

YEAR IN REVIEW

Developments in the field of allergy mechanisms in 2015 through the eyes of Clinical & Experimental Allergy

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Clinical & Experimental Allergy

Summary

In the first of two papers we described the development in the field of allergy mechanisms as described by Clinical and Experimental Allergy in 2015. Experimental models of allergic disease, basic mechanisms, clinical mechanisms and allergens are all covered. A second paper will cover clinical aspects.

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In this first of two reviews, we discuss the new findings in the field of allergy in 2015 focusing on mechanisms. Here, we look at experimental models of allergic disease, clinical mechanisms, basic mechanisms and allergens and their interaction with the immune system. In the next issue, we will focus on clinical aspects.

Experimental models of allergic disease

Allergen function in allergic sensitization

A large proportion of airborne allergens contain proteases (serine, cysteine and aspartate proteases), and emerging evidence from clinical and animal-based

models suggests that these allergen-derived proteases act on epithelial cells to (i) activate protease-activated receptors and stimulate production of epithelial-derived pro-T helper type 2 (Th2) cytokines [thymic stromal lymphopoietin, interleukin-25 (IL-25) and IL-33] and (ii) disrupt the epithelial barrier, which facilitates allergen delivery across the epithelial layer [1]. Dysregulation of barrier function and increased allergen absorption in the presence of pro-inflammatory cytokine production is thought to lead to direct activation of inflammatory cells (dendritic cells and pulmonary macrophages) that exacerbate an inflammatory allergic response and allergic phenotypes [2–5]. An example of an airborne allergen that contains proteases is the

filamentous fungus *Aspergillus fumigatus*. This filamentous fungus possesses a number of allergens that are proteases and has been linked with positive reactivity in severe asthma phenotypes in adults as well as the recently described severe asthma with fungal sensitization [6, 7]. The clinical importance of the individual *A. fumigatus* protease allergens is largely unknown; however, studies suggest that fungal protease allergens play a crucial role in disrupting epithelial integrity and subsequent initiation of inflammatory and airway remodelling responses [1].

Namvar et al. [8] investigated the role of individual *A. fumigatus* allergen proteases, Asp f 5 (a matrix metalloproteinase) and Asp f 13 (a serine protease) in airway inflammation, hyper-reactivity and remodelling in an experimental murine model of asthma. Studies utilized a clinically isolated parent strain of *A. fumigatus* and derived mutants lacking Asp f 5 (UniProt, AFUA_8G07080) and Asp f 13 (UniProt, AFUA_2G12630). The authors showed that Asp f 5 ($\Delta 5$) deletion had little effect on the proteolytic activity, as total $\Delta 5$ *A. fumigatus* protease activity was comparable to that of the parent wild-type (WT) strain; however, Asp f 13 ($\Delta 13$) deletion had a dramatic effect on proteolytic activity as very little substrate conversion was observed in $\Delta 13$ *A. fumigatus*. Assessing the disease phenotype in mice sensitized to the parent WT strain or the $\Delta 5$ or $\Delta 13$ *A. fumigatus* strains revealed that Asp f 5 and Asp f 13 were dispensable for development of the Th2 response and airway hyperreactivity (AHR).

Interestingly, loss of Asp f 5 and Asp f 13 did have a dramatic effect on the level of cellular inflammation and airway remodelling [8]. Moreover, the levels of neutrophils, eosinophils and lymphocytes were attenuated in these mutant strains compared to the WT parent strain, as was the level of airway remodelling [i.e. goblet cell (GC) hyperplasia and subepithelial collagen deposition]. The molecular basis of this modulatory activity was not delineated; however, the comparable antigen-driven Th2 response among all groups ($\Delta 5$, $\Delta 13$, WT) suggests that Asp f 5 and Asp f 13 are likely acting during the effector phase to modulate inflammatory cell (eosinophil) recruitment and downstream leucocyte-dependent histopathologic features such as airway remodelling. These observations indicate that the onset of an *A. fumigatus*-mediated allergic airway inflammation does not require the protease allergens Asp f 5 and Asp f 13; however, these allergens can act as modifiers and amplify the allergic inflammatory response and onset of the manifestations of the asthma phenotype.

T cell function in allergic inflammation

Hallmark features of the allergic asthmatic inflammatory response are CD4⁺ Th2 cells and elevated type-2

cytokines (IL-4, IL-5 and IL-13) [9, 10]. Th2 cells orchestrate the allergic inflammatory response through driving immunoglobulin E (IgE) production by B cells and acting in concert with innate lymphoid type 2 (ILC2) cells to produce the type 2 cytokine signature (IL-4, IL-5 and IL-13) and subsequent mast cell and eosinophilic inflammation and clinical manifestations of asthma [11, 12]. A family of transcription factors known as nuclear factor of activated T cells (NFAT) play an important role in T cell receptor-mediated, early gene transcription in T cells [13, 14]. Studies in the late 1990s employing various NFAT member-deficient mice revealed a complex interaction between the NFAT transcription factors in regulating IL-4 and IgE levels and allergic responses [15–17]. Moreover, NFATc1-deficient T cells showed decreased IL-4 production [16], whereas NFATc2- and NFATc3-deficient mice showed enhanced Th2 cytokines, IgE and allergic disease [17]. Koch et al. [18] generated a mouse with selective deletion of NFATc1 in the CD4⁺ T cell compartment by crossing CD4^{Cre} with NFATc1^{flox/flox} mice (CD4^{Cre}/NFATc1^{flox/flox}). The authors revealed that loss of NFATc1 in the CD4⁺ T cells did not affect Th2 development, as levels of the Th2 signature transcription factors GATA3, c-MAF and BATF were unaffected in Th2-polarizing conditions *in vitro*; however, there was decreased production of CD4⁺ T cell-derived cytokines (IL-2, IL-4, IL-5 and IL-13). Not surprisingly, the CD4^{Cre}/NFATc1^{flox/flox} mice had a reduced capacity to develop Th2-driven responses including reduced antigen-specific IgE and some asthma manifestations, namely GC hyperplasia, after allergen challenge. Unexpectedly, pulmonary levels of type 2 cytokines (IL-4, IL-5 and IL-13), airway inflammation (eosinophilic inflammation) and AHR were comparable in CD4^{Cre}/NFATc1^{flox/flox} and WT mice. The reduced antigen-specific IgE levels may be a result of reduced expression of CD4⁺ T cell-derived IL-4 or BTAF, given that both play important roles in immunoglobulin class-switching of plasma cells [19].

The perceived conflicting observation between reduced CD4⁺ T cell-derived type 2 cytokines production and the observed normal pulmonary type 2 cytokine levels, eosinophilic inflammation and AHR is likely attributable to the presence of a normal ILC2 compartment, which is not targeted in the CD4^{Cre}/NFATc1^{flox/flox} mice. This possibility is consistent with the emerging evidence that indicates that ILC2-derived cytokines are sufficient to drive allergic airway inflammation and the asthma phenotypes [20].

In contrast to the role of Th2 cells in exacerbating allergic inflammatory responses, T regulatory cells (Tregs) are thought to suppress the onset of allergic inflammation [21]. Consistent with this function, levels of peripheral, naturally occurring Tregs (nTregs) and

IL-10⁺ Tregs, are decreased in patients with asthma and have reduced suppressive capabilities [21]. Experimental analyses have elegantly demonstrated the suppressive capability of CD4⁺ CD25⁺ nTregs, as transfer of these cells into asthmatic mice reversed an established asthma phenotype [22, 23]. These data suggest roles for the suppressive cytokines IL-10, transforming growth factor β (TGF- β) and IL-35, as well as surface molecules CTLA and PD-1, in Treg-suppressive function; however, the underlying molecular basis of Treg suppression of allergic responses is not yet fully delineated. A recent study by Li *et al.* [24] revealed a requirement for CD39, an ectonucleoside triphosphate diphosphohydrolase 1 (ENTPDase 1), in Treg-suppressive activity and suppression of allergic inflammation. CD39 hydrolyses adenosine triphosphate and adenosine diphosphate to adenosine monophosphate and is expressed on nTregs in mice and the majority of nTregs in humans [25, 26]; notably, the loss of CD39 function in mice leads to loss of nTreg-suppressive activity [25, 26]. Li *et al.* showed that pharmacologic or genetic inhibition of CD39 activity leads to increased allergic airway inflammation and an asthma phenotype. The authors showed that increased disease was associated with a reduced frequency of nTregs and an impaired Treg capacity to limit allergic airway inflammation. The reduced Treg function was linked with adenosine and adenosine A2A receptor-suppressive function. These studies reveal an important role for CD39 in the protective function of CD4⁺ FoxP3⁺ Tregs in allergic airway inflammation. Notably, other purinergic receptors (P2Y2, P2Y4 and P2Y6) have also been implicated in several aspects of the asthma phenotype, including inflammatory cell recruitment (DCs and eosinophils), bronchospasm and airway remodelling, suggesting that purinergic signalling regulates multiple components of the allergic inflammatory cascade [27–29].

Mechanisms of inflammation, airway remodelling and airway hyperreactivity

The hallmark features of the asthma phenotype are allergic airway inflammation associated with eosinophils and neutrophils, airway remodelling (GC hyperplasia and fibrosis) and AHR [30]. Over the last two decades and with the development of murine models of experimental asthma, investigators have identified several underlying immune pathways that contribute to the clinical manifestations asthma [31–33]. A study by Asaduzzaman *et al.* [34] revealed an important role for proteinase-activated receptor 2 (PAR-2) in the induction of allergic airway disease. The authors revealed that pharmacologically inhibiting PAR-2 by drug antagonism and neutralizing antibody approaches significantly attenuated the development of the asthma phenotype

and importantly reversed established airway inflammation and asthma manifestations. These studies corroborate previous experiment-based analyses with PAR2-deficient mice showing decreased airway inflammation and AHR response after antigen sensitization and challenge [35, 36]. Intranasal administration of PAR-2 monoclonal antibodies and inhibitors was sufficient to inhibit the asthmatic phenotype, suggesting that the effects were due to blockade of local PAR-2 activity. PAR-2 is expressed by numerous pulmonary cells, including epithelial cells, smooth muscle cells and pulmonary macrophages, and can be activated by a variety of allergens and endogenous proteases to drive inflammatory reactions [37]. Although several serine proteases have been identified in the bronchoalveolar lavage of allergic mice, the proteases responsible for activating PAR-2 and augmenting the allergic inflammatory cascade are yet to be determined. Collectively, these studies indicate that PAR-2 is a key signalling step involved in the induction of allergic inflammation and the clinical manifestations of asthma and may be a therapeutic option for asthma.

Another molecule important in the onset of the clinical manifestations of asthma is IL-13 [33]. IL-13 has a broad spectrum of action in asthma including the following: isotype switching to IgE production; regulating leucocyte recruitment via chemokine expression and adherence protein activation; GC hyperplasia and increased mucus production; collagen deposition; and AHR [38, 39]. A recent study by Vock *et al.* [40] revealed a possible intrinsic mechanism within the distal lung that protects the alveoli from excessive GC hyperplasia and mucus plugging. The authors identified a difference in the level of GC hyperplasia and mucus production between the proximal and distal airways of mice with the allergic asthma phenotype. The authors noted that reduced GC hyperplasia in the distal airway was associated with reduced levels of the transcription factors SAM-pointed domain-containing ETs-like factor (SPDEF) and forkhead box A3 (FoxA3), major regulators of GC function. IL-13 is a potent inducer of SPDEF and FoxA3 and is required for GC hyperplasia. The authors revealed lower expression of IL-13R α 1 in distal than proximal airways of healthy mice. The authors speculate that the reduced expression and activation of IL-13R α 1 in distal airways may contribute to the absence of GC hyperplasia in mice with acute and chronic allergic airway disease and that this may be an intrinsic mechanism to protect from distal airway mucus plugging and impaired alveolar function.

Immune tolerance

An intriguing phenomena that derived from studies using murine-based models of allergic airway disease is that

exposure of mice to innocuous antigens under non-inflammatory conditions induces tolerance to that antigen. Moreover, subsequent exposure to the antigen does not promote sensitization or allergic responses [41–43], which is thought to be dependent on Treg activity [44]. A recent study by Ather et al. [45] examined whether activation of the transcription factor NF κ B in airway epithelial cells can overcome antigen tolerance and promote the allergic airways disease phenotype. The authors tolerated the airway epithelium of WT mice and mice capable of expressing constitutively active I κ B kinase β (CAIKK β) to the inhaled antigen ovalbumin (OVA) and then subsequently exposed the mice to OVA after epithelial CAIKK β activation. The authors showed that mice in which NF κ B expression was activated in the airway epithelium developed allergic airway disease. This phenotype was associated with increased myeloid DC maturation, increased ILC2s and the development of a Th2 and Th17 response. The authors also showed that the onset of the Th2/Th17 response and allergic airway disease was dependent on the cytokines IL-1 and IL-4. These studies identified the myeloid DC IL-1:IL-4 axis as an important initial step in inhalational antigen sensitization and the onset of clinical manifestations of asthma. Consistent with this concept, increased myeloid DCs have been observed in patients with asthma [46] and previous murine-based studies employing *Il1r1*^{-/-} and *Il1a*^{-/-} and *Il1b*^{-/-} mice have revealed a requirement for the IL-1RI/IL-1 pathway in allergic Th2 responses and development of the asthma phenotype [47]. Although animal-based studies clearly show that IL-4 is not required for the Th2 response and AAD phenotype [48], the presence of IL-4 significantly amplifies the Th2 cytokine signature and asthmatic response [11]. The study also suggests that activation of epithelial-derived NF κ B is sufficient to overcome tolerance to inhalational antigens. Activation of the NF κ B pathway in airway epithelial cells can occur after several exogenous (bacterial, viral) or endogenous (cytokines, endoplasmic reticulum stress and unfolded protein response) stimuli. Although there is significant clinical and experimental evidence that activation of an airway epithelial NF κ B response can provoke sensitization [49], there is evidence in mouse and man that this is not sufficient to promote allergic disease phenotypes. This would suggest the requirement of additional pathways, possibly including those associated with the myeloid DC:IL-1:IL-4 pathway, to overcome tolerance and drive the clinical manifestations of asthma.

Molecular understanding of immunotherapy

There is high-level evidence that specific immunotherapy reduces symptoms in people with allergic rhinitis (AR), allergic conjunctivitis, asthma and insect sting allergy [4–8].

Specific allergen immunotherapy, particularly sublingual immunotherapy (SLIT), has emerged as a treatment of allergic diseases [50], and there is significant clinical evidence that SLIT reduces symptoms in people with AR, allergic conjunctivitis, asthma and insect sting allergy. Liu et al. [51] examined the efficacy of SLIT with *Dermatophagoides farinae* vaccine in a murine model of atopic dermatitis (AD). The authors showed that SLIT treatment comprising repeated administration of *D. farinae* extract markedly reduced Th2 cytokine production, eosinophilic inflammation and clinical and histopathologic symptoms of AD. Notably, the clinical improvement was associated with a reduced *D. farinae*-specific IgE/IgG ratio, and the SLIT was proposed to inhibit symptoms of AD through balancing the Th2 vs. Th1 cytokine predominance. A recent Cochrane systematic review revealed no consistent evidence that SLIT is effective for treating AD; however, more research is required [52]. Given the evidence from the preclinical mouse model studies, further clinical assessment of SLIT in treating AD is warranted.

An alternate approach for treating allergic diseases such as asthma is the use of allergen-free immunotherapy approaches such as the *Mycobacterium leprae* 65-kD heat-shock protein (HSP65) or Toll-like receptor (TLR) 9 ligand [CpG oligodeoxynucleotides (ODNs)]. These microbial proteins and TLR agonists are potent negative regulators of Th2-driven immune responses, and treating mice with CpG ODNs or *M. leprae* HSP65 inhibits the development of the asthma phenotype [53–55]. A study by Prado et al. [56] demonstrated that treating mice with the combination of HSP65 and CpG ODNs more markedly suppresses the allergic inflammatory response than treating with HSP65 or CpG ODNs alone. In particular, levels of Th2 cytokines (IL-4 and IL-13) were suppressed by > 75% with HSP65 and CpG ODNs combined compared to ~50% with either HSP65 or CpG ODNs alone. The authors showed that the HSP65/CpG ODN-suppressive effect required activation of the TLR9:CCR2:IFN- γ axis and was associated with an increased CD11c⁺ Cd11b⁺ Ly6C⁺ pulmonary monocyte response [53]. Genetic deletion of the TLR9:CCR2:IFN- γ axis led to a loss in the CD11c⁺ Cd11b⁺ Ly6C⁺ myeloid cell-driven skewing of the Th1 response, suggesting that HSP65/CpG ODN-mediated suppression of the asthma phenotype involved the myeloid cell compartment [56]. Previous studies by Rha et al. [53] revealed that *M. leprae* HSP65 attenuation of the asthma phenotype involved gamma delta ($\gamma\delta$) T cells. Unfortunately, the possible involvement of myeloid cell: $\gamma\delta$ T cell communication in the enhanced inhibitory activity of HSP65/CpG ODN treatment was not examined. These studies suggest that a combined HSP65/CpG ODN treatment approach may be a more efficacious

approach than individual HSP65 or CpG ODN for treating allergic disease phenotypes.

Clinical mechanisms

Understanding the development of allergic sensitization

The view that the microbiome influences the development of allergic disease has gained traction in recent years, although the mechanisms are not clear. Using 16S ribosomal RNA sequencing of DNA extracted from fecal samples, Azad *et al.* [57] observed a relationship between low gut microbiota richness in early infancy (3 months of age) and subsequent food sensitization among infants at 1 year of age. Work by West *et al.* [58] echoed these findings in the context of IgE-associated eczema, but went a step further to implicate a deficiency in anti-inflammatory bacteria in the development of disease. A similar concept reported by Oriuori *et al.* [59] described a link between higher levels of the inflammatory marker, calprotectin, in the stool of infants and lower abundance of IL-10-inducing *Escherichia*, along with an increased risk of asthma and AD. While microbial dysbiosis may promote inflammatory pathways in the gut, work by Wang and Wang [60] in children with AD suggested that probiotic treatment might redress the microbial imbalance, if administered sufficiently early in life.

Several studies sought pre- and post-natal cellular mechanisms that might protect or else predispose to allergies. The observation that maternal intake of vitamins D and E impacted the responsiveness of neonatal epithelial cells to house dust mite and LPS, hinted at 'conditioning' of the innate response *in utero* [61]. In other work, infants with higher cord blood levels of the active vitamin D metabolite, 1 α ,25-dihydroxyvitamin D, displayed decreased Th2 responses in allergen-stimulated PBMC cultures at 6 months of age, again implying a protective effect of vitamin D [62]. Other studies focused on the theory that dysregulated adaptive immunity in early life might promote allergic sensitization. T regulatory T cells express the transcription factor, Foxp3. Bullens *et al.* [63] reported lower ratios of Foxp3/CD3 γ consistent with deficient Tregs, in the cord blood of children who developed allergy before 2 years of age. Subsequent exposure to pollutants during childhood might also contribute, as evidenced by the report of its association with increased methylation of *FOXP3* and impaired Treg function [64].

Diagnosis of food allergy and mechanisms of sensitization and tolerance

The continued rise in food allergies presents a major problem, and the need for improved diagnostic

approaches that mitigate the risks of oral food challenge is clear. Allergen component analysis, as well as basophil allergen threshold sensitivity, may hold value, as was shown for the diagnosis of hazelnut allergy, by discriminating IgE sensitization from symptomatic allergy [65]. Beyond diagnosis, there remains a lot to learn about how sensitisation to foods evolves. The phenomenon of liver transplant-associated food allergy presents a unique opportunity for study in this area. De Bruyne *et al.* [66] reported increased serum IgA, as well as higher food allergen-specific IgA and IgE, in children who developed food allergy post-transplant compared with those who did not. Given that IgA⁺ memory B cells were higher in *all* children who received transplants compared with both allergic and non-allergic children who did not, the findings point to disruption of intestinal immune homeostasis.

It is generally acknowledged that Tregs are central to the development of tolerance to foods. Qamar *et al.* [67] reported increased Tregs, including those that express IL-10, in children with egg or peanut allergy who had previously been allergic but since acquired tolerance. However, to what degree immunological tolerance is achieved may depend on the context, a consideration that has important clinical implications. For example, following oral immunotherapy (OIT), low numbers of peanut-specific Th2 cells with a pathogenic signature were identified in peanut-allergic children, despite clinical desensitization and a rise in peanut-specific IgG4 antibodies [68].

Towards a systems biology approach

New technologies provide powerful tools for identifying biomarkers of disease and clinical outcomes. In particular, the use of bioinformatics platforms that analyse complex data sets in an unsupervised fashion has gained momentum. Martinez-Botas *et al.* [69] used an allergen peptide microarray coupled with machine learning analysis to identify combinations of IgE-binding peptides derived from cow's milk proteins that predicted safety and efficacy of cow's milk OIT. In another study, Wu *et al.* [70] used model-based clustering to link cytokine expression patterns in dust mite-stimulated cultures to clinical outcomes in children. These types of analyses are fundamentally exploratory and do not rely on investigator bias or *a priori* hypotheses. Transcriptomics is also informing new research directions and holds promise for diagnostic purposes. For example, Lexmond *et al.* [71] reported mRNA profiling of 79 target genes in oesophageal tissue to create a diagnostic model built on only ten differentially expressed genes that distinguished eosinophilic oesophagitis from reflux oesophagitis. Together, these studies underscore the potential clinical applications of these new experimental tools.

Basic mechanisms in allergic disease

The year 2015 represented an exciting year for studies that advanced our understanding of the underlying basic mechanisms that regulate allergy. These discoveries ranged across a breadth of aspects of allergic mechanisms, including initiation of allergic priming, regulation over inflammatory processes and early pre-clinical assessment of new therapeutic targeting.

Early life influences

It has been appreciated for quite some time that genetics of allergic diseases provides only a partial explanation for what is seen clinically [72] and so the mechanisms related to environmental and societal influences have become an important area of research. Two manuscripts published during 2015 have shone new light onto these aspects. The work by Bundhoo et al. [73] addressed the important biology of how maternal IgE and IgE immune complexes can be actively transported across the placenta and was the topic of an editorial focus [74]. Previous studies in *Clinical & Experimental Allergy* had demonstrated how FcRn, the neonatal Fc receptor, regulated the absorption of immune complexes in murine models [75] and that this included IgE as well as more established transport of IgG molecules. Importantly, this IgE transport was shown to be capable of triggering basophil degranulation. Bundhoo et al. have now extended this important mechanism into human FcRn and demonstrated that much of the IgE found in cord blood was in a hetero-complex with IgG against IgE. In agreement with the murine study, this cord blood IgE correlated with IgE-positive basophils in the infants.

The environment as a factor in allergic development was also addressed by Ajamian et al. [76], who demonstrated the ability of respiratory syncytial virus (RSV) to tip the balance of immunity towards Th2. The influences of early microbial exposures to development of allergy have been well-studied and yielded both protective and pathogenic associations [77], with pathogenic links particularly for infection with respiratory viruses such as RSV. The study by Ajamian et al. helps to define the mechanism through which RSV might exert this pathogenic influence, with the authors showing elegantly that the virus induces the enzyme indoleamine 2,3-dioxygenase (IDO) in DCs and that IDO-expressing DCs suppressed T cells from adopting a Th1-associated phenotype but left Th2-associated responses intact.

Regulating allergic responses

Understanding the mechanisms that regulate the progression of allergic responses was another area that

Clinical & Experimental Allergy made significant impact in during 2015. In the 'Experimental models of allergic disease' section, the importance of Toll-like receptor 2 (TLR2) was highlighted as being important in regulating the balance between oral tolerance and allergy by a study by Tunis et al. [78]. Previous studies had concluded that signals via the TLR2 pathway were important for enhancing tolerance [79, 80]. However, through the use of TLR2-deficient mice, Tunis et al. demonstrated that TLR2 is not required for oral tolerance. Instead, TLR2 functions as a modulator of the B cell production of IgA vs. IgE, and low-dose TLR2 ligands are particularly protective. The authors highlight how this has potential impact for future vaccine and immunotherapy development strategies.

New discoveries about the TLR family of receptors were furthered by exciting data from Suurmond et al. [81] on how this family of innate sensing receptors also modulates the responses of IgE-primed mast cells. By looking at how concurrent activation by TLR ligands impacts cell function during the IgE-mediated activation process, the authors found dramatic differences in the production of several cytokines and chemokines. This was most notable for stimulation by the TLR4 and TLR6 pathways. Importantly, most allergen exposures likely include additional danger signals such as those that activate the TLR family, and so this work might very well reflect a more physiological interrogation of mast cell activation to IgE cross-linking than typically considered. In particular, the contribution of endotoxin (LPS) during allergen exposures, shown to be a modulating factor in house dust mite responses by the authors, seems to be an important factor worthy of further investigation.

In the lung, recent discoveries have helped to delineate the mechanisms regulating inflammation, airway function and remodelling events critical in understanding asthma biology. The remodelling process was investigated by Fang et al. [82], and the importance of resistin-like molecule- β (RELm- β) clearly demonstrated. Utilizing a potent model of airway inflammation to *A. fumigatus* and RELm- β -deficient mice, the authors demonstrated that, while collagen and procollagen increased in both control and RELm- β -deficient mice, expression of other fibrosis-associated molecules, including Laminin- α 1, was ablated in the absence of RELm- β . The authors furthered this work into human asthma airways and found significant effects over fibroblast responses and the deposition of extracellular matrix proteins, helping to add to the growing understanding of the important role remodelling and fibroblasts play in asthma [83].

Two interesting papers, one in 'Experimental Models of Allergic Disease' and one in 'Basic Mechanisms in Allergic Disease', addressed the importance of the

Rho-kinase pathway in allergic airway biology. Rho-kinase is a serine/threonine protein kinase that has previously been shown to regulate eosinophil recruitment, mucus hypersecretion and airway hyperresponsiveness in a murine model of asthma, because blocking its functions with a selective inhibitor (fasudil) prevented these hallmark responses [84]. Furthering these initial proof-of-concept studies into mechanistic understanding, Xie et al. [85] now demonstrate that fasudil exerts its antimucus effects by decreasing the expression of MUC5AC in the lung and were able to link this to alteration of STAT6 functions on the promoter of this gene. Importantly, this links Rho-kinase inhibition to suppression of the key signalling pathways for both IL-4 and IL-13. Using genetically deficient mice, Kasahara et al. provided a more in-depth understanding of the biology of this pathway in allergic airway processes. As Rho-kinase possesses two different isoforms (ROCK1 and ROCK2), the authors were able to demonstrate that ROCK2, but not ROCK1, was the key regulator of mucus hyperplasia [86]. Interestingly, the authors demonstrated that both ROCK1 and ROCK2 contributed to AHR, suggesting divergent regulation over mucus and lung contractility, and further supporting the efficacy of fasudil. Taken together, these studies provide crucial mechanistic insights into a potential new therapy that has already gone into clinical trials.

Allergens and their interaction with the immune system

The starting place for an allergic reaction is the allergen [87] and its interaction with the immune system. Our understanding of allergens and their interactions is developing.

Otsu et al. [88] have demonstrated that patients with more severe clinical reactions to peanut have specific IgE that recognizes less linear epitopes of Ara h 2 and Ara h 6 than for patients with milder reactions. This observation might provide a way of better predicting which patients are at risk of severe reactions. Many patients with sensitization to aeroallergens only present with either asthma or rhinitis. Multiple T cell epitopes are implicated in an allergic response [89]. Dillon et al. [90] looked to see whether CD4⁺ T helper responses to cockroach might explain these differences. Sensitized patients with and without asthma exhibited similar Th2-polarized responses, but individuals with asthma had larger

responses with different allergen specificity. To understand the immunological mechanisms underlying allergic responses, both the T cell epitopes and B cell epitopes need to be elucidated. Garcia Alonso et al. [91] describe these for the *Anisakis simplex* allergen, Ani s 1.

Local allergic rhinitis (LAR) with typical AR symptoms without systemic evidence of allergic sensitization is increasingly being recognized. Campo et al. [92] have assessed their immunological responses to *Olea europaea*. All AR and most LAR patients had a positive nasal allergen challenge with raised ECP levels in nasal lavages plus positive basophil activation test. A key question is why LAR patients do not have systemic evidence of allergic sensitization?

To make an allergy diagnosis, appropriate allergens are required. For example, high levels of ragweed were identified in Leicester (U.K.) in September [93]; this is a novel allergen for the UK. It was not clear whether it was produced locally or blown in from continental Europe. Not all whole allergen preparations contain adequate amounts of all allergen components, and Der p 3 is one such component. Bouaziz et al. [94] described a synthetic form of this allergen for use to improve the diagnosis of house dust mite allergy. Other cross-reactive allergens can also impede the diagnostic value of a test, and one example was described in a research letter from Walton et al. [95]. In a scabies-endemic population, specific IgE levels to house dust mite were elevated; however, only high Der p 1 and 2 levels indicated actual house dust mite allergy. Cross-reactive carbohydrate determinants can also cause confusion for delineating wheat flour and grass allergy. Howarth et al. [96] suggest that this is only seen in a minority of UK bakers assessed in an occupational lung disease clinic for possible occupational asthma. Finally, component resolved diagnostics may not be the answer for every food allergy as shown by Thalayasingam et al. [97], whose findings suggest that specific IgE to shrimp tropomyosin does not improve diagnosis in a Singapore population.

Conclusions

The year 2015 was another interesting year in the field of allergy with advances across our understanding of the mechanisms underlying allergic reactions and allergic diseases. In the next issues, we will review 2015 from the view point of clinic allergy.

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