

Allergic Inflammation in *Aspergillus fumigatus*-Induced Fungal Asthma

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Abstract Although fungi are pervasive in many environments, few cause disease in humans. Of these, *Aspergillus fumigatus* is particularly well suited to be a pathogen of the human lung. Its physical and biological characteristics combine to provide an organism that can cause tremendous morbidity and high mortality if left unchecked. Luckily, that is rarely the case. However, repeated exposure to inhaled *A. fumigatus* spores often results in an immune response that carries significant immunopathology, exacerbating asthma and changing the structure of the lung with chronic impacts to pulmonary function. This review focuses on the current understanding of the mechanisms that are associated with fungal exposure, sensitization, and infection in asthmatics, as well as the function of various inflammatory cells associated with severe asthma with fungal sensitization.

Keywords Fungi · *Aspergillus fumigatus* · *A. fumigatus* · Fungal exposure · Infection in asthma · Fungal sensitization · Allergic asthma

Introduction

Allergic airway diseases are thought to arise as a result of aberrant immune cell responses to non-infectious environmental

antigens [1–3]. Asthma has emerged as a major public health problem affecting over 300 million individuals worldwide [4]. The increasing incidence and the need to develop effective therapies have been a major driver for asthma research, with the aim being to better understand the mechanisms of disease pathogenesis and progression.

Although over 1.5 million fungal species have been described, only a handful cause disease in animals, and of those, only a few can routinely circumvent the barriers and innate immune responses within the human lung. In normal exposures, spores are barred by the barriers of sinus turbulence, temperature, mucus, and cilia or eliminated through phagocytic surveillance. However, in situations where innate barriers fail to eliminate the fungi, very serious disease can ensue. Sensitivity to the fungus, *Aspergillus fumigatus*, has been associated with severe persistent asthma in adults [5, 6].

The clinical spectrum of *Aspergillus*-associated respiratory disorders includes *Aspergillus*-induced asthma, allergic bronchopulmonary aspergillosis (ABPA), and allergic *Aspergillus* sinusitis (AAS) [7, 8]. ABPA is the most complex allergic manifestation caused by *A. fumigatus*, and is characterized by severe lung eosinophilia, increased mucus production, increased serum IgE production (total and antigen-specific), and chronic lung fibrosis [7]. Though ABPA is routinely under-diagnosed, particularly in asthmatics, its prevalence has been estimated at 25 % [7, 9].

Although it has been speculated that conidia and mycelium from *Aspergillus* can persist long enough in the airways to release potent antigens that inhibit ciliary movement and/or disrupt the lung architecture, the precise mechanism of *A. fumigatus*-induced allergic airway disease is not known. This review focuses on the current understanding of the mechanisms that are associated with fungal asthma and their role in inflammatory cell recruitment in the asthmatic lung affecting immune regulation in physiological and pathological conditions.

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Experimental Allergic Airways Disease and Asthma

Severe asthma with fungal sensitization (SAFS) is a relatively new designation in pulmonary diagnostics and treatment [10], and experimental models using *A. fumigatus* are being used to explore the mechanisms at play in fungal interactions. Animal models of fungal exposure have provided an understanding of the pulmonary immune mechanisms that mediate fungal pathogenesis and clearance [11, 12]. There are a variety of animal models that have been used to explore SAFS. Most are acute protocols that sensitize the lung with the repeated administration of a soluble antigen extract [13, 14]. Other models use intratracheally (IT) administered conidia to induced airway disease in animals that have been previously sensitized with *A. fumigatus* fungal extracts [15, 16]. While this type of model provides a closer representation of fungus-induced human airway disease, the route by which the conidia are introduced does not replicate inhalation that allows the spores to be inhaled deep into the lung. In a modification of the IT model, an inhalational route has also been employed to expose the allergen-sensitized animal to the live conidia of *A. fumigatus* to more closely mimic human exposure [17, 18•, 19]. Recently, an inhalational model of conidia exposure has been developed in which the real-time particle mass concentration in the animal's breathing zone can be continuously monitored [20•].

Fungal Asthma-Associated Inflammation

Fungal spores are generally cleared quickly and successfully from the lung via innate and mechanical mechanisms, but when they are encountered in large numbers or routinely, the innate response may not be sufficient to remove the spores and allergic sensitization takes over. Allergic model systems utilizing *A. fumigatus* have added considerably to our understanding of the orchestration of both the innate and acquired pulmonary responses to fungal spores.

Bronchial Epithelial Cells Airway epithelial cells contribute to the overall immune response to *A. fumigatus*, as they are the first cells to encounter the inhaled conidia. Bronchial biopsies from asthmatic patients demonstrate irregular tight junctions between airway epithelial cells with increased permeability of the epithelium pointing to a pathogenic role of epithelial dysregulation in asthma [21]. Epithelial cells secrete soluble antimicrobial compounds that play a direct role in airway defense [22], as well as expressing a broad repertoire of pathogen recognition receptors (PRRs) including toll-like receptors (TLRs), C-type lectins, protease-activated receptors (PARs), and the NOD-like receptors (NLRs), which can detect both fungal pathogen-associated molecular patterns (PAMP) and

damage-associated molecular patterns (DAMP) produced by local cellular death or stress [23–25].

Fungal factors, such as sialic acid residues on the conidial coat, contribute to the ability of *A. fumigatus* to bind and modulate the airway epithelium [26•, 27, 28]. Pathogenic species of *Aspergillus* display more conidial sialic acid than non-pathogenic aspergilla [27, 29, 30], supporting better adhesion to fibrinogen, laminin, and fibronectin that is partially mediated by sialic acid residues and other proteins of the conidia surface [31]. In conditions of lung injury or tissue distress when these matrix components are exposed, the risk factor for invasive aspergillosis (IA) is heightened. Fibrinogen and fibronectin attach to wounded surfaces such as the surface of the damaged epithelia, and laminin is exposed upon epithelial injury or detachment [9]. Thus, sialic acid, and perhaps other conidial factors that bind to alveolar components, could contribute to pathogenicity by enhancing adhesion to and colonization of the epithelia and components of injured tissue [9, 30].

The activation of epithelial PRRs leads to the release of cytokines, chemokines, and antimicrobial peptides in vivo that attract and activate the innate and adaptive immune cells [26•]. Members of the defensin family of antimicrobial peptides, which have broad-spectrum activity against multiple microbes, are produced by epithelial cells in vitro following incubation with *A. fumigatus* [9]. *Aspergillus* germinating conidia and hyphae are recognized in vitro by host PRRs on epithelial cells and induce the production of cytokines and chemokines such as IL-6, TNF- α , IL-33, IL-25, thymic stromal lymphopoietin (TSLP), monocyte chemoattractant protein-1 (MCP-1), and IL-8 [9]. In particular, IL-25, IL-33, and TSLP produced and released by airway epithelial cells induce and promote T_H2-type airway inflammation and cause remodeling and pathological changes in the airway walls, suggesting pivotal roles in the pathophysiology of asthma [32, 33]. Airway epithelium is not merely a structural barrier, but must be considered an active player in the pathogenesis of asthma and other allergic disorders.

Macrophages Current evidence suggests that macrophages have a wide range of immunoregulatory functions in fungal asthma beyond that of a barrier to pathogens intruding into the alveolar tissue. The three main classes of macrophages found in the lung tissue are the bronchial macrophages (BM), interstitial macrophages (IMs), and alveolar macrophages (AMs) [34, 35]. Alveolar macrophages are the primary resident phagocytic cells of the respiratory tract and a critical component of the host defense against *Aspergillus* conidia. When AMs are primed with allergen, they can also help expand the allergen-specific T cell response [36]. Macrophages activate multiple immunological pathways and serve as the first line of defense, while manifesting an anti-inflammatory response to protect against excessive tissue damage.

Alveolar macrophages phagocytose *Aspergillus* conidia in an actin-dependent manner, a process mediated by the recognition of pathogen-associated molecular patterns of host cell PRRs. PRR engagement of *A. fumigatus* ligands generates a proinflammatory response characterized by the production of cytokines and chemokines, including TNF- α , IL-1 β , IL-6, IL-8, macrophage inflammatory protein 1 α , and MCP-1 [37, 38, 39]. TLR2, TLR4, and the C-type lectin receptor dectin-1 are the well-characterized PRRs involved in the recognition of *A. fumigatus* and the activation of host cells [9, 39]. Both in vitro and in vivo studies have demonstrated that conidia and hyphae activate macrophages through TLR2 and TLR4 [27, 39]. TLR2 recognizes both conidial and hyphal morphologies, whereas TLR4 recognizes only the hyphal form.

Unlike TLRs, dectin-1 is essential for host defense against *Aspergillus* in both immunocompromised and immunocompetent hosts [40]. Dectin-1 is specific for the fungal carbohydrate β (1,3)-glucan, which is normally masked on resting *A. fumigatus* conidia by the proteinaceous hydrophobin layer [9, 41]. Following conidial swelling, β (1,3)-glucan becomes exposed and is present on swollen conidia, germlings, and hypha morphotypes. Dectin-1- β (1,3)-glucan engagement results in phagocytosis, macrophage activation, and a strong induction of proinflammatory responses [40, 41]. Thus, dectin-1, with additional contributions by TLRs, enables innate immune cells to phagocytose and kill conidia as well as elicit proinflammatory responses.

In adoptive transfer experiments, allergen-induced AHR and inflammation is ameliorated when AMs from unsensitized mice are transferred to macrophage-depleted sensitized mice [42]. It was apparent that AMs from unsensitized mice, which are not antigen-primed and have better regenerative capacity, provided protection against asthmatic symptoms [43, 44]. Visser et al. [45] further demonstrated that intravenously administered allergen-loaded macrophages accumulate in the spleen and induce indirect, but long-term, allergen-specific immunosuppressive effects through regulatory T cells (Tregs). Similar observations have been made in human asthmatic studies where suppression of T cell proliferation by AMs is thought to be mediated through cell-cell interactions [46]. Further, AMs from non-asthmatic subjects express fewer costimulatory molecules (CD80 and CD86) compared with AMs from asthmatics, decreasing their capacity to induce the production of IL-4 and IL-5 [47, 48]. In contrast, AMs from asthmatic subjects produce more IL-10 than healthy counterparts, suppressing the inflammatory response [35, 49].

Dendritic Cells Under inflammatory conditions, CD11b⁺ monocytes are recruited to the lungs and rapidly differentiate to dendritic cells (DCs) [50, 51]. In steady-state conditions, DCs line conducting airways of the lung [52], but are also found in interstitial compartments deeper in the lungs [50]. DCs initiate and maintain adaptive T helper 2 (T_H2) cell

responses to inhaled conidia in asthma. However, various functions such as antigen uptake, migration to the draining lymph nodes (LNs), and induction of tolerance and adaptive immunity are not equally shared by all subsets of DCs, adding considerable complexity to the immunology of allergic sensitization [53]. DCs sample the airway lumen by forming tight junctions with epithelial cells by expressing occludin and claudin family members as well as zona occludens-1 (ZO-1) [53]. In addition, a subset of DCs that probe the airway lumen attaches to the airway epithelial cells via E-cadherin and CD103 [52].

DCs that become activated by *A. fumigatus* conidia and hyphae mature and produce inflammatory cytokines, such as TNF- α , IL-12, IL-6, and IL-10 [54, 55, 56]. Mature DCs clear *A. fumigatus* conidia and hyphal elements, indicating their importance before and after the onset of fungal disease [27, 57]. DCs also participate in the adaptive immune response: when conidia-activated DCs are adoptively transferred, they activate IFN- γ -producing T cells, leading to fungal resistance [57]. Recent studies demonstrate the role of Dectin-1 and Dectin-2 in the induction of a proinflammatory cytokine response in immature DCs and plasmacytoid dendritic cells (pDCs) [55, 56], bridging innate and adaptive immune mechanisms during fungal infections.

Neutrophils Polymorphonuclear neutrophils (PMNs) are the predominant inflammatory cells that infiltrate airways during acute exacerbations of asthma and have been associated with the severity of asthma [58]. Moreover, studies in humans have demonstrated neutrophil recruitment in response to allergen challenge coincides with the peak of CD4⁺ T cell recruitment [59, 60]. However, PMNs in the sputum of asthmatics have been shown to produce lower levels of IL-8, IL-1 β , and TNF- α , and show reduced TLR4 mRNA expression as compared to controls [13, 27, 61]. Recent studies have also demonstrated that IL-17-producing neutrophils increase in fungal asthma [62], suggesting that the alteration of neutrophil functions in asthma may be a trigger for a tolerance-to-sensitivity shift.

Neutrophils play an essential role in antifungal defense [63]. PMNs were initially thought to act exclusively on hyphae of *A. fumigatus* [27]. However, several studies have shown that they are also able to ingest and kill resting or swollen conidia not previously killed by macrophages [30, 61]. They rapidly migrate to the infected lungs and suppress conidial germination through phagocytosis or extracellular trap formation [13]. Nevertheless, neutrophils remain responsible primarily for hyphal, not conidial, killing [64]. Contact between neutrophils and hyphae triggers a respiratory burst, secretion of reactive oxygen intermediates, and degranulation [65]. In contrast to the killing of conidia by macrophages, hyphal damage by PMNs is rapid. In one study, 50 % of the hyphae were killed after a 2-h incubation [27], although

in vivo spatial conditions may be considerably different than in vitro.

Given the correlation of airway neutrophilia with severity of airway obstruction in humans, neutrophils have been postulated to promote more severe airway inflammation by mediating direct tissue injury or by elaborating proinflammatory mediators [66, 67]. Conversely, it is possible that the accumulation of neutrophils is an epiphenomenon that does not directly contribute to pathogenesis. Finally, neutrophils could conceivably serve to attenuate the inflammatory response; for instance, in some inflammation models, neutrophils have skewed acquired immune responses to a T_H1 phenotype [68]. Neutrophils may also serve to clear some antigenic stimuli from the lungs by virtue of their antimicrobial properties; this may be particularly relevant in allergic airway responses to fungal antigens (Ags) because neutrophils are recognized as being important in innate immunity to fungi [69].

Eosinophils Eosinophils are recruited to the lung through the orchestration of maturation factors, chemoattractants and differential expression of receptors for homing [70, 71]. Eosinophils regulate the allergen-dependent T_H2 pulmonary immune responses mediated by dendritic cells and T lymphocytes, as well as suppress T_H1 responses [71]. Eosinophils can mediate inflammation in the lung compartment by releasing their granule proteins with cytotoxic, immunological, and remodeling-promoting properties [72].

Activated eosinophils participate in the modulation of the immune response by inducing AHR and releasing leukotrienes, growth factors, and metalloproteinases, which play a role in airway remodeling [73]. The leukotrienes liberated from eosinophils are potent bronchoconstrictors and perpetuate the migration of eosinophils to the airways [70, 73, 74]. It is little wonder that many successful treatments for reducing asthma symptoms in either human or animal models correlate with a reduction in eosinophil counts.

The cytotoxic compounds contained in the granules of eosinophils are cytotoxic to bronchial epithelium and are associated with the immunopathology of fungal asthma [75]. The release of eosinophilic granules increases vascular permeability in vivo at physiologic concentrations that are observed in pathological conditions [76]. They also activate mast cells to release proinflammatory mediators including histamine, eicosanoids, and cytokines [77]. Eosinophils produce stem cell factor (SCF) and nerve growth factor (NGF), which support the growth and survival of mast cells [76]. Eosinophils also produce the T_H2 -type cytokines IL-4 and IL-13, which potentially stimulate the release of eotaxin and the production of CCL2 and CCL5, further enhancing eosinophil recruitment [70]. Eotaxin can induce respiratory burst and actin polymerization in eosinophils, directly contributing to tissue damage and coordinating the continued recruitment of both eosinophils and T cells [78].

Eosinophils can also recognize the products of adaptive immunity. Sepharose beads coated with IgG, IgA, and secretory IgA (sIgA) stimulate eosinophil degranulation, with sIgA being the most effective. Of these, sIgA is the most potent as eosinophils possess binding sites for the secretory component, and interaction with sIgA increases eosinophil proinflammatory function [79–81]. The role of IgE in mediating eosinophil activation is controversial. Some studies have shown that eosinophils isolated from patients with eosinophilia degranulate in response to anti-IgE antibody [82] and that a high-affinity IgE receptor is present on eosinophils from patients with eosinophilia and various effector functions are mediated through this receptor [82, 83]. Other studies have shown that the number of high-affinity receptors expressed on the surfaces of eosinophils from patients with allergic diseases is minimal and that ligation of FcεRI does not result in eosinophil degranulation [84, 85].

In the context of fungal asthma, eosinophils have proven to be a successful target; however, eliminating eosinophils may not be a risk-free therapeutic strategy. Eosinophils contribute to the defense against respiratory viruses, which may impact both viral disease and, in turn, predispose the asthmatic patient to virus-associated exacerbations. Given the importance of eosinophils in the pathogenesis of asthma, and also in lung defense mechanisms, the best asthma treatments must include partial control of eosinophilic responses.

T Lymphocytes Substantial evidence supports the notion that allergic asthma is an immune-mediated disease linked to a spectrum of T helper (T_H) responses, including T_H1 , T_H2 , and T_H17 cell activation [70, 86•]. Local allergen challenge with *A. fumigatus* has been shown to induce an influx of $CD4^+$ T cells into the airways [47•, 48, 87]. $CD4^+$ T_H2 cells that secrete IL-4, IL-5, IL-9, and IL-13 contribute to many pathophysiological features of fungal asthma, including airway inflammation, mucus secretion, and AHR [63, 87, 88]. The identification of transcription factors controlling T_H1 and T_H2 development further support the T_H2 hypothesis because GATA3 is overexpressed and T-bet is under expressed in the asthmatic airway [89]. Much of the current research is aimed at understanding how T_H2 cytokines act on resident lung cells to induce the asthmatic phenotype, and signaling pathways and transcription factors involved in this process are rapidly being elucidated.

More recently, T_H17 cells have been implicated in the development of pulmonary inflammation and AHR [12, 48]. IL-6 promotes inflammation and pulmonary fibrosis [90, 91], further suggesting that the “ T_H2 theory” is inadequate to explain the diverse forms of asthma in humans and the various inflammatory responses seen in animal models of allergic asthma. Over the last few years, a number of research groups have demonstrated a more direct role for IL-17 in exacerbating eosinophilia and neutrophilia [12, 48, 92, 93]. Similarly,

neutralization of IL-6 has been associated with decreased inflammation in allergic mice, which occurs in conjunction with IL-17A, indicating that IL-6 can regulate IL-17A production in models of lung injury [90]. Consistent with these observations, our laboratory has demonstrated a role of IL-17 and IL-6 responses in mediating eosinophilic and neutrophilic inflammation in *Aspergillus*-induced asthma [94•]. This is supported by other recent work that demonstrates a role of IL-17 in driving pulmonary eosinophilia [48], suggesting a need for future studies determining the role of IL-17 in the initiation of the inflammatory response in fungal asthma.

Effector functions of T helper cells are regulated by Tregs. Treg-mediated suppression of allergic airway inflammation is governed largely by IL-10 and TGF- β . IL-10 is essential for effective suppression of allergic responses in the lung [95]. Adoptive transfer of antigen-specific CD4⁺CD25⁺ Tregs suppresses allergic inflammation and AHR via an IL-10-dependent mechanism [96, 97]. When delivered after the onset of disease, Tregs producing IL-10 were able to downregulate established inflammation and prevent airway remodeling [94•]. This dependence on IL-10 highlights an important feature of how Tregs control pulmonary responses.

Like IL-10, TGF- β also induces the peripheral expression of the transcription factor Foxp3, promoting the generation of CD4⁺ CD25⁺ Tregs that are able to inhibit allergic airway disease [98–100]. TGF- β influences the lineage specificity of effector T cell subsets. It is instrumental in driving the ROR γ T-dependent differentiation pathway in CD4⁺ T cells, resulting in either T_H17 or Tregs depending on the concurrent presence of maturation and polarization factors such as IL-6, IL-21, retinoic acid, IL-23, and IL-10 [101–103]. Although TGF- β has been associated with CD4⁺ Foxp3⁺ Treg-mediated regulation of allergic airway disease, the majority of studies have described a central role for IL-10, with or without TGF- β , in the control of allergic airway inflammation. A major reason for this is because TGF- β plays a vital role in promoting the structural changes of tissue remodeling associated with severe asthma [104, 105].

Innate Lymphoid Cells Innate lymphoid cells (ILCs) are non-T, non-B effector cells that show high levels of effector cell functions (mainly cytokine production) upon activation [106]. ILCs are involved in tissue homeostasis, repair, and remodeling, as well as innate immunity [107]. Unlike B and T cells, ILCs lack recombined antigen-specific receptors, providing instead a nonspecific, rapid response to a wide range of innate signals [108]. ILCs have been classified into three subsets—type 1 (ILC1s), type 2 (ILC2s), and type 3 (ILC3s)—based upon cytokine production patterns [108]. The prototypic ILC1s is the NK cell, which produces IFN- γ and expresses T-bet [109]. ILC2s, which were previously called nuocytes, produce IL-5, IL-9, and IL-13, a set of cytokines similar to those produced by T_H2 cells [110]. ILC2s require the

transcription factors ROR α and GATA3 [111]. ILC3s have been divided into three subtypes: (a) lymphoid tissue inducer (LTi) cells, which produce both IL-17 and IL-22; (b) IL-17-producing cells, which are active in the gut of patients with colitis and in obesity-related forms of asthma; and (c) IL-22-producing cells, which are present in the skin and gut [112••].

IL-5- and IL-13-secreting ILC2s have been the most closely linked to allergy [113], but ILC3s may add additional nuance when allergy is overlaid with the response to fungi. A series of intriguing studies from the Steele research group have demonstrated that, once triggered by the engagement of the Dectin-1 beta glucan receptor, IL-22 mediates both the innate response to fungi [114, 115] and the increased mucus and cytokine/chemokine expression associated with the pathophysiology of the response to multiple fungal exposures [116]. This cytokine expression includes IL-33 production [116], which was shown to induce proliferation of Lin[−]CD25⁺CD44^{hi} ILC2s in an *Alternaria* model [117]. The production of IL-5 and IL-13 by ILC2s in response to this fungus was abrogated in *Il1rl1*^{−/−} mice, suggesting that ILC2s mediate mold-induced allergic response in an IL-33-dependent manner [117]. Although it is important to note that the source of IL-22 was not identified in the *Aspergillus* studies, it is tempting to hypothesize that a ready supply of pulmonary IL-22 from ILC3s may contribute to the varying reactions in the fungus-sensitized lung through both antifungal and allergic responses.

B Lymphocytes B cells in all stages of activation and differentiation are found in increased numbers in the blood and bronchial mucosa of clinical patients undergoing an asthma attack [87, 118, 119]. The role of B cells in allergic asthma has largely been consigned to their production of Abs, which mediate mast cell degranulation [120, 121]. This pathophysiological role of allergen-specific Ab is supported by the successful use of anti-IgE therapy [122, 123]. While allergen-specific antibodies are recognized as contributing factors in the immunopathology of an aberrant response to an innocuous allergen such as pollen or animal dander [124], they have also been suggested to be a component of the successful clearance of fungal elements from the airways [125, 126].

In the allergic lung, a number of factors support the relative importance of B lymphocytes in the development of T cell responses and cytokine production. These include antigen receptor specificity, antigen uptake and processing, and anatomical co-localization in the lymph organs and inflammatory zones of the airways. B cells present in the lymph nodes can present antigen at a capacity that approaches that of DCs [87]. DCs and B lymphocytes may work in conjunction, as published work shows DCs passing off antigen to B lymphocytes [87]. Such a cooperative scenario may also occur in the inflammatory zone of the parenchyma in the allergic lung, where concentrations of T and B cells congregate [87, 127].

B cells have been implicated in the control of the allergic response through the regulation of T_H2 -type cytokines and chemokines [87, 128, 129, 130•], and a steady supply of antigen from inhaled conidia, coupled with the dramatic influx of B lymphocytes to the lungs, may favor a B lymphocyte-centric APC scenario that helps to explain why T_H2 -type responses are blunted after sensitization and fungal challenge in the absence of B lymphocytes in $J_H^{-/-}$ and μ MT mice [87, 94•].

Although there are clearly aspects of the B cell's role in allergy that are not well understood, using different models of sensitization and challenge may help clarify important pathways in the B cell's regulation of pulmonary responses. For example, studies in our lab using a systemic antigen sensitization followed by an inhalational challenge with *A. fumigatus* conidia show significantly more granulocytic inflammation in the B cell-deficient lung when compared with normal controls [94•]. From this data, we hypothesized that in the absence of B lymphocytes, the development/survival of Tregs or inducible Tregs is impaired, perhaps explaining the more robust neutrophilic and eosinophilic inflammation in these mice [131, 132]. However, recently published work using a mixed allergen challenge of *A. fumigatus*, *Alternaria alternata*, house dust mite antigen, and ovalbumin (used as an allergic response tracker) in an inhalation-only sensitization/challenge model in the same B cell-deficient animals ($J_H^{-/-}$) showed a marked reduction in eosinophilia [130•] when B cells were not present: virtually the opposite response, ostensibly due to a change in the route of sensitization. Other work has shown that B cells may indirectly influence the T cell response by altering DC function and/or lymph node organization, modifying the resulting phenotype at another level of immune organization [133–135]. Taken together, these results suggest multiple points where B cell regulation impact systemic outcomes. These findings have important implications for understanding normal B cell activation in health and disease, as well as our understanding of appropriate animal models to mimic human disease.

ECM Components Hyaluronan (HA) is a major component of the ECM that undergoes dynamic regulation under conditions of inflammation. In its native state, HA exists as high molecular mass (HMM HA), usually in excess of 10^6 Da [136]. However, under conditions of inflammation and tissue injury, HA is more polydisperse with a preponderance of low molecular mass HA (LMM HA) [136]. A number of studies have shown that LMM forms of HA (<500 kDa) induce inflammatory responses in various cell types, supporting the concept that HA fragments generated at sites of inflammation, but not native HMM HA, can stimulate the production of inflammatory mediators by many cell types [137, 138].

Increased amounts of HA and its degradation products are observed in many allergy models of asthma [47•, 139–143]. While it is unclear whether the generation of HA fragments is

the result of HA catabolic enzymes (HYAL1-4), ROS, a truncated product of the HA biosynthetic enzymes (HAS1-3), or a combination of these mechanisms, it is clear that HA polymers of specific sizes contain distinct biological properties and can impact cellular inflammation.

Some of the cell types that are known to interact with HA are T cells, eosinophils, macrophages, fibroblasts, and smooth muscle cells [141, 144]. LMM HA may function as a signaling molecule for B lymphocyte chemotaxis and cytokine production, which are critical to the maintenance of allergic inflammatory responses [145]. Similarly, other research groups have demonstrated a role of LMM HA in impacting eosinophil function and movement [138]. Further studies examining HA's role and other ECM components in mediating cellular responses may help to develop targets for treatment in patients with SAFS.

Conclusions

Epidemiological studies on the risk factors and genetics of asthma, as well as mouse models of the disease, have revealed a crucial interplay between inflammatory cells and disease pathogenesis. Both anecdotal accounts and clinical research indicate a growing risk for individuals in industrialized countries to develop fungus-related allergic diseases. The bias for this trend is unknown, yet it provides incentive to expand our knowledge of the protective and pathological roles that the lung's immune response plays in allergic asthma. Allergic asthma is a chronic inflammatory disease that comprises multiple phenotypes and inflammatory triggers. The different phenotypes share common inflammatory features, though their specific immunologic pathways may differ mechanistically. As we dissect these mechanisms at the molecular and cellular level, the treatment of patients with allergic asthma can be tailored to specific phenotypes. Targeting the cells, and mediators produced by them, is the basis of current asthma treatment and highlights the potential for the development of novel therapeutic agents in the future. Moreover, a greater understanding of the early-life origins and factors leading to disease consolidation and persistence will open new doors for both asthma prevention and treatment.

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Compliance with Ethics Guidelines

Conflict of Interest Drs. Ghosh, Schuh, and Mr. Hoselton declare no conflicts of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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- Of major importance

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