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The use of biological agents for the treatment of fungal asthma and allergic bronchopulmonary aspergillosis

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Allergic bronchopulmonary aspergillosis (ABPA) is a virulent manifestation of the Th2 asthma endotype that includes asthma with fungal sensitization, raising the feasibility of biological therapies targeting Th2 pathway molecules or cells. The first molecule amenable to clinical intervention with a biological was IgE. Omalizumab, a humanized monoclonal antibody (Mab), targets the same epitope on the IgE CH3 region that binds to and crosslinks high-affinity receptors on mast cells and basophils, thereby initiating the allergic inflammatory cascade. Omalizumab is licensed for allergic asthma and has been beneficial in uncontrolled studies of ABPA, reducing exacerbations and steroid requirements. Trials of several Mabs directed against the Th2 cytokine IL-5 show clinical benefit in patients with a severe refractory eosinophilic asthma phenotype, while a Mab against IL-13 is effective in asthma patients with a Th2-high endotype. Immunodulation is also feasible with small molecule biologicals, such as antisense oligodeoxynucleotides and cholecalciferol. Controlled trials of Th2-inhibiting biologicals in patients with ABPA and severe asthma with fungal sensitization appear warranted.

Keywords: asthma; ABPA; phenotype; endotype; cytokine; omalizumab

Asthma is a chronic inflammatory disease of the airways characterized clinically by intermittent episodes of wheezy shortness of breath, chest tightness, and cough. Pulmonary function tests show bronchoconstriction that is at least partly reversible with acute bronchodilator administration. The airways of people with asthma are hyperresponsive to bronchoconstrictive stimuli. Asthma is one of the most prevalent chronic diseases of humankind, with an estimated 300 million cases worldwide, including 26 million Americans (35% of whom are below 18 years of age). The social cost of asthma is staggering: about $20 billion in the United States in 2010, including over $5 billion in hospital costs, not to mention missed school or work and restricted activity. Acute asthma can be fatal. It is estimated that over half of the total costs of asthma are incurred by the 10–20% of asthmatics with severe disease. Depending on age, between half and three quarters of asthmatics are thought to have an allergic contribution or cause of their disease.[1]

Fungi have long been known to be among the causative agents of acute asthma in atopic patients with fungal sensitization. Fungal exposure has been linked to loss of asthma control, and more recently as a cause of asthma onset in both children and adults. A wide variety of fungi have been implicated, but the most common agents are several Ascomycota, including *Alternaria, Aspergillus, Penicillium*, and *Cladosporium* spp.[2] Recently the connection between fungal exposure, sensitization, and increased severity of asthma has become clearer.[3,4] *Aspergillus fumigatus* in particular has been associated with more severe asthma,[5] with pooled prevalence of sensitization in 28% of asthmatics seen in specialty clinics.[6] Sensitization to *A. fumigatus* is associated with lower lung function in asthma,[7] and antifungal therapy improves symptoms in severe asthmatics with fungal sensitization (SAFS).[8]

Allergic bronchopulmonary aspergillosis (ABPA) is the most severe manifestation of fungal asthma, occurring in ∼2% of asthmatics, and is also a major complication in cystic fibrosis.[9] In addition to fungal sensitization (to *A. fumigatus* in >90% of cases), ABPA is characterized by colonization and fungal growth in the airways, a florid allergic and

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mixed granulocytic local inflammatory response, and progressive structural destruction of the airways (bronchiectasis and fibrosis) unless treated. Systemic corticosteroids and azoles are mainstays of ABPA therapy but treatment is impeded by difficulties in diagnosis, side effects of treatment, and the chronic relapsing natural history of this disease. The global burden of ABPA is estimated at ∼4 million cases, with >500,000 in the United States.[10] Given the current limitations of conventional therapy for fungal asthma and ABPA and the severity of the asthma seen in this group, we propose that new therapies are needed to improve control and outcomes, with a significant role for emerging biological drugs. Before discussing these it is important to frame the approach in the context of our evolving understanding of asthma.

Asthma phenotypes and endotypes

Clinicians have long been used to characterizing people with asthma according to whether they had associated allergies and were sensitized to common aeroallergens, such as pollens, dust mite, animal danders, cockroach, and fungi. The distinction of allergic asthma from nonallergic (or intrinsic) was given a mechanistic underpinning by the elucidation of a $CD4^+$ T cell Th1/Th2 cytokine differentiation dichotomy in murine models, which was soon successfully applied in clinical asthma to show that a substantial element of Th2 polarization is present in the airways of many asthma patients.[11] However, in the 1990s further research revealed that this simple Th1/Th2 dichotomy was inadequate to encompass and adequately explain the broad range of clinical asthma and associated adaptive immune responses.[12] In the last decade, therefore, there has emerged a major effort to reassess asthma and define subgroups from the viewpoint of clinically observable characteristics, or phenotypes (Table 1). Some have gone so far as to plea to abandon the term *asthma* altogether, as it seems more of a conceptual hindrance than a diagnostic or therapeutic aide.[20] In a 2006 review, Wenzel extended the clinical view of phenotypes in persistent adult asthma to include categories based on clinical characteristics, triggers and predominant inflammatory granulocytic cell type.[16] Similar distinctions have been made in childhood asthma. Notably, phenotypes based on simple criteria involving one or few clinical features have been criticized as "one dimensional," and a more sophisticated multidimensional approach using statistical cluster analysis was proposed and has recently been applied.[21] Using this methodology Haldar *et al.* identified three clusters in mild–moderate asthma and four clusters in severe asthma.[13] Moore *et al.*, examining asthma over the entire severity

Table 1. Asthma phenotypes, as organized by clinical presentation/features, precipitating factors, and character of cellular inflammation[13–19]

Clinical presentation/features
Severity
Hereditary, early onset allergic asthma
Poorly reversible, very severe, neutrophilic asthma
Late onset eosinophilic asthma
Late onset, symptom dominant, obese minimal inflammation
Exacerbation proneness
Chronic airflow restriction
Poorly steroid responsive
Age at onset
Pediatric
Adult
Cluster analysis[13–15]
Early onset atopic (mild–moderate/severe)
Late onset obese female noneosinophilic
Early onset noneosinophilic
Late onset eosinophilic
Reduced lung function (more/less reversible)
Precipitating factors
Nonsteroid anti-inflammatory agents
Environmental allergens
Occupational allergens or irritants
Menses
Exercise induced
Ozone
Cigarette smoke
Diesel particles
Infection
Aspirin
Cold air
Obesity related
Character of cellular inflammation
Eosinophilic
Neutrophilic
Mixed
Pauci-granulocytic

spectrum, identified five clusters in which atopy was present in >75% of the total cases and severe asthma was present in about a third.[14] McGrath *et al.* reported that about half of mild–moderate asthmatics do not have a persistent eosinophilic phenotype.[22] The data suggest that increasing asthma severity is associated with allergic sensitization. This conforms well with studies demonstrating the association of fungal sensitization with increasing asthma severity.[4,5,7,8,23]

The most recent development in this effort to dissect asthma into meaningful subgroups has been the identification of distinct pathophysiologic mechanisms underlying the emergence of particular asthma phenotypes, first proposed by Anderson in 2008 with the introduction of the term *endotype*.[24] A consensus report from the European and American Academies of Allergy cited six examples of asthma endotypes (aspirin-sensitive, allergic bronchopulmonary mycoses [ABPM], adult allergic, early-onset allergic, severe late-onset hypereosinophilic, and asthma in cross-country skiers).[17] In this conception, adult allergic asthma and ABPM endotypes, for example, exist within at least two phenotypes, eosinophilic asthma and exacerbation-prone asthma. The complex genetic, molecular, and cellular basis of the endotypic heterogeneity of asthma is being slowly, but surely, elucidated.[18] The Th2 pathway (Fig. 1) is perhaps the most well-studied and best understood of these asthma endotypes, and particularly useful in severe asthma.[19]

The usefulness of this endotypic approach to discernable asthma phenotypes is that it begins to allow rational targeted therapeutic interventions to be defined not only theoretically by disease mechanism but also practically as selection criteria in clinical trials using biomarkers associated with a particular endotype.[25,26] This approach has now begun to be applied, as will be discussed later. It can be seen that fungal asthma and ABPA (as *A. fumigatus* is by far the most common cause of ABPM, accounting for well over 90% of cases) are Th2 endotypes based on extensive examination of their pathophysiologic features, including demonstrable involvement of Th2 cytokines, IgE, eosinophils, and basophils.[9,10,23,27] The Th2 pathway thus retains great explanatory power and therapeutic potential for a substantial number of people with asthma, especially those with fungal asthma and ABPA.[28]

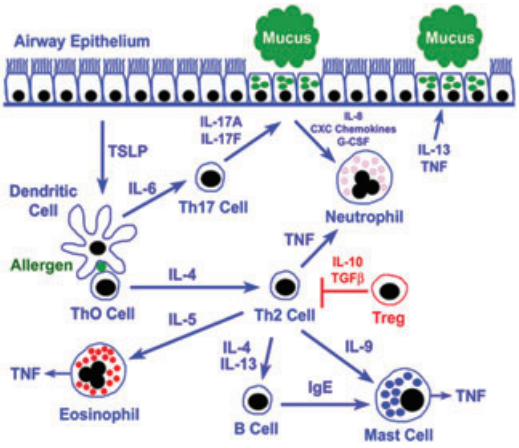

Figure 1. Cells and cell products of the Th2 immunoinflammatory asthma pathway. Respiratory epithelial cells (activated by interactions with products such as fungal pathogen-associated molecular patterns, allergens and proteases) secrete a variety of innate immune molecules including thymic stromal lymphopoeitin (TSLP), which in turn activate pulmonary dendritic cells to induce differentiation of naive $CD4^+$ T cells (Th0) into Th2 cells. Th2 cells are polarized to secretion of a discrete set of cytokines, including IL-4, IL-5, IL-9, IL-13, and tumor necrosis factor (TNF-α). Th2 cytokines orchestrate airway eosinophil and mast cell recruitment, B cell production of immunoglobulin E, mucus secretion, and mast cell (and basophil) priming for allergic response to encountered allergen. Dendritic cell–derived IL-6 promotes differentiation of Th2 and Th17 cells. IL-17 from Th17 cells promotes airway neutrophil entry by inducing epithelial cell production of chemoattractant cytokines, such as CXCL8 (interleukin-8) and granulocyte colony-stimulating factor (G-CSF), a survival and proliferation factor, by bronchial epithelial cells. IL-17 also induces epithelial mucus secretion. The process is downregulated by regulatory T cells (T_{reg} cells) secreting IL-10 and transforming growth factor-β (TGF-β). Reprinted with permission from Ref. 50.

Anti-IgE

Anti-IgE, or omalizumab, was the first and currently the only biological licensed to treat asthma. It is based on the differentiation of allergic from nonallergic asthma, at the distal end of the Th2 pathway where IgE acts upon cells bearing high-affinity IgE receptors to effect release of allergic mediators such as histamine, proteases, cytokines (TNF-α, IL-4, IL-13), and lipid mediators including prostaglandin D2 and leukotrienes B4 and E4.[29] Omalizumab is a humanized IgG1 kappa monoclonal antibody, with <5% murine complementarity-determining region, that binds to circulating free IgE with affinity comparable to the binding of IgE to its high-affinity receptor. The binding site for

omalizumab on IgE is the same third constant heavy chain domain epitope that is the site for IgE binding to its receptors; thus omalizumab does not bind to IgE that is already bound to either high- or low-affinity receptors on inflammatory cells, as the ligand epitope are hidden. Competitive binding of omalizumab to IgE results in formation of IgE–anti-IgE complexes, primarily 2:2 tetramers and 3:3 hexamers, that do not activate complement and are slowly cleared by the reticuloendothelial system. Omalizumab at a concentration of 2–100 times basal IgE level results in >99% of IgE being complexed, leaving <1% available for binding to IgE receptors, thereby ablating a trigger of the allergic reaction cascade. This process also results in eventual down-regulation of receptors and IgE production.[30] By reducing early- and late-phase reactions to allergic stimuli, omalizumab was posited to have the potential to prevent allergen-induced asthma exacerbations.[31] To reduce free IgE sufficiently, a dosing nomogram of omalizumab based on basal IgE level and weight was devised to ensure a minimum dose of 0.016 mg/kg/IgE/month.[32] Omalizumab is administered subcutaneously every 2–4 weeks.[33]

The early pivotal randomized double-blind placebo-controlled trials with omalizumab focused on adolescents and adults with established moderate or severe asthma and allergic sensitization (positive immediate skin test) to at least one perennial allergen (dust mite, cockroach, and/or cat or dog dander) requiring moderate or high doses of inhaled corticosteroids with or without long-acting bronchodilators.[34–40] Thus, fungal-associated asthma was not specifically assessed in these trials. In a meta-analysis of seven such trials lasting 6–12 months each, with pooled evaluation of 2,511 omalizumab and 1,797 control subjects in which asthma exacerbation was the primary endpoint in six trials, omalizumab was shown to reduce exacerbations by 38.3%. Health utilization decreased concomitantly (e.g., emergency room visits by 60% and hospital admissions by 51%) and quality of life scores improved.[40,41] Two pediatric trials in children 6–12 years old ($n = 609$ omalizumab, 301 placebo) with similar selection criteria and treatment length showed safety and comparable endpoint improvements.[42–44]

Recently, a 60-week controlled trial in young inner city 6- to 20-year-olds with predominantly moderate to severe asthma confirmed these benefits and also demonstrated a marked reduction in seasonal exacerbations, suggesting omalizumab reduces exacerbations and symptoms caused by seasonal changes that might be related to pollen or mold exposures (although these were not measured) and/or interactive effects on viral triggers.[45,46] This raises the possibility of seasonal rather than ongoing omalizumab therapy as a potential subject of study, as the maximum effect was observed within one month. A second recent study focused on inadequately controlled severe asthma in adults despite optimal current NIH guideline pharmacotherapy (National Asthma Education and Prevention Program Expert Panel Report 3E, steps 5 and 6);[47] in this 48-week study ($n = 427$ omalizumab, 423 placebo) exacerbations were reduced by 25% and other endpoints such as symptoms, rescue medication use, and quality of life scores also improved.[48] Interestingly in this study a noninvasive measure of pulmonary inflammation, exhaled nitric oxide concentration, also decreased with omalizumab, confirming cellular anti-inflammatory effects.[31,48,49]

Overall, the adverse effect profile of omalizumab is quite good, with rare anaphylactic reactions noted in 0.1–0.2% of recipients, which has led to recommendations that dosing be done under medical supervision with two hours of observation postinjection. There are also ongoing concerns about rare risks of malignancy (0.5% in recipients vs. 0.2% in controls) or possible cardiovascular/cerebrovascular adverse events.[50] The major limiting factors in its use, besides the specificity of its target, are the inconvenience of physician-supervised injections and the issue of pharmacoeconomic justification, that is, health benefits outweighing the high cost; thus, patient selection is key, and should focus on those with severe allergic asthma. To date omalizumab has not been specifically studied in SAFS, which appears to respond to add-on azole antifungal therapy.[8] However, because of the toxicity of prolonged systemic glucocorticosteroid therapy and often inadequate control of ABPA with combination steroid-azole therapy, omalizumab is increasingly used in treatment of ABPA.[51] To date 64 omalizumab-treated ABPA patients have been reported in abstracts and peer reviewed publications, with reduced exacerbations and systemic steroid burden being the main benefits of therapy.[52–60] However, no placebo-controlled trials have been completed. Two recent open-label

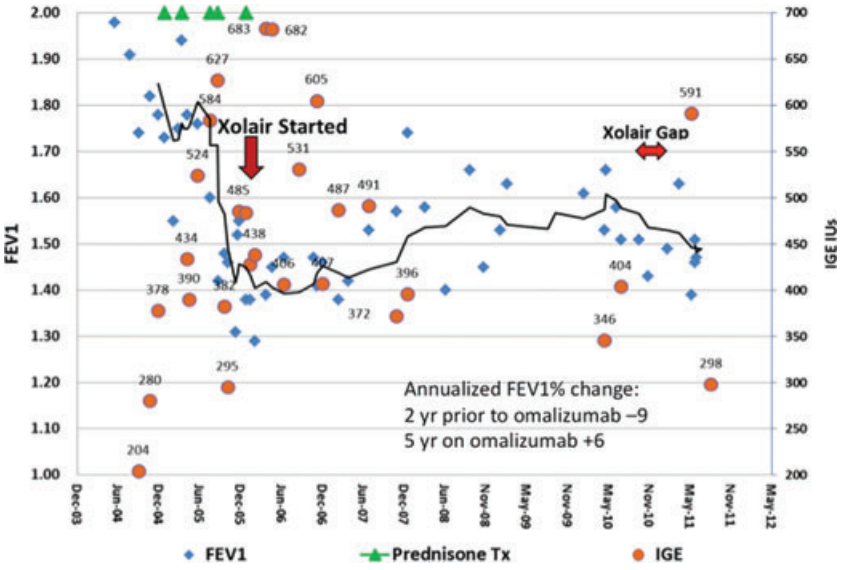

Figure 2. FEV1 and prednisone history. Long-term response to omalizumab in a patient with cystic fibrosis and allergic bronchopulmonary aspergillosis. This patient, currently 23 years old, had ABPA diagnosed in 2000 and was treated conventionally with prednisone and itraconazole, in addition to ongoing treatments for cystic fibrosis and asthma. After a period of accelerated lung function decline, despite several toxic courses of prednisone, she was started on omalizumab (Xolair®, Genentech). Subsequent lung function has been stabilized for six years, with a reduction in IgE and no further need for prednisone. An attempt to discontinue omalizumab in late 2010 resulted in an exacerbation, decline in lung function, and rise in IgE, which resolved with reinstitution of omalizumab. Green triangles = prednisone courses; orange circles = IgE (IU/mL) with values shown; blue diamonds = forced expiratory volume in one second (FEV1, L); solid line = rolling 6-month average FEV1.

series from Spain and France (pooled $n = 34$, 2 with CF-ABPA) showed significant reductions in exacerbations and oral steroid doses.[58,59] A major caveat for this approach, however, is the very high basal IgE levels in these patients, driving a need for concomitantly high doses of omalizumab—up to 600 mg weekly have been employed. Illustrative results of treatment of a patient with cystic fibrosis and ABPA are shown in Figure 2; in this patient accelerated decline in lung function and frequent exacerbations, despite steroid and azole therapy, were halted and stability was maintained, without adverse effects, by long-term omalizumab therapy.

Th2 cytokine inhibition

Although omalizumab is thus far the only biologic agent licensed for treatment of asthma, studies have shown that its real-world effectiveness is limited, with up to 40% of severe asthmatics being nonresponders—in terms of gaining asthma control.[61–63] However, the complexity of the Th2 pathway offers a rich variety of further potential targets for treatment, as well as potential limitations on highly specific agents (Fig. 1).[18,19,26,28,50] Only those biologics whose evaluation reached the stage of mid-to-late (i.e., phase 2 or 3) clinical trials—where multidose safety and at least exploratory clinical endpoint efficacy measures were obtained—are discussed below. Several comprehensive reviews, including other agents and approaches, are available.[64–69]

The Th2 pathway includes an important component of eosinophilic recruitment and activation.[70] Interleukin 5 is known to play a central role in eosinophil differentiation, maturation, and survival. Early controlled clinical trials of monoclonal anti-IL-5 antibodies in patients with mild or moderate asthma demonstrated reductions in blood and sputum eosinophils but little clinical effect.[71] Subsequent trials have focused on selection of a severe adult-onset asthma with persistent sputum eosinophilia despite high-dose inhaled or systemic corticosteroid therapy phenotype, probably representing ~5% of adult asthmatics.[70] Haldar *et al.* studied 61 such patients (29 active, 32 placebo) who received monthly anti-IL-5 for a year and showed that treatment lowered exacerbation rate and increased quality of life score but did not affect

pulmonary function.[72] Nair *et al.*, using the same antibody, studied 20 subjects (9 active, 11 placebo) in a 26-week protocol and also found that active treatment lowered exacerbation rate, improved symptoms and quality of life score, and allowed systemic steroid dose reduction.[73] Castro *et al.*, using another anti-IL-5 antibody, studied 106 patients (53 active, 53 placebo) for 16 weeks. In this shorter study, lung function and quality of life improved on active treatment, with a trend toward reduced exacerbations.[74] Thus, the current evidence suggests that there is a severe asthma phenotype that is responsive to anti-IL-5 therapy. Whether this group might include asthmatics with fungal sensitivity or ABPA remains to be seen.

A second major component of the Th2 pathway proximal to IgE induction is the action of IL-4 and IL-13 in furthering asthmatic pathology.[75] IL-4 and IL-13 have features both distinct and in common; both cytokines act in part via the IL-4 receptor alpha chain (IL-4Rα) of heterodimer receptor cell signaling ligands. Although IL-4 promotes differentiation and proliferation of $CD4^+$ Th2 cells and production of IgE from B cells, IL-13 appears crucial in inducing and sustaining airway hyperreactivity, mucus secretion, and remodeling. Earlier studies using either monoclonal antibodies to IL-4 or a soluble IL-4 receptor were disappointing, although the reasons remain obscure.[67] More recent studies have focused on inhibiting IL-13, either via anti-IL-13 antibodies[76] or interruption of receptor-mediated signaling. A monoclonal antibody to IL-4Rα has shown some activity in patients with more severe asthma phenotype.[77] A similar approach using a mutated nonagonistic IL-4 molecule that competitively binds the IL-4Rα also shows promise.[19] Most impressively, a 24-week controlled trial by Corren *et al.* of an anti-IL-13 monoclonal antibody examined subjects ($n = 107$ active, 112 placebo) with asthma poorly controlled on inhaled corticosteroids.[78] Importantly, in this trial before randomization, subjects were stratified for the Th2 endotype by total IgE level (>100 IU/mL) and blood eosinophil count (>140/mL). Later in the study, serum periostin levels were added as an additional surrogate to examine the Th2-high and -low groups (periostin being an IL-13–induced epithelial product that appears to contribute to airway remodeling).[25] Anti-IL-13 improved pulmonary function; this effect was attributable to positive responses in the Th2-high subgroup. This important study appears to validate a vital physiological role for IL-13 in the Th2 pathway in asthma, and thus offers an attractive and important target for further clinical trials.[75] Here, as with IL-5, the role of IL-13 inhibition in fungal asthma and APBA is currently undefined and merits investigation.

Unfortunately, amelioration of asthma by antibodies to TNF-α and IL-25Rα (CD25) has been outweighed by their toxic side effects, which precluded further development.[79,80]

Other strategies

Inhibition of Th2 cytokines is but one general approach to biological control of asthma. It is also possible to target cells directly. A promising approach is to use antisense oligodeoxynucleotides (ODNs) to target specific RNA sequences and downregulate transcription of specific proteins playing a role in asthma pathogenesis or pathophysiology. The eosinophil, as a major component of several asthma phenotypes, has been selected for study by development of ODNs against several proteins, including the CCR3 chemokine receptor, which has been correlated with asthma severity, and the common beta chain (CD131) of the heterodimeric receptors for GM-CSF, IL-3, and IL-5, all of which are eosinophil growth

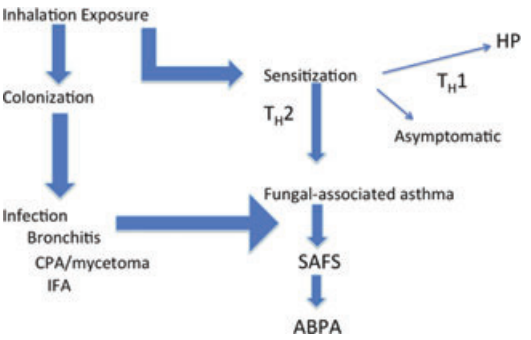

Figure 3. A hypothetical schematic representation of a Th2 pathway spectrum of clinical respiratory disease associated with fungi. Inhalation of fungal conidia or fragments can lead to allergic sensitization and simple fungal asthma. It is likely that in cases of poorly controlled asthma as well as cystic fibrosis, defects in airway host defense lead to germination of conidia and exposure of the host innate defenses to hyphal allergens and proteases that lead to more severe adaptive Th2 responses and granulocytic inflammation, manifesting clinically in severe asthma with fungal sensitization and allergic bronchopulmonary aspergillosis.

factors (in addition to having other activities).[81] An inhalable formulation of a combination small molecule containing two ODNs for CCR3 and CD131 has shown, in a controlled crossover four-day trial, the ability to inhibit sputum eosinophilia and allergen-induced asthmatic responses.[82,83] Further studies seem warranted to determine if these early observations translate into a safe and clinically effective approach.

Finally, it is vital to note that technologically advanced solutions may not necessarily provide the only, or even best, paths to biologic therapy. Fungal allergy and ABPA have been shown to be dependent upon respiratory epithelial cell activation and secretion of innate mediators (such as thymic stromal lymphopoeitin, IL-17, IL-25, and IL-33) that influence dendritic cells to secrete Th2-polarizing chemokines, such as CCL17 and CCL22.[18,84,85] Kreindler *et al.* demonstrated that dendritic cell orchestration of the Th2 pathway occurs via an OX40 ligand-dependent process that is downregulated by vitamin D.[86] Thus, vitamin D supplementation may prove beneficial in preventing or treating fungal allergy and ABPA, a possibility that is currently being tested in patients with CF and ABPA (ClinTrial.gov identifier NCT01222273).

In conclusion, asthma is heterogeneous. Fungal asthma, severe asthma with fungal sensitization, and ABPA are forms of an allergic or Th2 asthma endotype that manifest clinically along a phenotypic severity spectrum (Fig. 3). Omalizumab, or anti-IgE, is an effective biological approach to allergic asthma, including ABPA. Other biologicals targeting elements of the Th2 pathway, such as IL-13, IL-5, and eosinophils, show promise for selected severe Th2 endotype fungal asthma and ABPA patients. Controlled trials of biologics are needed in fungal asthma, severe asthma with fungal sensitization and allergic bronchopulmonary aspergillosis.

Conflicts of interest

The author declares no conflicts of interest.

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