

SPECIAL ARTICLE

Developments in the field of allergy in 2013 through the eyes of Clinical and Experimental Allergy

M. A. Blankestijn¹, R. J. Boyle², R. Gore³, C. Hawrylowicz⁴, D. Jarvis⁵, A. C. Knulst¹ and A. J. Wardlaw^{6,7}

¹Department of Dermatology and Allergology, University Medical Center Utrecht, Utrecht, The Netherlands, ²Department of Paediatrics, Imperial College London, London, UK, ³Manchester Academic Health Science Centre, University Hospitals of South Manchester and East and North Herts NHS Trust, Stevenage, UK, ⁴Department of Respiratory Medicine and Allergy, Guy's Hospital, King's College London, London, UK, ⁵Respiratory Epidemiology, Occupational Medicine and Public Health, National Heart and Lung Institute, Imperial College, London, UK, ⁶Department of Infection Immunity and Inflammation, Institute for Lung Health, University of Leicester, Leicester, UK and ⁷Department of Respiratory Medicine, University Hospitals of Leicester NHS Trust, Leicester, UK

Clinical & Experimental Allergy

Summary

2013 was another exciting year for allergy in general and Clinical and Experimental Allergy in particular. In the field of asthma and rhinitis, there continued to be a focus on heterogeneity and phenotypes with increasing use of biostatistical techniques to determine clusters of similar populations. Obesity- and aspirin-associated disease are intriguing associations with asthma which were explored in a number of papers. We published a number of excellent papers on mechanisms of airway inflammation and how this relates to physiology, pathology, genetics and biomarkers in both human and experimental model systems. In terms of mechanisms, there is less on individual cell types in allergic disease at the moment, but the immunology of allergic disease continued to fascinate our authors. Another area that was popular both in the mechanisms and in the epidemiology sections was early life events and how these lead to allergic disease, with an increasing focus on the role of the microbiome and how this influences immune tolerance. In the clinical allergy section, oral immunotherapy for food allergy is clearly a major topic of interest at the moment as was *in vitro* testing to distinguish between sensitization and allergic disease. There was less on inhalant allergy this year, but a good representation from the drug allergy community including some interesting work on non-IgE-mediated mechanisms. In the allergen section, important new allergens continue to be discovered, but the major focus as in the last couple of years was on working out how component-resolved approaches can improve diagnosis and management of food and venom allergy.

Correspondence:

Professor Andrew J. Wardlaw,
Institute for Lung Health, Glenfield
Hospital, Groby Road, Leicester LE3
9QP, UK.

E-mail: aw24@le.ac.uk

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Asthma and rhinitis

Obesity and asthma

There continues to be considerable interest in the relationship between asthma and obesity [1]. A question of great practical importance in management is whether weight reduction results in improved asthma control. Scott et al. [2] found that in a randomized study of weight reduction due to dietary or exercise interventions, both clinical and inflammatory parameters of control improved, emphasizing the importance of a weight reduction strategy as part of any management protocol, particularly for severe asthma. In a similar weight reduction study from the same group in obese

children, relatively little weight loss led to improvements in lung function although in this study, which was set in context in an editorial by Holguin, there was no change in markers of inflammation [3, 4].

Obesity-related obstructive sleep apnoea is common in a difficult asthma population. Lavigne et al. [5] found that intranasal topical corticosteroids improved control of obstructive sleep apnoea (OSA) in patients with asthma particularly those with documented allergic rhinitis emphasizing once again to 'always ask about the nose' in asthma clinics.

Obesity and physical activity (or inactivity) are closely linked. In ISAAC, asthma and eczema were more common in overweight and obese children and adolescents [6]. Children and adolescents who watched

television for more than 5 h per day reported more allergic disease. Parents/children were also asked how frequently their child/they 'engaged in vigorous physical activity long enough to make your child/yourself breathe hard?' This question may not accurately capture children's highly variable and intermittent activity patterns, and no association with disease was seen. However, there was a *positive* association of allergic disease with physical activity in teenagers. While it is possible that adolescents with respiratory disease have lower exercise tolerances and report breathlessness at lower levels of exercise, this does not readily explain the higher rates of rhinitis and eczema in those teenagers reporting more frequent vigorous exercise. There are reports of vigorous exercise having adverse effects on lung health, particularly in elite athletes, but the mechanism for these associations is not clear [7]. The associations and interactions of allergic disease with vigorous exercise, sedentary lifestyle and obesity may be more easily investigated now that relatively cheap and versatile accelerometers are available.

Treatments for asthma and rhinitis

In the last three decades, at least in the United Kingdom, there have only been two new classes of medicines licensed for asthma. One class are the leukotriene receptor antagonists (LTRA), montelukast and zafirlukast which antagonize the receptors for the sulphidopeptide leukotrienes LTC₄ and LTD₄ [8]. These have an established place in asthma therapy with both bronchodilating and anti-inflammatory properties, demonstrating, at least in the subset of asthmatics who respond to these drugs, the importance of the 5-lipoxygenase (5-LO) pathway to asthma pathogenesis. Two problems with the LTRAs are firstly that they do not prevent the actions of the chemoattractant LTB₄ which may be important in neutrophil recruitment into the airways, and secondly, they do not inhibit the actions of LTE₄ which is the most stable sulphidopeptide leukotriene and binds only weakly to the CysLT1 and CysLT2 receptors with strong circumstantial evidence that it acts through an alternative receptor. 5-LO requires an activating protein to become functional (FLAP). Kent et al. [9] reported the activity of a drug which inhibited FLAP in a human allergen challenge study. The drug was significantly better than placebo at inhibiting both the early and the late response as well as reducing the sputum eosinophilia generated after allergen challenge. This was promising data to support further development of this cousin of the LTRAs.

Overuse of salbutamol has long been considered to be a marker of poor asthma control including the risk of severe exacerbations. This was objectively confirmed by Patel et al. [10] who found that increased salbutamol

use predicted an increased risk of severe exacerbations although the effect size was relatively modest and did not inform whether this was a cause or effect relationship.

Rhinitis and chronic rhinosinusitis

Considerable attention has been given to poor compliance as a cause of treatment failure in asthma, but this topic has been given less prominence in allergic rhinitis where it is just as important, both in the use of topical treatment and sublingual immunotherapy. This area was comprehensively reviewed by Passalacqua et al. [11]. A clinical assessment of the severity of rhinitis and response to treatment can be difficult in the clinic. Demoly et al. [12] have provided a useful service to the allergy community in validating the visual analogue score as a simple tool for use in the clinic both for primary and secondary care.

The triggers for non-allergic rhinitis (as with non-allergic asthma) remain uncertain although there is increasing interest in the role of locally generated specific IgE. A small subset of patients may have mastocytosis which should be added to the list of conditions that needs to be sought in this setting [13].

El-Shazly et al. [14], using primarily immunohistochemistry, demonstrated significantly more NK cells infiltrating the nasal tissue of patients with allergic chronic rhinosinusitis than non-allergic chronic rhinosinusitis or healthy controls. A single nasal aeroallergen provocation challenge increased the expression of CX3CR1 on NK cells in allergic rhinitis patients. The study highlights a potentially novel role for CX3CR1, and the ligands CX3CL1 and CCL26, in trafficking of NK cells to the upper airways.

Baumann et al. investigated whether Th2 cytokines would be increased in nasal lavage after allergen challenge and natural allergen exposure. They found increased concentrations of IL-5, IL-16 and soluble ST-2 in both settings providing further evidence that the paradigm of Th2-mediated inflammation holds sway in allergic rhinitis [15].

Nasal polyposis is an unpleasant and poorly understood condition, where sinus infections, chronic inflammation and increased vascularity, are each implicated. The role of the bacterial product lipopolysaccharide (LPS) in stimulating vascular endothelial growth factor (VEGF) expression and the mechanism underlying VEGF production in nasal polyps were studied [16]. LPS stimulated the production of VEGF through the TLR4-Akt signalling pathway in nasal polyp-derived fibroblasts, leading the authors to conclude that bacterial infection can mediate the marked remodelling of the nasal mucosa seen in this disease.

Wang et al. [17] investigated the roles of IL-17 and IL-23 in allergic nasal inflammation. In an

ovalbumin (OVA) challenge murine model, they observed that IL-17 inhibition resulted in less neutrophil and eosinophil nasal infiltration, suggesting a role for this cytokine in the accumulation of both cell types in allergic airways disease. Inhibiting IL-23 had similar effects, possibly because IL-23 may act partly through the IL-17 pathway, but this also resulted in an attenuation of allergen-specific IgE production and allergic symptoms, suggesting a role for this cytokine in the allergic humoral response.

Mechanisms of inflammation and airway remodelling in asthma

Efferocytosis is the process by which apoptotic bronchial epithelial cells are removed from the airway by phagocytosing macrophages. Simpson et al. [18] studied macrophages isolated from the sputum of adult asthmatics. Efferocytosis was reduced in those asthmatics with sputum neutrophilia, when compared to those with eosinophil-predominant airways inflammation. More work is needed to determine whether targeting this process will improve asthma control, and to establish how and when such a defect arises during the natural history of the asthma sufferer.

Eosinophilic airway inflammation is linked to fatal asthma, asthma exacerbations and steroid responsiveness. However, the relationship between the presence in the airways of inflammatory cells and the structural changes that constitute airway remodelling is unclear.

Wilson et al. [19] explored this relationship. In a cross-sectional study of control subjects and mild steroid-naïve asthmatics (thereby removing the potential confounding factor of steroid treatment), patients underwent full airways inflammatory profiling and airways biopsy. The asthmatic patients were divided into two groups: tissue eosinophil^{HIGH} and tissue eosinophil^{LOW}. Patients in the former group demonstrated more pronounced changes of remodelling, with increased epithelial damage, reticular basement membrane thickening, expression of the mucus protein MUC2, increased airway smooth muscle (ASM) thickness and, notably, an increase in airway smooth muscle infiltration by T cells and eosinophils. There may therefore be a causal role for the eosinophil in epithelial damage and basement membrane thickening. The presence of increased numbers of eosinophils within ASM is intriguing, although how they influence ASM pathophysiology is as yet unknown. The study is also of methodological interest; the authors used epithelial EGF-R receptor expression as a measure of epithelial damage, avoiding the problem of interpreting the effects of crush artefact on epithelial damage. These findings were set in context by an accompanying editorial [20].

Airway chitinases and chitinase-like proteins are evolutionarily conserved proteins that appear to contribute to the Th2-allergic airway response in mouse models. Levels of the chitinase-like protein YKL-40 in both the human lung and serum have been shown to correlate with asthma disease severity and specifically to subepithelial basement membrane thickening [21]. YKL-40 may therefore be involved in the airway remodelling process. Gavala et al. [22] showed that human airway allergen challenge induced airway chitinase activity and YKL-40 expression. CCL18, an important lung-derived Th2-related chemoattractant (reviewed by Tsicopoulos et al. [23]), was also increased by allergen challenge. The correlations among each of these three factors induced by allergen challenge support an allergen-mediated role in the mechanisms of airway remodelling in the human asthmatic lung [24].

There is increasing interest in the role of fungi in causing tissue damage and inflammation in both the upper and lower airways. Fungi can cause problems in allergic disease by acting as seasonal aeroallergens and by causing low-grade infection of the bronchial tree. Fungi such as *Alternaria alternata* and *Cladosporium herbarum* cause the former, whereas thermotolerant fungi, many belonging to the *Aspergillus* and *Penicillium* genera, which can grow at body temperature, can cause both problems [25, 26]. Two papers addressed controversial issues in fungal allergy; firstly the role of fungi in causing sinusitis and secondly the criteria used to define a subendotype of asthma (and cystic fibrosis), called allergic bronchopulmonary aspergillosis (ABPA) [27, 28]. Callejas and Douglas have undertaken a comprehensive review of all aspects of fungal sinusitis describing the various patterns of upper airway disease, how to diagnose them and the current state of the art in management, emphasizing the central role that surgery plays. They highlight the lack of good clinical trial data to support the use of antifungal therapies. Agarwal and his colleagues from the International Society for Humans and Animal Mycology as part of a very comprehensive review of the literature have tackled the vexed question of the diagnostic criteria for ABPA. Their opinions on this subject are essential for reading for anyone with an interest in allergic fungal disease.

The human asthmatic airway manifests a heterogeneous T cell profile that cannot be described accurately by reference to the Th1/2 paradigm alone. Seys et al. [29] set out to map this diversity by evaluating the gene expression of a wide range of cytokines in induced sputum from patients with asthma and healthy control subjects. In doing so, they explored relationships among cytokine gene expression and a range of clinical characteristics. Intriguingly, the asthmatics fell into three distinct groups, each with a different sputum cytokine profile: IL-4-high; IL-5-high; and IL-5/IL-17A/IL-25

high. The latter group had worse lung function and poorer asthma symptom control than the other groups.

In the same issue, Chien reported that children with moderate persistent asthma had increased numbers of CD4+ve IL-17A +ve T cells, increased fractional exhaled nitric oxide (FeNO) and serum IL-17 when compared to those with mild intermittent asthma [30]. There were excellent correlations among serum IL-17, FeNO and a worsening childhood asthma clinical score. FeNO is increased through the activation on bronchial epithelial cells of inducible nitric oxide synthase (iNOS). Increased iNOS expression and FeNO despite corticosteroid therapy appear to identify a severe subset of asthma, where the strongest correlation between FeNO and airway eosinophilia exists [31]. In the study by Chien, the strong correlation between serum IL-17, FeNO and worsening childhood clinical asthma score suggest a role for IL-17 in iNOS expression, a conclusion supported by the use of an IL-17 antagonist which resulted in decreased expression of iNOS in sputum cell studies.

Smoking has been shown to increase airway neutrophilia in the asthmatic airway and to increase peripheral blood eosinophilia in the general adult population [32]. In studies of young asthmatics, the effects of smoking on airway eosinophilia are less clear-cut. In a study of newly diagnosed steroid-naïve asthmatics by Nagasaki et al. [33], patients spanned a broad range of age, thereby allowing a robust investigation of the effects of age and smoking on serum total IgE and blood eosinophils. Serum IgE, blood eosinophilia and FeNO decreased with age in never smokers, but the decrease in serum IgE with age was not seen in current smokers. In atopic asthmatics, both serum total IgE and blood eosinophils increased with age in current smokers, yet declined with age in ex- and non-smokers. These relationships were not seen in non-atopic asthmatics. The results suggest that current and ex-smoking promotes blood eosinophilia and attenuates the age-related decline in IgE production, especially in atopic asthmatics. Kontogianni found that asthmatics who smoke had increased sputum concentrations of cysteinyl leukotrienes and prostaglandin E_2 (PGE₂), the former associated with sputum eosinophilia and the latter associated with sputum neutrophilia [34]. These observations may explain the recently observed enhanced effect of leukotriene receptor antagonists in mild-smoking asthmatics when compared to non-smoking asthmatics as well as suggesting a role for PGE₂ in the increased airways neutrophilia in this group [35].

Asthmatics showed significantly lower levels of apolipoprotein 1 (Apo1) in BALF, than healthy controls. The anti-inflammatory properties of ApoA1 were investigated in a house dust mite (HDM)-induced model of

allergic airway disease, where ApoA1 treatment decreased lung IL-25, IL-33 and thymic stromal lymphopoietin (TSLP) [36]. The authors suggested that Apo1 acts via effects on bronchial epithelial cells, including promoting recovery of disrupted epithelial tight junctions.

Morin et al. [37] examined the effects of eicosapentaenoic acid monoacylglyceride (MAG-EPA), a newly synthesized EPA derivative, on the resolution of lung inflammation in guinea pigs. MAG-EPA treatment reduced OVA-induced airway hyperresponsiveness and lung inflammation and decreased IL-4, IL-5, IL-13, COX-2 and MUC5AC expression levels in the lung. The authors propose that MAG-EPA represents a stable, non-toxic compound that could serve as a precursor to generate a variety of PUFA-derived mediators, such as resolvins and protectins, which are known to mediate anti-inflammatory and pro-resolving effects.

Aspirin-exacerbated respiratory disease

A diagnosis of aspirin-exacerbated respiratory disease (AERD) rests on identifying aspirin hypersensitivity, asthma and chronic rhinosinusitis with nasal polyps. Overproduction of cysteinyl leukotrienes (CysLT) by the 5-lipoxygenase pathway with a reduction in activity of the COX pathway, alongside increased airways eosinophil inflammation are thought key to the pathogenesis of AERD. Choi et al. [38] explored these mechanisms in more detail. They used a nasal lysine aspirin challenge model and compared nasal lavage fluid (NLF) responses in patients with AERD to those with aspirin-tolerant asthma. The eosinophil protein, eosinophil cationic protein (ECP), was increased during both early and late challenge responses. Cysteinyl leukotrienes were increased only during the early airway response and were not increased in the baseline NLF of patients with AERD, despite other studies showing elevated levels of CysLT in serum, urine and sputum compared with aspirin-tolerant asthmatics. This study supports the notion that CysLT is an acute mediator of eosinophil chemotaxis after aspirin challenge. Eotaxin-2 may be associated with a more basal level of eosinophil activation, as it was elevated in the AERD group compared to the ATA group, but was not elevated by aspirin challenge. Furthermore, the novel mediators apolipoprotein a1, ceruloplasmin and alpha-2 macroglobulin appear to be biomarkers of chronic rhinosinusitis in AERD.

The exact incidence of aspirin hypersensitivity in asthma is not clear, and assessment is not part of routine practice, partly because of concerns about safety and partly because the outcome usually does not greatly alter management. Miller et al. reported their experience with lysine aspirin challenge in an outpatient setting. They found it straightforward to undertake

with a good safety profile and informative outcomes including a high rate of positive results [39].

Genetic studies in asthma

Interferon regulatory factors (IRFs) control interferon- β 1 and interferon- γ expression and T cell differentiation. Genetic associations in interferon regulatory factor 4 (IRF-4) were found to be associated with recurrent bronchitis in two paediatric cohorts [40]. Notably, the authors were able to genetically dissociate asthma from recurrent bronchitis within these cohorts, with the implication that factors that control airway responses to viruses in childhood are distinct from those associated with asthma. Associated polymorphisms were localized in a putative regulatory element in the 3'UTR region of *IRF-4*. These findings suggest a putative role of *IRF-4* in the development of bronchitis.

The thromboxane A2 receptor TXA2R is expressed within the lung on bronchial epithelial cells. Ligation of TXA2R leads to bronchoconstriction and occurs as part of the airway inflammatory response. Two SNPs in the TXA2R gene were previously reported to be associated with asthma, although these SNPs are not thought to be functionally relevant. In April, Takeuchi et al. [41] described how they set out to identify SNPs within the TXA2R gene which resulted in a direct alteration in gene transcription, and an association with asthma. They firstly investigated genetic variation within in the TXBA2R gene and subsequently investigated the associations between 4 haplotypes on the transcriptional activity of TBXA2R, blood eosinophil concentration, lung function and blood TBXA2R mRNA in a cohort of Japanese children with asthma. One haplotype, which was associated with increased TBXA2R transcription *in vitro*, was associated with increased TBXA2R mRNA in blood, a higher level of peripheral blood eosinophilia and worse lung function. The genetic variation in TBXA2R identified by Takeuchi could have downstream effects on airway smooth muscle contraction, on lung function, on airway remodelling pathways as well as on eosinophil accumulation in tissues.

Micro RNAs are attracting increasing interest as an important mechanism controlling gene expression in asthma. Plank et al. [42] reviewed this fast-moving area and the potential for their use in therapeutics.

In the May edition of the Journal, Mougey et al. [43] described a promoter polymorphism in the 5-lipoxygenase gene that was associated with poorer lung function, increased urinary cysteinyl leukotriene LTE₄ and a trend towards increased symptoms in children with asthma. The proportion of children with variant alleles was increased in African American children, and this may explain why this population has a lower FEV₁ than Caucasian asthmatics. We do not yet know whether

identifying this polymorphism in the individual patient can help target treatment with leukotriene receptor antagonists. In the same vein, Hoskins et al. [44] found that polymorphisms in the glutathione-s-transferase gene modulated the inflammatory response to local allergen challenge.

Vascular endothelial growth factors (VEGF) are implicated in airway remodelling in asthma. Kreiner-Muller analysed 13 SNPs in the *VEGFA* gene in 411 children from a birth cohort and replicated in another cohort. They found that several VEGF variants were associated with lung function at 6 years but not birth although not with asthma suggesting a role in post-natal development [45].

One explanation for the strong relationship between asthma and obesity has been that there are genes common to both. A study of over 23 000 children and adults, recruited into several different cohorts, showed little consistent evidence for common genes, even though initial analysis suggested *DENND1B* variants may be associated with both [46]. There was considerable heterogeneity in associations between the studies, and even larger studies may be required [47].

Biomarkers in asthma and rhinitis

The anti-IL-5 monoclonal antibody mepolizumab has been proven to reduce exacerbation frequency in patients with difficult asthma [48], yet at the lowest therapeutic doses, its use is not accompanied by a reduction in sputum eosinophilia [49]. If the mechanism of action of this antibody is not by simple depletion of eosinophils from the airway, are there changes in eosinophil activation that explain the clinical benefits? IL-5 induces the expression on eosinophils of the active form of β_2 integrins, a step necessary for eosinophil trafficking into the lung. In March, Johansson et al. [50] investigated the effects of mepolizumab on eosinophil integrin expression in mild atopic asthmatics. Eosinophils from both blood and BAL were studied, both before and after allergen challenge. The results demonstrated that allergen challenge caused an increase in the expression of highly activated β_2 integrins on circulating blood eosinophils, although there was no change on eosinophils from BAL, which maintained a high level of surface activated β_2 integrin expression. Anti-IL-5 attenuated the induction of β_2 integrin expression seen on blood eosinophils after allergen challenge. No such attenuating effect was seen on BAL eosinophils. The results suggest that a mechanism of action of anti-IL-5 is by down-regulation of β_2 integrins on blood eosinophils before they traffic into airway tissues.

Jung et al. [51] used a metabolomic approach (1H-NMR spectroscopy), to provide a global profile of

sera from 39 patients with asthma and 26 controls. Sera from patients with asthma showed changes in metabolites associated with hypermethylation, response to hypoxia and immune reactions that correlated with asthma severity. Future studies will need to increase numbers of subjects studied to allow evaluation of the clinical implications of these findings, including the impact of medication, and the potential application of this methodology in clinical practice.

Baron-Bodo et al. evaluated whether antibody responses can be used as biomarkers for the efficacy of sublingual grass pollen immunotherapy (SLIT) using samples from a previously reported clinical trial [52, 53]. A comprehensive analysis of changes in IgA, IgE, IgG and IgM in peripheral blood, saliva and nasal washed was performed using a panel of purified grass pollen allergens or grass pollen extract. The study concludes that SLIT is efficacious in patients exhibiting different IgE sensitization profiles; however, antibody profiles cannot readily be used as a marker of SLIT efficacy at an individual patient level.

An attractive approach to measuring airway inflammation is to detect mediators in exhaled air. Progress in developing an 'electronic nose' was reviewed by Fens et al. [54], and exhaled breath profiling was found to predict steroid responsiveness in asthma and finding discussed in an accompanying editorial [55, 56]. On the same theme, Elliot et al. [57] investigated various physiological markers of future poor asthma control in wheezy infants and found that a single breath exhaled nitric oxide was the best predictor of exacerbations, wheeze and impaired lung function at 3 years. The findings were set in context in an accompanying editorial [58].

Eosinophilic oesophagitis (EoE) is generally considered a Th2-type condition. As CysLTs contribute to Th2 responses, a study by Lexmond et al. [59] addressed whether mRNA levels of LTC₄ synthase (LTC₄S) served as a marker of EoE, using digital mRNA expression profiling. The study identifies a subpopulation of EoE patients who are characterized by elevated oesophageal mRNA levels for LTC₄S and Th2-associated TSLP, with reduced IL-23 mRNA. Serum IgE was significantly higher in LTC₄S high as compared to LTC₄S low individuals. These data may offer future opportunities for optimization of personalized patient treatment regimens.

Simms et al. investigated whether peripheral blood mononuclear cells would contain useful information regarding asthma phenotypes. They stimulated the cells with allergens and measured a range of cytokines. Interestingly, not only were there no differences between different phenotypes of asthma but also there was no difference from healthy controls emphasizing the lung-specific nature of asthma and a lack of a systemic signal in many patients [60].

Lastly, lipocalins are an emerging class of compounds that can both act as allergens and also as mediators of inflammation. Their potential role as mediators was reviewed in detail by Dittrich et al. [61].

Mechanisms of allergic disease

Sensitization and allergic inflammation

A cross-sectional study design of 66 children with active atopic dermatitis (AD) was used to analyse age-related differences in IgE antibodies and relation to wheeze. IgE antibodies to food persisted at a similar prevalence and titre throughout childhood [62]. In contrast, IgE antibodies to aeroallergens rose sharply into adolescence. Early sensitization to cat allergen was strongly associated with wheeze in this cohort. Differences in cat and HDM sensitization in young children with AD may aid identification of those at increased risk for disease progression and the development of asthma.

Some allergens have been shown to initiate sensitization and Th2 responses in a manner dependent on their native protease activity [63]. For example, in the mouse, sensitization to cockroach allergen is dependent on activation of the protease-activated receptor 2 (PAR₂) pathway [64]. PAR₂ is expressed on human airway epithelial cells and is up-regulated in the human asthmatic airway, although the mechanism by which PAR₂ influences sensitization to allergens is unclear. Davidson et al. explored these mechanisms further. Using a mouse model of house dust mite sensitization, a PAR₂ receptor knockout mouse and blocking antibodies administered before the sensitization, the group showed that airway PAR₂ activation is required for the development of allergen sensitization, Th2 polarization and airway hyper-reactivity [65]. Whether PAR₂ on epithelial cells acts directly or indirectly to influence T cell activity or whether the effects also depend upon direct activation of PAR₂ on T cells is not yet known.

Traffic-related air pollution (TRAP), of which the main constituent is diesel exhaust particles (DEP), affects the severity of the allergen immune response worsens lung function and induces markers of neutrophilic inflammation [66]. In children, epidemiological studies have linked DEP exposure to wheeze, yet the effects of such exposure on airways inflammation in this age group are unknown. In the Journal, Acciani et al. [67] explored the effects of DEP exposure on the inflammatory profile in the immature lung using a murine challenge model. Interestingly, while low-dose DEP exposure elicited no effect at all, when combined with HDM exposure, a clear synergistic activation of airways inflammation was demonstrated. Both Th2 and Th17 pathways were activated. Given that Th17

responses may be a marker of neutrophil-driven severe asthma, the data from this murine model suggest relevant mechanisms for the effects of TRAP on childhood airway health seen in epidemiological studies.

Do other environmental exposures skew the airway inflammatory profile towards neutrophilia? In a thought-provoking paper, Kim et al. [68] examined the effects of exposure to extracellular vesicles (EV) on airways inflammation. Extracellular vesicles are bacteria-derived spherical bilayered phospholipids of 20–200 nm in diameter. Kim et al. showed that airways exposure to EV from house dust was shown to cause an increase in neutrophilic airways inflammation with an associated Th17 cell infiltration. Fifty percent of the content by weight of the extracellular vesicles was lipopolysaccharide (LPS); administration of the LPS-blocking agent polymyxin B attenuated the increase in Th17 lung inflammation. A cohort of children had serum drawn: when compared to healthy children, children with asthma had elevated levels of IgG1 to extracellular vesicles. Further work is needed to determine whether exposure to EV from environmental house dust is the main source of LPS for the human airway and whether there is a significant relationship between airborne EV exposure and asthma severity in humans.

In the same vein, Wang et al. noted that exposure to environmental factors, such as cigarette smoke, *in utero* is linked to disease susceptibility. The effects of prenatal smoke exposure on DNA methylation profiles of cord blood cells showed that only the methylation status of TSLP 5'-CpG island was significantly associated with cigarette exposure. The degree of methylation correlated with TSLP protein levels, which enhances disease-associated Th2 responses [69]. This study, together with several referenced therein, suggests that prenatal tobacco smoke exposure is associated with changes in DNA methylation, plausibly linked to disease.

Intestinal absorption of food proteins can trigger an allergic reaction. In a model system employing Caco-2 cells, the peach allergen Pru p 3 was transported at a high rate to the basolateral chamber, in an intact form, and without disturbing the integrity of tight junctions of the model epithelial layer [70]. By contrast, transport of the non-allergenic peach LTP1 was much lower. Pru p 3 induced the gene expression of the Th2-promoting cytokines TSLP, IL-25 and IL-33 by the epithelial cells, and in Caco-2:PBMC cocultures, increased mRNA for pro-inflammatory cytokines and IL-10. The authors suggest that the high rate of Pru p 3 transport may be responsible for its sensitizing capacity.

Mast cells

High concentrations of stem cell factor (SCF), an important growth and survival factor for mast cells, are seen

in the asthmatic lung. Lewis et al. [71] demonstrated that mast cells isolated from human lung resection tissue responded to SCF, in a c-kit dependent manner, to drive high levels of mediator (histamine, CysLTs, prostaglandin D₂) release. The response to SCF was weaker than to anti-IgE; however, the combination of stimuli induced the greatest response. These data suggest a role for SCF in mast cell activation in disease.

Prostaglandin E₂ (PGE₂) can inhibit IgE-dependent human lung mast cells, an effect mediated via EP receptors, of which four have been identified. Using PGE₂, and selective EP₂ and EP₄ agonists or antagonists, Kay et al. [72] demonstrated that the principle mechanism by which PGE₂ inhibits mediator release in mast cells is by activating EP₂ receptors.

Ion channels control many cellular activities including activation and mediator release and are attractive therapeutic targets. The important ion channels involved in mast cell activation were reviewed by Ashmole and Bradding [73].

Immune tolerance

An inverse correlation exists between helminth infections and allergies, even though both are characterized by dominant Th2 responses. The capacity of certain helminths to generate an immunosuppressive environment, allowing them to escape host defence mechanisms and establish a chronic infection, may explain this relationship. Aranzamendi et al. [74] show that *Trichinella spiralis* infections protects against experimental allergic airway inflammation (EAAI). Splenic CD4⁺ T cells from infected mice contained more immunosuppressive CD4⁺CD25⁺Foxp3⁺ Treg than uninfected animals, and adoptive transfer of these cells reduced multiple aspects of airway inflammation (e.g. lung eosinophilia and IL-5, cellular infiltration into the lungs and reduced cytokine production by lung draining lymph node cells). The subject of immune tolerance was reviewed by Wisniewski et al. [75].

Statins possess anti-inflammatory functions distinct from their lipid-reducing actions and enhance the anti-inflammatory actions of corticosteroids. Maneechotesuwan et al. [76] assessed the effects of the statin simvastatin and fluticasone on cytokine profiles of asthmatic, monocyte-derived dendritic cells (mDC) and in mDC:T cell cocultures. The combination of simvastatin and low concentrations of fluticasone enhanced the frequency of Treg and reduced Th17 cells, comparable to high concentrations of fluticasone alone. This effect was linked to up-regulation of IL-10 and indoleamine 2, 3-dioxygenase and down-regulation of Th17-promoting IL-6 and IL-23 in mDCs. These data contrasted with other reports showing high levels of IL-17A in cultures from patients with severe steroid-refractory asthma, and

that corticosteroids fail to inhibit or enhance Th17/IL-17A responses *in vitro* and *in vivo* [77]. Further studies using primary cells from the periphery and respiratory tissues are needed to identify potential steroid-sparing properties of simvastatin.

Studies in mice suggest that plasmacytoid dendritic cells (pDC) are tolerogenic. Conversely, pDCs are activated by viral infections, a common cause of disease exacerbations, to produce pro-inflammatory mediators. Bratke et al. used flow cytometry to demonstrate increased expression of activation markers, Th2-associated molecules and an increased migratory potential of circulating pDCs in asymptomatic patients with mild seasonal allergic asthma as compared to healthy controls. Inhaled corticosteroids favourably modulated some of these parameters. Further work is required to investigate distinct pDC subpopulations and whether these exhibit different functions in allergic inflammation. Parallel studies of pDC in human respiratory tissue, although challenging, are also needed to contribute to this debate.

Observational studies link vitamin D deficiency with worse asthma control and lung function. The effects of vitamin D deficiency, sufficiency or supplementation were examined in a murine model of allergic airway inflammation [78]. Deficiency was associated with higher airway hyperresponsiveness and marked airway remodelling. In BALF, higher eosinophilia, increased pro-inflammatory cytokines but reduced anti-inflammatory IL-10 were reported. Blood Treg was also reduced. Vitamin D supplementation attenuated but did not completely reverse the features of allergic airway disease. As discussed in the accompanying editorial, these data resolve some misunderstandings arising from earlier studies in animal models and are broadly in agreement with human observational data [79]. In the same issue, the popular topic of vitamin D was also discussed in relation to food allergy in a review by Vuillermier et al. [80].

Regulatory T cell (Treg) cell function was assessed during a 3-year prospective, placebo-controlled, double-blind clinical trial of grass sublingual immunotherapy (SIT) [81]. Grass pollen SIT effectively reduced rhinitis severity and airway inflammation, as assessed by exhaled nitric oxide. In the second year of treatment, SIT reportedly increased the frequency of ZAP70+ Treg (CD4+ CD25hiCD127low) cells and their intensity of ZAP70 expression especially at the height of the pollen season. The authors interpret these data that SIT not only increases the frequency of Treg but also restores Treg intracellular signal transduction, although no assessments of suppressive function were made.

Farmyard exposure in early life protects children from developing atopic disorders. This is associated with increased TLR2 expression in these children, and

ligands for TLR2 are present in farmyard dust. A novel synthetic TLR1/2 ligand, LPGerD, was poorly stimulatory compared to native LPS. LPGerD was protective against sensitization and airway inflammation in an OVA-induced mouse model of asthma [82]. Pre-treatment of bone marrow-derived dendritic cells with LPGerD led to a state of LPS tolerance, and these cells no longer primed mice for an allergic response. The authors suggest that induction of a LPS-tolerant state in antigen presenting cells may contribute to the protective effect of the farmyard environment and that these properties of LPGerD make it a promising candidate to mimic farmyard-induced LPS tolerance.

Cow's milk allergy is one of the most common food allergies in children. Although many children outgrow this condition, it predisposes to other allergic reactions. Consumption of long-chain n-3 polyunsaturated fatty acids (n-3 LCPUFA), present in fatty fish, is associated with a reduction in milk allergy. A murine model of cow's milk allergy was employed to study mechanisms associated with n-3 PUFA consumption [83]. Dietary fish oil given from 3 weeks of age reduced allergic sensitization by suppressing the humoral response, enhancing intestinal and systemic Treg and reducing acute allergic symptoms. The challenges in translating such observational and experimental findings into the public health arena are discussed in an accompanying editorial [84].

Insect bite hypersensitivity (IBH) is the most common allergic manifestation in horses. CD4+CD25high Treg from healthy, but not IBH-affected, horses inhibited the proliferative responses of peripheral blood effector T cells to *Culicoides* allergen [85]. This impaired IBH Treg activity was associated with a higher proportion of IL-4+ T cells and reduced IL-10+Foxp3+ cells in culture and was antigen specific. The suppressive activity of *Culicoides*-specific Treg could be restored by rIL-2/rTGF- β 1 or retinoic acid plus rapamycin in culture. These data demonstrate an imbalance between Th2 and Treg in equine IBH and suggest an important role for inducible allergen-specific Treg in control of IBH, highlighting clear parallels with human studies.

The microbiome and allergic disease

The microbiota in the gut influences immune maturation in early life with implications for the long-term health of the child. Gosalbes et al. [86] demonstrated that the meconium microbiota of 20 healthy term babies differs from that of adult faeces, vagina and skin. The meconium samples clustered into two distinct phenotypes. Lower diversity, and domination by enteric bacteria associated with a history of atopic eczema in the mother, while domination by lactic bacteria associated with respiratory problems in the infant. The

authors suggest that further studies may enable use of specific meconium bacterial profiles as biomarkers of increased risk of immune disease and as probiotics for early immune modulation and suggest other approaches to favourably modulate the infant gut microbiota and immune system.

Perinatal supplementation with probiotics may reduce the risk of developing allergic disease. The allergy-protective effects of viable *Lactobacillus rhamnosus* GG (LGG) and LGG-derived soluble mediators were demonstrated *in vitro* and *in vivo* by treatment of neonatal mice from 2 days of birth and OVA sensitization at 6 weeks [87]. Both treatments led to a reduction in allergic airway inflammation and a reciprocal increase in BAL IL-10 although no effect on AHR- or OVA-specific serum IgE was observed. The data demonstrate protective probiotic-derived soluble factors, and follow-up studies need to determine whether these offer a safer, more controlled and effective means of probiotic delivery. The significance of these observations in what is a complex area was set in context by Jacquet [88].

A previous clinical trial of probiotics in late pregnancy and the first year of life showed a reduced risk of eczema in the children at 2 years [89]. Here, Forsberg *et al.* [90] investigated allergen-induced cytokine responses in PBMC samples from these children and demonstrated a reduction in both Th1 and Th2 cytokine responses to both cat and food (ovalbumin) allergens up to 2 years, which could explain the reduction in eczema in these children post-treatment. These data imply a reduced overall responsiveness to allergen, although no direct evidence for induction of specific regulatory mechanisms (e.g. mRNA for Foxp3, or the Ebi3 subunit of IL-35; or IL-10 secretion) was observed.

In another follow-up report of a probiotic trial, Wickens *et al.* found that the benefits conferred in a previous study in terms of lower rates of eczema and atopy were maintained up to the age of 6 years. Interestingly, benefits were found with only one of the two lactobacilli strains investigated a point discussed in an accompanying editorial [91, 92]. The whole area of dietary interventions before or after birth is very complex with mixed signals from the literature. To aid the reader interested in this area, the literature was reviewed with great clarity by Jenmalm and Duchon [93].

Enterovirus infections are transmitted via the oral-faecal route and replicate primarily in the gut. Korhonen *et al.* [94] proposed that cumulative exposure to several different enterovirus infections in early childhood is associated with a reduced risk of atopic disease. Further studies are required to address whether this effect is specific to enteroviruses or reflects the total burden of infections or other environmental factors.

Epidemiology

Phenotypes

It is argued that there will be improved scope for prevention of asthma and allergy if we understood risk factors for specific phenotypes of disease. There was only limited evidence that risk factors differed between phenotypes in two studies published in this journal. In the Prevention and Incidence of Asthma and Mite Allergy (PIAMA) cohort in the Netherlands [95], about 25% of 3000 participants had a history of wheeze by the age of 8 years. The majority (almost 70%) had 'early transient wheeze', and this phenotype was associated with male sex, parental allergy, low maternal age, maternal smoking in pregnancy, shorter pregnancy duration, high maternal body mass index and increased exposure to other children in infancy (older siblings and day care attendance). Associations were less easy to detect for the less frequent phenotypes, but in general, the direction of association was similar for many of the risk factors. Formal tests for differences in associations were not presented although there may be differences for maternal age. Children born to young mothers were more likely to have 'early transient wheeze', but those born to older mothers more likely to have 'late-onset wheeze'.

We may discover more by collecting even more detailed information to determine phenotypes. The Copenhagen Study of Asthma in Children (COPSAC) described investigations ('deep phenotyping') undertaken during the follow-up of 690 infants, recruited before birth [96]. Pregnant mothers and later, their offspring, had regular clinical assessments with the study team for collection of health information and biosamples, supplemented with daily diary cards, web-based questionnaires and additional checks if the child became ill. In addition, mothers were given opportunities to take part in nested randomized controlled trials (for example on the effect of vitamin D supplementation in the third trimester). The commitment required to take part in this study was substantial. This may explain, at least in part, why only 1876 of the 54358 pregnant women approached, went on to express an interest in the project, and why those who themselves had some history of allergic disease were more likely to take part. Such low response rates with some evidence of 'non-representativeness' are, sadly, typical of ongoing studies in Europe, and raise concerns about generalizability of findings to the wider population. While there will be debate on the 'external validity' of such studies, 'internal validity' can only be maintained if studies maintain high participation rates. The COPSAC team have done admirably so far (98% of participants attended year 1 visit, 95% attended year 2 visit).

Weinmayr used latent class analysis to define phenotypes of asthma in children based on the data from the ISAAC phase II study in Spain [97]. They found seven classes: one of healthy subjects, three related to wheeze and three to bronchitis. Atopy as expected mapped to the wheeze category. This is one of a number of studies that have investigated phenotypes of asthma in children using semi-objective biostatistical techniques, and the findings were set in context in an editorial by Poon [98].

Studies looking at the role of atopy

Garden et al. [99] identified phenotypes of atopy using latent class analysis and information on skin prick test positivity collected at ages 1.5, 3, 5 and 8 years in about 500 Australian children in the Childhood Asthma Prevention Study (CAPS). Participants had at least one parent with either asthma or wheeze. Four phenotypes were identified: non-atopic (68%), 'late mixed inhalant' (11%; children who developed more sensitization with age), 'mixed food and inhalant' (4%; children who were positive to peanut and egg early in life, but who with age became less sensitized to egg and increasingly sensitized to inhalant allergens) and 'HDM monosensitized' (17%; sensitized to HDM with less marked sensitization to other allergen). All phenotypes were associated with allergic disease and bronchial reactivity, and associations of phenotype with each of the examined risk factors (birth order, maternal smoking, breast feeding, parental asthma, parental educational status) appeared similar. Of note, mothers who smoked in pregnancy were less likely to have children with atopy. The strengths, limitations and implications of these data are discussed in an accompanying editorial [100].

Sensitization to aeroallergens is clearly a risk factor for allergic disease. Substantial increases in sensitization to most of the tested allergens were seen in a large sample of school children aged 13–14 years living in Guangzhou in 2002 and 2009 [101]. At the second survey, almost 64% of children had a positive skin prick test to at least one of the allergens tested, with almost 55% being positive to house dust mite (compared to 46% and 42%, respectively in 2002). Standardization of measurement of skin prick tests over time is challenging because of the high interobserver variability in assessments and possible changes in skin prick test reagents over time, but, even with these caveats, the results suggest major changes in allergy prevalence in China over the last 20 years.

The association of atopy with symptoms was examined within the Childhood Origins of Asthma Study (COAST) in the United States, a follow-up of children at high risk of atopy [102]. By the age of 9 years, over 53% of children had measurable serum-specific IgE to

at least one of the 8 measured allergens. Sensitization to each of the allergens was, in general, associated with an increased risk of asthma, but effect estimates were consistently higher for sensitization to dog than for some other allergens (e.g. *Alternaria alternata*). Previous reports from this cohort suggest dog exposure in infancy was associated with reduced wheeze symptoms and there was some evidence that dog exposure was protective for symptoms irrespective of whether individuals were sensitized to dog. Although the association of dog exposure in early life with sensitization to dog was not given, data presented in one of the tables suggest that dog exposure at birth is associated with less sensitization to dog.

Eosinophils are more abundant in the blood and airways of people who are atopic. The National Health and Nutrition Examination Survey (NHANES), a large annual cross-sectional survey of representative samples of the population in the United States, found that the prevalence of current physician-diagnosed asthma was 4.6% in those who were non-atopic and had normal eosinophil count and more common in those with positive skin prick tests (7.6%) and in those with a high eosinophil count (6.9%) [103]. However, it was most common in those with both (17.2%). This 'excess prevalence' in the latter group was largely seen in the younger participants. The authors argue that this provides further evidence that asthma is a heterogeneous disease and that, in children, atopy and raised eosinophil count act jointly through one common causal pathway (i.e. synergistically), while in adults, the effects are acting through two independent pathways. It is difficult to assess the extent to which these findings could reflect differing diagnostic patterns in children and adults (the outcome is 'physician-diagnosed asthma') and the extent to which it reflects genuine differences in 'asthma'. Some 'deep phenotyping' would clarify these relationships further.

Studies looking at maternal and early life factors

Maternal diet likely influences lung growth and asthma development, and there is interest in the role of vitamin D on allergy and lung health throughout the life course. The Avon Longitudinal Study of Parents and Children (ALSPAC) recruited a population-based sample of children born in 1991/1992 living in one area of the United Kingdom and showed there was no link between allergic or respiratory outcomes by age 8 and pre-natal maternal levels of Vitamin D [104]. This study comprehensively examined the data seeking nonlinear and threshold effects, but failed to show any associations. Randomized controlled trials are needed to fully test the hypothesis – but, on the basis of these results, there is little reason to think such trials would show an

effect. Nevertheless, this is an area of considerable debate, and not all studies or investigators agree with these conclusions [105].

Early life factors were investigated in the 'Healthy Growth Study' in Athens [106]. Reporting 'ever asthma' was more common in children aged 9–13 years who were conceived through IVF (compared to natural conception) and in those born by Caesarean. However, numbers were relatively small to fully investigate the former (only 4 of the 59 children conceived by IVF reported current wheeze). The positive association of asthma with Caesarean delivery has been attributed to lack of normal microbial colonization that occurs as babies progress through the birth canal. However, the Dunedin Multidisciplinary Health and Development Study which has followed over 1000 to the age of 32 years showed that asthma and atopy at 13 years and at 32 years were more common in those delivered by forceps [107]. Only the association with asthma at age 13 years survived controlling for other factors. Caesarean section was uncommon (< 5%) and showed no associations with asthma. As discussed in the accompanying editorial [108], it seems likely that the relationship of asthma with 'non-vaginal' birth is confounded by the indications for such interventions.

Where a child lives may influence their risk of asthma. Using the detailed national birth registry databases and hospital admission records, admissions for asthma in almost 900 000 2- to 11-year-olds [109], Swedish researchers showed that the proportion of children who had ever been hospitalized with asthma was higher, and admission rates were higher, in more deprived areas. These associations were seen even after controlling for maternal factors (not married or cohabiting, fewer years in education, maternal smoking) suggesting it is not just the characteristics of immediate families which influence asthma risk – it is also the characteristics of the neighbourhood. Deprivation may lead to more stressful environments, with fewer support structures and, in general, be less conducive to healthy living (e.g. fewer open spaces to exercise). However, such areas may also have lower quality primary care services (leading to more asthma admissions). People living in deprived communities often have poor housing – and these factors were not examined. Results from the International Study of Asthma and Allergies (ISAAC), an international multi-centre, cross-sectional survey, suggest this may be important [110]. In 8- to 12-year-old children, reported damp and mould were associated with wheeze, rhinitis, eczema, cough with phlegm, and with higher mattress HDM levels. Mattress HDM level was associated with sensitization to HDM, but increased symptoms with damp were seen in children irrespective of HDM level and irrespective of sensitization status, suggesting damp does not cause

symptoms solely through its (weak) association with HDM levels. This study has all the limitations of a cross-sectional study relying on reported exposures, but the study estimates are similar to those in recent meta-analyses of other observational studies on this topic, and further strengthen the evidence that home characteristics influence children's respiratory health.

In Australia, infantile eczema was not related to socio-economic status [111]. It was related to ethnicity. The HealthNuts study in Melbourne, Australia, showed that by 12 months, about 18% of children had a history of an itchy rash treated with topical steroids and about 20% had evidence of current eczema on physical examination. It was more common in boys, those with parental history of eczema and those whose parents were of East Asian descent. This is of some interest as the reported prevalence of eczema by mothers and fathers of East Asian descent was much lower than in the mothers and fathers born in other regions.

Exposure to pollens in early life may contribute to allergic sensitization to grasses and to the development of asthma. The association of month of birth (a proxy for pollen exposure) with subsequent asthma and rhinitis has been explored in the past, but the Melbourne Atopy Cohort Study (MACS) – a high-risk cohort study – based early life exposure to average daily pollen counts [112]. Higher pollen exposure during the first 3 months was associated with an increased risk of sensitization by 2 years of age, and increased hay fever by age of 6 years. Higher exposure in the first 6 months was associated with an increased risk of asthma by age 6.

Infant feeding, particularly the age of introduction of solid foods, was explored in over 1900 children recruited at birth as part of the Study of Eczema and Asthma To Observe the influence of Nutrition (SEATON), of whom 900 were followed to the age of 10 years [113]. Twelve-month period prevalent wheeze at age 10 years was less common in those who never had formula milk, and was less common in those introduced to formula milk after 2 weeks than those introduced after 2 weeks. Effect estimates were similar (although $P > 0.05$) for asthma at the age of 10 years. However, breast feeding did not appear to protect from development of asthma. The authors used a very conservative method (Bonferroni) to adjust for multiple testing (they examined introduction of a range of foods), and it is possible that with equally valid but less conservative methods, they may have felt more confident to conclude some of the associations within this analysis were not chance findings, and may warrant continued investigation.

Increasingly, what adults can and do eat is influenced by the impact of food allergy and food intolerance. Skypala *et al.* [114] reported findings from a large

questionnaire-based survey of pollen food syndrome (PFS). At least 2% of responders had PFS, but the estimate was based on reported symptoms applied to a diagnostic algorithm, and there was no assessment of the impact of response bias on the prevalence estimates. However, of note, PFS was more common in women, often began in the teenage years, and there were 26 foods that were reported as triggers by more than 5% of those affected. Apples, hazelnuts and kiwi fruit were the most frequently cited.

Asthma and comorbidities

Assessing comorbidities in allergic disease is complex due to biases that may be introduced by sampling and the over-reporting of symptoms and conditions by 'health aware' individuals who already have one diagnosis. An extensive review of the association of allergy with cancer argued that there is biological plausibility for an association [115]. Available studies were mainly cohort and case control studies relying largely on self-reports and on hospital discharge summaries, but suggest that such relationships, if they exist, are different for different cancers.

Disrupted sleep may be a symptom of asthma and allergy, but it may also be a cause of disease [116]. In the Swedish Twin Study of Child and Adolescent Development (TCHAD) [117], children who were 'over-tired' at 8 years were more likely to develop rhinitis in the following 5 years. Children with asthma at age 8 years were more likely to report being 'over-tired', 'sleeping less than other children' and to 'have trouble sleeping' at age 13 years. This report is consistent with previous cross-sectional studies, but as disrupted sleep limits short-term performance, quality of life, and may have long-term impacts on health, this longitudinal investigation is likely to prompt further work in this field.

Clinical allergy

Food allergy

Several interesting papers described safety issues in relation to food allergy, which is the commonest trigger for anaphylaxis. First, Parisi et al. investigated 4 cases of anaphylaxis to a routine vaccine (oral polio vaccine; OPV) in Argentina during a 2009 vaccination campaign. They used clinical assessments of the individuals who suffered anaphylaxis to show that all 4 patients had cow's milk allergy. They then used a competitive inhibition ELISA to show convincing evidence that cow's milk derivatives, presumably added as a stabilizer to the vaccine, were the likely cause of anaphylaxis [118]. OPV is the latest addition to a long list of non-food sources of food allergens which food-allergic

people may encounter, and better regulation of such products with clearer allergen information is needed to protect food-allergic people. The authors suggest that use of an alternative polio vaccine (the Salk vaccine) should be considered when vaccinating milk-allergic people against polio, especially if skin prick test to the OPV is positive.

At least 50% of people with egg allergy tolerate baked egg in a muffin or cake, and knowledge of this can lead to significant liberation of dietary restrictions [119]. The issue for those who first diagnose egg allergy, commonly paediatricians, is how to simply identify those egg-allergic patients who can tolerate baked egg. Tan et al. [120] reported a series of 143 egg-allergic children undergoing supervised challenge to establish whether they were tolerant or allergic to baked egg. They found that in 15% of those who reacted to baked egg, there were symptoms of anaphylaxis, suggesting that advice to egg-allergic people to try baked egg products at home may not be safe. They did however find that patients with a skin prick test (SPT) reading < 2 mm to a muffin slurry were highly likely to tolerate baked egg. The implications were discussed in an accompanying editorial [121].

An important question in food allergy is how best to predict severity and threshold of reaction. A second group studying egg allergy assessed whether the fat content of food challenge meals influenced this [122]. Previous work has found that the fat content of challenge meals is an important factor in peanut allergy, with high-fat meals leading to delayed reactions, which may therefore be more severe due to increased cumulative intake [123]. Libbers et al. found no such evidence for egg allergy, with similar threshold and severity of reaction seen in egg-allergic children having 23% (vanilla pudding) and 53% (minced meat) fat content in their food challenge meals. Potential insights into differences between peanut and egg allergy are discussed by the authors.

Finally, Vazquez-Ortiz et al. [124] provide a detailed analysis of safety issues in the exciting area of food immunotherapy. Like others, they found that a high proportion of milk-allergic individuals can be successfully desensitized using oral immunotherapy. The main issue preventing oral immunotherapy (OIT) from becoming a part of routine clinical practice is the high rate of adverse reactions to therapy – much higher than the rate seen in individuals on avoidance diets, and similar to the rates of adverse reaction seen in subcutaneous immunotherapy to venom or pollen where each dose is usually administered under supervision. Vazquez-Ortiz et al. show convincingly that most allergic reactions during therapy, and particularly reactions occurring beyond the up dosing phase, occur in a restricted group of individuals – in their series compris-

ing 25% of subjects enrolled. This high-risk group can be predicted with moderate accuracy by higher baseline milk IgE and milk skin prick test readings, and increased severity of reaction at baseline milk challenge. Interestingly, these are very similar to the variables which best predict persistence of milk or egg allergy, suggesting that the patients whose food allergy is most likely to persist are those who have most difficulty tolerating OIT. Improving the safety of OIT is an important goal if it is to be developed into a useful food allergy treatment.

Eczema is closely associated with food allergy particularly in children. Its role was discussed in the context of the overall management of eczema in a state of the art review of the management of this distressing condition [125].

Food allergy is a common cause of anaphylaxis, but the risk of death is not clear. Umsunthar et al. undertook a systematic review of the literature and found 240 reports of death from 13 studies. They concluded that the risk of death from food-induced anaphylaxis in food-allergic people was lower than the risk of accidental death in the general population [126].

Inhalant and venom allergy

Immunotherapy for inhalant allergy is of course a well-established treatment, and the sublingual route has now become the preferred mode of delivery in many settings due to improved safety and convenience for patients. There is a paucity of immunotherapy studies in older adults, perhaps due to the lower prevalence of allergic rhinitis in this age group. Bozek et al. [127] contributed to filling this gap by demonstrating efficacy for a house dust mite droplet SLIT preparation in a randomized controlled trial of adults aged 60–75 with allergic rhinitis. In general, tablet SLIT is preferred where available, due to improved convenience and allergen delivery. Didier et al. [128] advanced the field of tablet SLIT for grass pollen by demonstrating that pre-/co-seasonal grass pollen tablet SLIT can lead to reduced symptoms 1 year post-treatment. This approach can lead to cost savings compared to continuous grass pollen tablet SLIT, and this publication provides some reassurance that discontinuous treatment can still lead to efficacy beyond the treatment period as seen with continuous grass pollen tablet SLIT.

There is relatively little information on allergic reactions to venom in children. Stritzke and Eng described their experience with 83 children referred for venom allergy. They found that re-stings were common particularly in children under the age of six and that immunotherapy offered good but not perfect protection particularly against bee stings [129].

Drug allergy

Allergy to medications is the commonest cause of fatal anaphylaxis, with beta-lactam antibiotics the leading agents. This means that accurate diagnostic assessment and post-diagnostic information is important. Richter et al. [130] reported a disconcerting variability in approaches to diagnosis and management of beta-lactam allergy across the United Kingdom, with frequent deviation from European guidelines. Some patients are being put at risk of anaphylaxis due to exclusion of beta-lactam allergy after inadequate testing, for example no skin testing or no drug provocation testing. The authors argue that specific IgE testing for drugs should only be available in specialist allergy centres, which seems a very sensible recommendation to prevent unreliable exclusion of drug allergy using specific IgE alone. They also note significant differences in paediatric and adult practice and suggest the need for separate diagnostic protocols in each age group. These recommendations have now been included in recent guidelines from the UK National Institute for Care Excellence [131].

Recent studies have tried to reduce the burden of detailed diagnostic workups for drug allergy – for example, children with delayed onset rashes after beta-lactam use have a very low risk of reproducible reaction and may not need detailed diagnostic work-up. Blanca-Lopez et al. [132] showed in the *Journal* that it is difficult to diagnose reproducible urticarial/angioedema reactions to non-steroidal anti-inflammatory drugs (NSAIDs; COX-1 inhibitors) on history alone – only in patients with a clear history of typical symptoms on multiple occasions, including reactions to 3 or more classes of NSAIDs, which occurred within 60 min of exposure, could the diagnosis be reliably made without proceeding to drug provocation testing. So the drug provocation test remains a key component of the diagnostic work-up for allergy or intolerance to NSAIDs, as for other drugs [133]. NSAIDs are one of the commonest causes of hypersensitivity drug reactions which can present in a number of different ways which are reviewed by Ayuso et al. [134].

There is increasing interest in the role that proton pump inhibitors have in altering the allergenicity of foods and drugs, and this was brought into focus by a case control study from Spain showing that patients taking proton pump inhibitors (PPIs) were more than four times more likely to have a hypersensitivity reaction than those not taking these medications [135]. The implications of this important observation were discussed in an accompanying editorial [136].

Biological agents are becoming widely used for a variety of conditions. Anaphylaxis is a rare but important complication. Matucci et al. [137] report their experience of investigating patients who have had

hypersensitivity-like reactions to infliximab and show that skin prick testing is a specific though not very sensitive test to identify those with more severe reactions.

Stevens–Johnson syndrome (SJS) or toxic epidermal necrolysis (TEN) are mucocutaneous reactions, typically to medications. Given the seriousness of these conditions, provocation tests can be too dangerous for use in determining drug sensitivity, while the lymphocyte transformation test is often negative. PBMC from SJS/TEN patients were cultured with culprit drugs to investigate immune parameters for improved sensitivity of tests [138]. The authors recommend a set of three end-points (granulysin expression in CD4+, granzyme B ELISpot and IFN γ secretion in cell culture supernatant) as the first choice to determine causality in SJS/TEN patients, although validation in other groups of patients and by other investigators is now desirable.

Allopurinol is prescribed for the treatment of gout, but hypersensitivity reactions occur in around 2% of patients causing severe cutaneous adverse reactions. Using the lymphocyte transformation test (LTT), only 1 of 26 patients was shown to respond weakly to allopurinol, while 46% responded to its metabolite oxypurinol, with no evidence for cross-reactivity [139]. Allopurinol-allergic patients are likely to develop oxypurinol-specific T cell responses because of rapid metabolism to the latter *in vivo*; the T cell response is dose-dependent and is most pronounced in patients with the HLA-B*58:01 allele. The implications of this study and the complexities of this poorly understood area of drug hypersensitivity are discussed in an accompanying editorial [140].

IgE-mediated allergic reactions to neuromuscular blocking agents (NMBAs) are the main cause of immediate hypersensitivity reactions in anaesthesia. Previous environmental exposure to apparently unrelated compounds is thought to underlie the NMBA response. The prevalence of specific IgE to quaternary ammonium ions was assessed in two populations professionally exposed to quaternary ammonium compounds, hairdressers and baker/pastry makers, as well as in healthy controls [141]. This study suggests that apprentices subjected to repetitive exposure to quaternary ammonium compounds used in hairdressing over a 2-year period were 4.6 times more likely to be sensitized compared to control groups, suggesting that exposure to quaternary ammonium compounds used in hairdressing is a serious risk factor for NMBA sensitization.

Urticaria and angioedema

Idiopathic urticaria and angioedema which often occur together are common and distressing conditions. Isolated angioedema is potentially more complex. Bradykinin-mediated angioedema, including the common ACE

inhibitor induced and the rare hereditary (HAE) forms, is potentially life-threatening and difficult to treat. The spectrum of angioedema, particularly the bradykinin associated forms, was reviewed in April by Busse and Buckland, and new developments in treatment were reviewed by Xu et al. [142, 143]. In addition, we reported a study by Saule et al. accompanied by a commentary by Hilary Longhurst which described the potential benefit of progesterone in women with mainly HAE [144, 145]. This group identified over 50 women who had been given progesterone treatment and found a convincing improvement in the frequency and severity of attacks after being started on this hormonal therapy. Time we felt for a double-blind placebo-controlled trial.

Allergens

Introduction

The use of component-resolved diagnostics (CRD) in allergy diagnostics is rapidly expanding. The focus lies on both discovering new allergens and improving the knowledge of existing allergens and the way they could enhance diagnostics of allergies. In addition, there is an increased interest in characterization of specific allergen epitopes to serve as a possible targets for immunotherapy. Last year, *Clinical & Experimental Allergy* published a series of articles with a focus on allergens.

Newly discovered allergens

Two articles covered newly discovered allergens in fish. Parvalbumin is considered the major fish allergen and is present in most commonly consumed (salt water) fish species. The considerable homology between the different parvalbumins across different species could be responsible for the clinical cross-reactivity often found. However, allergenic potency of parvalbumins seems to differ between fish species [146]. Sensitization can be also limited to only parvalbumins from certain fish species [147]. Kuehn et al. [148] investigated three commonly consumed fish species, cod, salmon and tuna, to identify new allergens. Two newly discovered proteins were identified as beta-enolase and fructose-bisphosphate aldolase A. Like parvalbumin, both fish enolases and aldolases showed interspecies cross-reactivity, though limited. In patients suspected for fish allergy, but not sensitized to parvalbumin, these proteins could help to provide evidence of sensitization. Much less is known about allergens of freshwater fish, although parvalbumins have been described in carp. Liu et al. [149] identified a tropomyosin protein, Ore m 4, in the Mozambique tilapia (*Oreochromis mossambicus*) showing IgE binding in immunoblotting, ELISA and

ELISA inhibition with sera of tilapia allergic patients. Tropomyosins are well known as major allergens in shellfish, such as shrimp and crab, as well as house dust mite and cockroach. Although parvalbumin in tilapia has been previously described, it was not significantly recognized within the studied population of Liu et al. They highlight that it is the first report of tropomyosin from vertebrates acting as an allergen. Tropomyosins from vertebrate species were generally considered non-allergenic.

Fruit allergy remains one of the most rewarding research topics, because of its prevalence. Cross-reactive protein families are generally considered responsible, but fruit-specific allergens also play a role. The two best studied protein families are the pathogenesis-related type 10 proteins (PR-10) and the non-specific lipid transfer proteins (nsLTP). In peach, the nsLTP Pru p 3 has been associated with severe allergic symptoms, especially in the Mediterranean area. Analysis of peach pulp and peel extracts by Tuppo et al. [150] led to the identification of a new allergen, Peamaclein. This heat-stable, digestion-resistant protein was able to trigger positive skin prick test reactions in patients with severe peach-allergic symptoms similar to that of Pru p 3, without *in vitro* sensitization to known peach components on the ISAC 103 microarray, including Pru p 3. Peamaclein was also able to bind IgE in the allergen micro-beads array assay. Tuppo et al. demonstrated the presence of Peamaclein in (Pru p 3-enriched) peach extracts, including commercially available extracts and an in-house-made extract. Although Peamaclein belongs to a different protein family, it shows biochemical behaviour similar to Pru p 3 and it has a similar molecular mass and isoelectric point, explaining why it can go undetected in Pru p 3 preparations. This may lead to attribution of clinical symptoms to sensitization to the wrong peach component. This new peach allergen was labelled Pru p 7 by the WHO/IUIS Allergen Nomenclature Sub-committee.

While for culinary purposes it is considered a vegetable, botanically tomato is a fruit, with many cultivars available for consumption. Because of its world-wide adoption in gastronomy and known allergenicity, it has also become a prevalent plant food allergen. A large individual variability in allergic reactions to tomato was observed by Welter et al. in previous studies. The hypothesis they wanted to test is whether this individual variability is due to specific allergens with different levels of expression based on the genetic background or cultivation method of the tomato [151]. Three different tomato genotypes were used of which one was fertilized in four different ways with different amounts of nitrate and organic nitrogen. A total of six samples were derived from the genotypes and used to perform 2-D immunoblots with sera of nine tomato-allergic subjects.

Skin prick tests were performed with the same samples and subjects. No significant correlations were found between tomato genotypes, growth conditions and spot quantity on the immunoblots. Positive spots on the immunoblots were analysed by mass spectrometry, resulting in ten putative allergens. Each subject showed IgE binding to nearly every identified putative allergen, making it impossible to determine candidate proteins responsible for the large differences in clinical reactivity. Nonetheless, five new putative allergens were identified, which will be further analysed to assess their allergenic potency.

Allergens for food allergy diagnosis

In 2013, the World Allergy Organization published an international consensus document on the use of molecular-based allergy diagnostics, also commonly referred to as CRD [152]. In this document (multiplex), CRD is considered a third-line approach, behind allergen extract-based IgE tests, and useful 'for selection of specific immunotherapy, evaluation of cross-reactivity and assessment of severity of reaction associated with various allergens'. Additional evaluation is needed to determine the value of sensitization to single allergen components in diagnosing a food allergy and assessing its severity. Klemans et al. [153] investigated the discriminative ability of individual peanut allergens Ara h 1, 2, 3 and 8 between different diagnostic techniques. They assessed the sensitization to the specific allergens using multi-plexed microarray immunoassay, single-plexed IgE assay, SPT and immunoblot in a population of 22 peanut-allergic adults and 15 children as determined by double-blind placebo-controlled food challenge (DBPCFC). Comparable sensitivity values were found with all four techniques compared with each other and between adults and children. Ara h 2 showed the highest sensitivity, varying from 76 to 96%. A correlation was seen between specific IgE (sIgE) titres to Ara h 1, 2 and 3 and clinical severity, but only in adults. A high area under the receiver-operating characteristic curve (AUC) value of was found for Ara h 2 (0.92–0.96), indicating a strong discriminative ability. Because there was no diagnostic study design, the AUC values do not accurately represent Ara h 2's diagnostic value. Also last year, Lopes de Oliveira et al. [154] discussed the role of Ara h 2 as a diagnostic tool. Lopes de Oliveira et al. warned clinicians for solely using Ara h 2 in diagnosing a peanut allergy. In their German population of 61 children, they only found a positive predictive value of 72% for Ara h 2 when using a previously proposed cut-off value of 15 kU/L due to multiple false positives.

While the use of CRD in the diagnosis of peanut allergy is a hot topic, articles on the diagnosis of tree

nut allergy are still scarce. The prevalence of self-reported tree nut allergy however is similar or even higher to that of peanut in adults in Western Europe and North America. In the European EuroPrevall study, sensitization to hazelnut was the most common food sensitization and also in the top three most common foods containing allergens not cross-reacting with pollens [155]. Similar to fruit, tree nuts are known to contain both cross-reactive and tree nut-specific proteins, where sensitization to non-pollen-related allergens is linked to more severe symptoms. Last year, Masthoff et al. [156] demonstrated that sensitization to Cor a 9 and Cor a 14 is strongly associated with more severe hazelnut allergic symptoms in both children and adults in the Netherlands. Together these data illustrate that CRD might significantly improve the diagnostics of peanut and tree nut allergy.

Inhalant and insect venom allergens

Several indoor and outdoor mould species are associated with clinical allergies or asthma, such as *Alternaria alternata* and *Aspergillus fumigatus*. Sensitization for individual mould species can be assessed using the skin prick test. Although it is acknowledged that standardization of skin prick test extracts is necessary, there is still a large heterogeneity of allergen extracts. Kespohl et al. [157] analysed commercially available mould SPT extracts of 4 indoor mould species, each from several different manufacturers, with respect to quantity and quality of protein, allergen and human IgE binding capacity. Extracts from *Alternaria alternata*, *Aspergillus fumigatus*, *Cladosporium herbarum* and *Penicillium chrysogenum* were used, as well as two in-house extracts from *Aspergillus versicolor*. Quantitatively and qualitatively the tested extracts were highly heterogeneous, indicating limited comparability between the extracts from the different manufacturers. A multi-centre trial is being conducted to test and compare the diagnostic value of each SPT extract.

Correctly diagnosing a hymenoptera venom allergy using sIgE can be difficult in some patients. Although double sensitization with both honeybee and yellow jacket wasp venom is possible, serological cross-reactivity is often seen, probably caused by cross-reacting carbohydrate determinants (CCD) epitopes present in venom extracts. Component-resolved diagnostics (CRD) could be one way to overcome this issue. Ebo et al. [158] used non-glycosylated recombinant allergen components from yellow jacket (rVes v 1 and rVes v 5) together with bromelain as CCD marker to determine their value in diagnosing wasp venom allergy. They included patients with a convincing history of allergy to venom of the yellow jacket wasp. Sensitization was confirmed by either sIgE

against venom extract or positive venom skin test (VST) or a positive basophil activation test (BAT). Of the 26 patients with a sensitization for both venoms, 18 patients were positive for sIgE against bromelain (69%). The research team found that combining CRD with rVes v 1 and rVes v 5 resulted in a correct diagnosis for 94% of their population, concluding that combining rVes v 1 and rVes v 5 is a reliable method to diagnose yellow jacket allergy and 'can help to take the sting of difficult cases'. Because some patients were sensitized to rVes v 5, but had a negative test result for the traditional yellow jacket extract, Ebo et al. also investigated a novel rVes v 5 supplemented yellow jacket allergosorbent. Although an improved sensitivity was seen with Ves v 5 sensitized persons, larger studies are needed to confirm these preliminary results. A review by Brehler et al. [159], published elsewhere the same year, concurs with Ebo et al. regarding the role of CRD in diagnosing hymenoptera venom allergy. They emphasize however that the clinical relevance of anti-CCD IgE is still a matter of debate.

Allergen epitope characterization

Specific immunotherapy for peanut allergy is accompanied by a substantial risk of severe adverse reactions in some patients. These safety concerns take centre stage in the discussion whether desensitization by oral immunotherapy is ready for clinical practice. Short T cell epitope-based peptides of major allergens are already proven to be effective *in vivo* and *in vitro* desensitization without causing adverse IgE-mediated reactions in bee venom and cat allergy. Prickett et al. [160] aimed to select peptides of major peanut allergens, based on the most reliably recognized CD4⁺ T cell epitopes, for possible use in specific immunotherapy. Ara h 1-specific CD4⁺ T cell lines from 18 HLA-diverse peanut-allergic subjects were generated and used to assess recognition of 20-mer peptides collectively spanning the Ara h 1 sequence. Nine 20-mers containing the most frequently recognized epitopes were selected, and their recognition confirmed in 18 additional peanut-allergic subjects. Within these 20-mers, ten core epitopes were mapped, all being HLA-DQ and/or HLA-DR restricted, with each presented on at least two different HLA molecules. Seven short (≤ 20 aa) non-basophil-reactive peptides encompassing all core epitopes were designed and validated in peanut-allergic donor PBMC T cell assays. The authors conclude that, together with previously identified T cell epitope-based Ara h 2 peptides, these peptides would allow for a possible safe way to achieve desensitization in peanut-allergic patients [161].

Another method of investigating possible epitopes for peptide-based immunotherapy for peanut allergy is

performed using computer simulation, also known as *in silico*. Using MHC-class II-binding predictions, a method already frequently applied in epitope identification on infectious agents, Pascal *et al.* [162] focused on identification of Ara h 2 peptides for possible use in specific immunotherapy. Thirty-one CD4⁺ T cell epitopes of Ara h 2 in four regions of overlapping peptides were determined *in vitro* using blood samples from 80 peanut-allergic subjects. Two epitope prediction algorithms were to identify significant peptides from the peptides that were utilized in the *in vitro* assays and in the 107 additional theoretical 20-mers that Ara h 2 holds. In the overall analysis using both algorithms, Ara h 2 was predicted to have 21 significant putative 9-mer core epitope sequences. These epitopes were similar to those identified *in vitro*. The relevant peptides would cover virtually all subjects in the studied cohort. The authors conclude that MHC-class II-based T cell epitope prediction algorithms might be useful in establishing new peptide-based immunotherapy.

Reviews on allergens

The interaction of the innate immune system with allergens is discussed in a review by Thomas [163]. While the immunologic mechanisms mentioned in the review are covered elsewhere in this study, the article also discusses the dominance and abundance of allergens from certain allergen sources. For certain allergen sources, the majority of specific IgE was directed towards a select group of allergens or even a single allergen. This is the case with Bet v 1 and Ole e 1 for birch and olive pollen, respectively, and the group 1 and 2 allergens of HDM. These pollen allergens are also the most abundant proteins in their respective pollen grains. The amount of pollen present in the air during pollination roughly correlates with airborne allergen count, but subpollen particles, found before and after pollination, might also be important allergen carriers, especially in ragweed asthma. HDM extracts on the other hand, made from mites that are bred in optimal conditions, are abundant with allergens, while the natural ratio of different allergens in dust might be different. Several articles covered in the review by Thomas illustrate the difference in concentration of HDM allergens in multiple dust samples.

Subcutaneous allergen immunotherapy (SCIT) is a therapeutic option for patients with grass pollen allergy. Traditionally, the vaccines contain natural pollen extracts, but the use of recombinant allergen-based vaccines might provide a more targeted immunotherapy as discussed in a review by Gangl *et al.* [164], which provides an comprehensive overview of grass pollen allergens. To date, ten different groups of grass pollen allergens are officially recognized, based on their IgE binding frequencies and physicochemical properties in extracts. Group 1, which includes Phl p 1, the major allergen in Timothy grass pollen, and group 13 allergens are present in most allergenic grasses. As allergens from groups 1, 2, 5, 6 and 13 are specific for grasses, they can be considered markers of true grass pollen sensitization. Allergens from other groups occur not only in pollen from grasses, but also in pollen from other plants, such as trees and weeds. Pan allergens such as polcalcins (e.g. Phl p 7) and profilins (e.g. Phl p 12) act as a marker for broad sensitization against pollen from various sources. The prevalence of sensitization to allergens from the allergen groups varies in grass pollen-allergic patients, with about 90% recognizing group 1 allergens.

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Conflict of interest

The authors declare no conflict of interest.

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