cells in the pathogenesis of atopic allergic diseases are Th2 cells, which display an aberrant cytokine profile dominated by type 2 cytokines (46). Recent results obtained from numerous animal studies suggest that primary prevention of severe forms of allergy in humans might be possible in the near future. There is a growing awareness that in humans established effector Th2 cells can be reverted to predominant Th1 phenotypes. The most promising approaches include the induction of systemic or local allergen-dependent or -independent Th1 immune responses, through the use of killed bacteria (or components derived from them), CpG oligodeoxynucleotides or plasmid DNA. In fact, the Th1-driving cytokine IL-12 is the crucial factor in this respect. IL-12 is mainly produced by dendritic cells (DC), which can be primed for IL-12 production using CpG-ODN or gene vaccination (99-101). There are no coherent data demonstrating that SIT switches Th2 cells towards Th1 type (102-105). However induction of Th1 type of immune response to allergens has been shown to be harmless and protective in animal models of allergy (99-101).

6.1. CpG immunization

CpG DNA directly activates two important classes of APC, macrophages and DC. DNA from bacteria has stimulatory effects on mammalian immune cells, which depend on the presence of unmethylated CpG oligodinucleotides (CpG-ODN) in the bacterial DNA. In contrast, mammalian DNA has a low frequency of CpG-ODN, and these are mostly methylated; therefore, mammalian DNA does not have immunostimulatory activity (106,107). CpG DNA induces a strong Th1-like inflammatory response. Cellular response to CpG DNA is mediated by a Toll-like receptor, TLR9 (108). TLR9-deficient (TLR9-/-) mice did not show any response to CpG DNA, including proliferation of splenocytes, inflammatory cytokine production from macrophages and maturation of dendritic cells. Thus, vertebrate immune systems appear to have evolved a specific Toll-like receptor that distinguishes bacterial DNA from self-DNA. Accumulating evidences have shown that therapeutic potential exists for CpG DNA as adjuvants for vaccination strategies for cancer, allergy and infectious diseases. CpG-ODN have been shown to reduce eosinophilia, bronchial hyperreactivity, serum IgE, and Th2 cytokines and enhance the production of IFN-gamma and IL-12 levels in murine models of asthma (99-101). CpG can be used alone or in a combination with an allergen (109-111). When used alone there is a concern that administration of CpG-ODN may initiate or exacerbate Th1 type autoimmune reactions in susceptible individuals. Toxicity of CpG –ODN can be minimized by producing conjugates with immunogenic peptides from allergens. The CpG-allergen peptide conjugate has the potential to stimulate allergen-specific Th1 cells as opposed to a universal Th1 bias with unconjugated CpG-ODN.

Our study using CpG treatment in *Aspergillus* antigen sensitized mice demonstrated significant reduction in peripheral and lung eosinophils. IgG_{2a} antibody levels showed a two-fold increase, while IL-5 in the lungs showed a marked reduction. Histology of the lungs showed marked reduction in goblet cell hyperlasia and overall inflammation in CpG treated mice compared to mice sensitized with *Aspergillus* antigen alone. These findings suggest that CpG may be of value in reversing the response in ABPA (111).

6.2. Genetic immunization

The use of plasmid DNA alongside with CpG-ODN to elicit immune responses has greatly increased the ability to skew the desired immune response to a particular antigen. DNA immunization induces potent Th1 cell-mediated responses including humoral immunity as well as cytolytic T-lymphocyte immunity (112-117). Mice receiving DNA containing a dominant peanut allergen gene (pCMVArah2) produced secretory IgA and serum IgG_{2a}. Compared with non-immunized mice those treated with 'naked' DNA or with nanoparticles containing DNA and a natural biocompatible polysaccharide chitosan, showed a substantial reduction in allergen-induced anaphylaxis associated with reduced levels of IgE, plasma histamine and vascular leakage (113). The primary response in mice immunized with a plasmid encoding a major cow's milk allergen bovine beta-lactoglobulin (BLG), was of the Th1 type. Immunization with plasmid DNA inhibited the Th2 response induced by a subsequent immunization using BLG adsorbed on alum (114). Analogous results were reported for mice immunized with a plasmid encoding Dermatophagoides pteronyssinus allergen (115). These and other experiments demonstrated that both gene vaccination and coimmunization with protein and immunostimulatory DNA were effective in attenuating the development of anaphylactic hypersensitivity in mice previously sensitized and demonstrating a Th2 type of response (116). Taken together these results demonstrate that oral, mucosal, intradermal or intramascular allergen-gene immunization with DNA is effective in modulating murine anaphylactic responses, and indicates its prophylactic utility in treating food, sting, dust mite, fungus and likely other types of allergy (113-117).

6.3. Altered peptides

Peptides representing natural T cell epitopes of allergens are usually used for the peptide immunotherapy. The main characteristic of dominant T cell epitope peptide is its MHC groove binding anchors. There are usually 3 to 4 amino-acids in specific positions which are important for peptide-MHC class II molecule interaction (118). When a single amino-acid in this anchor position is substituted, a stronger binding between a peptide and MHC molecule is observed in some cases (119-122). These stronger binding peptides, termed altered peptide ligands (APL), were shown to induce Th1 immune response while the parental peptide induced a Th2 response (121). These APL can be considered as a tool to modulate immune response to allergens (122).

6.4. Allergen modification

Possible adverse side effects of SIT is the main concern when natural extracts or full-length recombinant proteins are used (123). The most severe side effects including anaphylaxis are induced via IgE mediated processes such as degranulation of mast cells and basophils and IgE facilitated antigen uptake by DC (124-127). IgE B cell epitopes are mostly conformational while IgG recognize both linear and conformational epitopes (126). Disruption of IgE B cell epitopes in allergen can decrease allergenic but preserve immunogenic properties of allergens used for SIT (128-130).

Deletion mutants or proteolitically modified proteins can be used for SIT if their IgE B cell epitopes are disrupted (130,131). This approach includes: i) recombinant allergen production; ii) IgE epitope analysis; iii) modified allergen production; iv) preclinical study. Experimental studies demonstrate that IgE B cell epitope removal decrease the risk of anaphylaxis [130-131]. Modified allergens can be safely used for rush SIT protocols decreasing time needed to induce stable remission. We have mapped the epitopes of Asp f 1, f 2 and f 3 and several linear B-cell epitopes have been identified. Th1 and Th2 specific epitopes have also been identified which may have significant importance in SIT (131-133).

7. PREVENTIVE IMMUNIZATION

We have discussed above whether anergy induction is the main mechanism of action during SIT. Experimental systems permit to study the activation and division of immune “allergic” cells after immunotherapy. Recently, Janssen and colleagues found evidence of strong systemic T cell activation during effective immunotherapy (134). Fluorescent labeled T cells from naïve transgenic OVA specific mice passively transferred in wild animals proliferate and produce cytokines protecting these mice from allergic response. T cell numbers below a certain threshold did not affect the effector function of Th2 cell response and did not influence the airway symptoms. These data are supported by the observation that when mice are immunized with pure Af antigens Asp f 1, Asp f 2, Asp f 4 and Asp f 6 they do not produce specific or total IgE, while mounting a high IgG response (135). On the contrary, when the same BALB/c mice were immunized with Af crude extract, containing 20 or more proteins in low concentration, the mice developed high level of total IgE and strong lung eosinophilia. Additional studies are needed to clarify the effect of preventive immunization on the development of allergy.

8. CONCLUSIONS

APBA is a severe complication of bronchopulmonary pathology in asthmatics and cystic fibrosis patients. In spite of the fact that Af related allergic response is most important in ABPA syndrome, hypersensitivity itself to Af does not seem to be the cause of ABPA. The incidence of ABPA in farmers highly exposed to Af conidia is not higher than in the normal population. The main pathology induced in this case is the hypersensitivity pneumonitis, a Th1 mediated lung disease (136-138). Although Af can induce diseases such as

"farmer lungs", asthma, allergic sinusitis, rhinitis and extrinsic allergic alveolitis, and other conditions such as protein rich microenvironment in the bronchopulmonary compartment, the quality of ciliary system of the mucosal epithelium, epithelial resistance to Af proteases, airway remodeling, the deficiency of innate immune system, which can be exhausted during chronic inflammation, all can serve as main contributing factors. Since ABPA involves a hyperreactivity to multiple proteins originated from Af and since IgE to other allergens can also take part in the pathology of ABPA specific immunotherapy can hardly ameliorate the disease. Patients with early forms of Af induced sensitivity and patients with cystic fibrosis as a risk group are the best candidates for Af SIT. Since the main allergens from Af are characterized and their recombinant forms are produced, different vaccines containing several molecules of allergen and CpG or DNA can be studied and further used to induce preventive Th1 or limited Th2 memory responses. However, additional studies are needed in these directions.

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