

SPECIAL ARTICLE

Developments in the field of allergy in 2012 through the eyes of *Clinical & Experimental Allergy*

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Clinical & Experimental Allergy

Summary

In 2012, we received 683 submissions and published 20 editorials, 38 reviews, 11 letters and 128 original articles. This represents an acceptance rate for original papers in the range of 20%. About 30% of original papers were triaged not to go out to review, either because the editors did not feel they had sufficient priority for publication or because the topic did not feel right for the readers of the journal. We place great emphasis on obtaining sufficient high-quality reviews to make our decisions on publication fair and consistent. Inevitably, however, there is a degree of luck about what gets published and which papers miss out, and we are always happy to receive an appeal on our decisions either at the triage stage or after review. This gives us the opportunity to revisit the decision and revise it or explain in more detail to the authors the basis for the decision. Once again in 2012, we were delighted by the quality of the papers submitted and the breadth and depth of research into allergic disease that it revealed. The pattern of papers submitted was similar in previous years with considerable emphasis on all aspects of asthma and rhinitis. We were particularly pleased with our special issue on severe asthma. Elucidating mechanisms using either animal models or patients has always been a major theme of the journal, and the excellent work in these areas has been summarized by Harissios Vliagoftis with a particularly interesting section on early-life events guiding the development of allergic disease, which understandably continue to be a major theme of research. Magnus Wickman summarized the papers looking at the epidemiology of allergic disease including work from birth cohorts, which are an increasingly rich source of data on risk factors for allergic disease, and two papers on the epidemiology of anaphylaxis. Giovanni Passalacqua discussed the papers in the clinical allergy section of the journal, and Adriano Mari who runs the excellent Allergome website discussed the papers looking at allergens including characterization and the relative usefulness of allergen arrays versus single extracts in diagnosis and management.

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Asthma and rhinitis

Asthma phenotypes

It has been recognized for many decades that asthma is heterogeneous [1, 2]. Phenotypes of asthma can be defined on a clinical basis (e.g. exacerbation prone, brittle, refractory), on a biological basis (neutrophilic, eosinophilic, paucicellular) or on an aetiological basis (allergic, occupational, viral-induced). In addition, asthma symptoms may represent either the disease itself

or the overt presentation of other diseases (e.g. aspergillosis or vasculitis). Nonetheless, phenotyping asthma still represents a challenge, because of the numerous and heterogeneous levels of investigational outcomes that should be considered at the same time, so that also the term 'endotype' has been proposed [3, 4].

Asthma associated with obesity has been an important topic of research, as it seems to be a distinct entity causing an increased risk of severe asthma [5–8]. The question that whether this is a cause or an effect was discussed in an editorial accompanying these papers

[9]. The interaction between mechanical and inflammatory factors has been characterized in detail [10]. In this context, the atopic status and the distribution of the adipose tissue seem not to be an incremental risk factor for asthma and its severity in obese subjects [7]. Otherwise, although a general inflammatory process has been repeatedly claimed in asthma–obesity, a recent trial failed to demonstrate in children the possible role of an inflammatory process [11].

Defining phenotypes and endotypes of asthma requires measurements of as many dimensions of the disease process as possible. Detection of inflammation is tremendously important, but difficult to routinely measure. A great deal of effort has gone into exploring the value of exhaled nitric oxide (eNO), in guiding management of asthma, and two studies addressed this issue in relation to severity of disease, the findings of which were discussed in an accompanying editorial [12–14]. A further study investigated that the relationship between eNO and exercise-induced asthma demonstrated a significant correlation between eNO and the magnitude of the exercise-induced fall in FEV₁ [15].

There continues to be a lively debate about childhood wheezing phenotypes. Van der Valk and colleagues sought to shed new light on this area by determining how eNO related to wheezing phenotypes in children aged 4 and 8 years. They found a relationship with some phenotypes, but only in atopic children [16].

An important phenotype of asthma is that caused by occupational agents. Challenge with the offending agent is a staple of the occupational asthma physician, and Malo *et al.* investigated whether low molecular weight allergens caused a different pattern of response to high molecular weight agents. They found significant differences in the timing of the recovery from the early response and the incidence of late responses [17].

The overlap between asthma and chronic obstructive pulmonary disease (COPD) is another important topic of research. There is a fraction of asthmatic subjects, with a history of early-onset asthma and with atopy, who have a fixed obstruction (thus functionally resembling COPD). Recent advances in our understanding of the genetics of fixed airflow obstruction were reviewed by Wain and colleagues [18]. In parallel, there is a proportion of (older age, history of smoking, cor pulmonale) patients with COPD, which maintain a significant reversibility [19, 20]. For these reasons, there have been attempts to redefine obstructive lung disease, for instance by considering the so-called 'A to E' model (airways hyperresponsiveness, bronchitis, cough, damage and extrapulmonary factors) [21, 22]. It is not still clear whether we should redefine the diagnostic criteria or the definition of the two diseases. The degree of involvement of the small airways in various patterns of

asthma is a popular topic for research, and this area was reviewed by Contoli *et al.* [23].

Severe asthma

In a special issue, a number of invited reviews and relevant original papers addressed the issue of severe asthma. Severe asthma is a challenging model from both the basic science and clinical point of view [24]. The main problem of severe asthma remains its definition. Certainly, in clinical practice, physicians are approximately able to identify a patient as having 'severe asthma' when symptoms (and exacerbations) persist despite optimal therapy, but an agreed characterization based on objective and standardized parameters still eludes us. This obviously biases the numerous studies that attempted to quantify and characterize severe asthma within large populations. The definition of severe asthma is further complicated by other factors such as adherence and comorbidities. In addition, the problem of severity versus control requires a strict identification [25]. As described by Blakey and Wardlaw, several definitions have been proposed, and overall, they agree on a common characteristic that is the persistent absence of clinical control despite optimal therapy. In some cases, the use of oral steroids for prolonged periods was introduced as a possible marker of severity. At this stage, the only robust point seems to be the definition of 'refractory asthma' that is the disease remaining uncontrolled with the maximal therapy, with ascertained optimal adherence and with comorbidities identified and properly treated [26]. Although severe asthma is a well-recognized clinical phenotype, its biological characteristics are not yet well defined, and it is not clear whether severe asthma is a distinct phenotype or a label to be applied to different phenotypes [27].

This is an exciting time with the potential for several new classes of drugs to be introduced for the management of severe asthma [28, 29]. In the special issue, approaches to treatment of severe asthma were addressed in three reviews looking at current state-of-the-art and future treatments particularly antagonists of IL-5 for eosinophilic asthma [30–32]. Poon *et al.* and Brightling and colleagues summarized current evidence regarding the pathogenesis of severe asthma and in particular the fixed airflow obstruction and bronchiectasis that is associated with it, an issue also addressed in an original article by Amelink *et al.* [33–35]. In a later issue, Bourdin and colleagues reviewed the literature regarding adherence in severe asthma, which is undoubtedly the single most important reason for treatment failure [36]. Finally, it has to be remembered that severe chronic asthma is not necessarily equivalent to severe exacerbations of asthma, which is distinct if

linked phenotype that is associated with risk of hospitalization and rarely death [37–40].

Biomarkers

Szczeklik et al. [41] reported 12-hydroxyeicosatetraenoic acid as a potential biomarker for Churg–Strauss syndrome having found higher concentrations in this group of patients compared with those with uncomplicated asthma. There is increasing interest in the role of thermotolerant fungi in driving the inflammatory process in asthma [42]. Goldman and colleagues added to this body of work by demonstrating increased concentrations of chitinases and antibodies for colonizing fungi in children with asthma compared with controls [43]. Consistent with this finding, a greater fungal burden was found in homes of asthmatics compared with controls [44].

The most well-established biomarker for asthma is the sputum eosinophil count. Munoz and colleagues asked whether patients with well-controlled symptoms still had an evidence of airway inflammation [45]. About a third had raised numbers of sputum eosinophils, and a third raised numbers of neutrophils. A sputum eosinophilia is a risk factor for severe exacerbations, and this finding probably explains why 50% of asthmatics with severe exacerbations have apparently mild background disease. Cysteinyl leukotrienes are also well established as important mediators in asthma, but some mystery still surrounds the nature of the LTE₄ receptor, an area reviewed in detail by Laidlaw and Boyce [46].

Ferreira et al. [47] examined TSLP expression in post-mortem specimens from fatal asthma compared with appropriate controls and found a significant increase in the epithelial layer.

Treatment of asthma

The chemoattractant receptor CRTH2 has long been thought to be potentially important in asthma through the recruitment of eosinophils, basophils and Th2 cells into the lung. A number of CRTH2 antagonists are in the later stages of development, but the first report of a clinical trial of this class of drugs in moderate asthma was published by Barnes et al. [48] who demonstrated a modest improvement in FEV₁ and quality of life in the per-protocol subjects. Further studies of drugs that block this receptor are eagerly awaited. Another class of drugs that are of potential value in allergic disease are chemokine antagonists including antagonists of the CXCR2 receptor. Nair et al. [49] reported on a study of a CXCR2 antagonist in severe asthma. They found that the drug was well tolerated and resulted in a significant although modest reduction in sputum neutrophils, which was associated with a reduction in mild exacerbations. In a

proof-of-concept study, Virtala et al. [50] demonstrated that an oral antagonist of this receptor significantly inhibited the neutrophil influx into nasal lavage fluid by about 50% in healthy subjects, paving the way for the use of this treatment in neutrophilic airway disease.

A more unorthodox approach to the treatment of asthma and other allergic diseases is to use the immune response to parasite infection to suppress the allergic response. The basis for this approach and the results of clinical trials were reviewed by Pritchard et al. [51]. Bourke et al. [52] found that *Trichuris suis* ova had no effect on cytokine responses in allergic rhinitis despite modulating parasite-related immune responses.

There is currently a vogue for using long-term macrolide therapy in a range of respiratory disease although whether these drugs perform any better than other broad-spectrum antibiotics remains uncertain. This area was reviewed by Cameron and colleagues [53].

Plopper et al. [54] studied the effects of inhaled corticosteroids in developing primate lungs using a rhesus monkey model of allergic airway disease. They treated allergic rhesus monkeys daily with inhaled budesonide from 3 to 6 months of age. They showed that inhaled corticosteroids had a significant benefit regarding manifestations of the disease, although they were not able to completely reverse lung pathology. However, inhaled corticosteroids disrupted some aspects of post-natal growth and differentiation of airways and lung parenchyma.

Allergic rhinitis and chronic rhinosinusitis

A perennial question in allergic disease is the relationship between sensitization and disease. Zidarn and colleagues addressed this question in the context of grass pollen allergy by studying the relationship between specific IgE and basophil sensitivity. They found that basophils from patients with allergic symptoms responded to a lower threshold of allergen dose [55]. Whether this can translate into a clinically useful test remains to be seen.

Katellaris et al. [56] reviewed the literature on allergic rhinitis in geographical areas other than Europe and the United States where most research has been undertaken, so providing a database of the prevalence and allergenic causes of rhinitis in these countries.

Kamekura et al. investigated the expression of the topical Th2-related cytokine IL-33 and its receptor ST2 in the nasal mucosa in allergic rhinitis. They found increased expression (or concentrations) of IL-33 in the blood and nasal mucosa of patients with allergic rhinitis as well as investigate the regulation of IL-33 production by inflammatory cytokines in vitro in epithelial cells [57]. Together, these data formed a persuasive case for the importance of this cytokine in the pathogenesis

of allergic rhinitis. In another study investigating the mechanisms involved in allergic rhinitis, Liu et al. [58] found that T regulatory cell numbers were lower in the nasal mucosa and that this was associated with higher levels of staphylococcal enterotoxin B and lower levels of expression of the integrin $\alpha v \beta 6$. Khoo et al. investigated expression and function of acid-sensing channels (ASC) in nasal epithelium and found that eosinophil peroxidase induced expression and enhanced the function of ASC-3, the implications of which were discussed in an accompanying editorial [59, 60]. Cho and colleagues studied the epigenetic regulation of myofibroblast differentiation in nasal polyp fibroblasts and demonstrated that inhibition of HDAC was associated with myofibroblast differentiation [61]. Another study investigating chronic rhinosinusitis found that TGF β 1 expression was significantly increased in the paranasal sinuses in early-stage disease [62].

Allergic mechanisms

New therapeutic approaches to allergic inflammation

A number of manuscripts studied new therapeutic approaches to treat allergic airway inflammation using animal models [63–66]. The approaches taken in these manuscripts ranged from the use of small molecule inhibitors to vaccination with peptides or plasmids expressing immunomodulatory proteins.

Kaminuma and colleagues [63] used a small molecule antagonist of CCR4 in mouse models of Th2-, Th1- and Th17-mediated airway inflammation. Their model consisted of adoptive transfer of *in vitro*-polarized T cells from OVA transgenic mice followed by challenge with intranasal OVA. The low molecular weight CCR4 antagonist, compound 22, prevented the development of airway hyperresponsiveness (AHR) and the accumulation of Th2 cells and eosinophils in the airways of these mice, but had no effect on goblet cell hyperplasia. Compound 22 had no effect on inflammation mediated by the transfer of OVA-specific Th1 or Th17 cells. CCR4 antagonism has been previously shown to prevent airway inflammation in other models, but in this study, the authors showed that CCR4 antagonism targets primarily Th2-mediated airway inflammation.

Shimizu et al. [64] showed that a novel NF- κ B inhibitor, dehydroxymethylepoxyquinomicin (DHMEQ), was very effective in a murine model of OVA-mediated allergic airway inflammation. DHMEQ inhibited AHR, accumulation of eosinophils and Th2 cytokines in bronchoalveolar lavage (BAL) fluid and also parameters of airway remodelling such as mucus production, peribronchial fibrosis and the expression of a smooth muscle actin. DHMEQ is a small molecule capable of penetrating cellular membranes, and although fairly

specific for NF- κ B, it also has the ability to activate Nrf2. Nrf2 activation can inhibit eotaxin release [67], which may have contributed to the effectiveness of this compound in murine models of allergic airway inflammation.

Severe steroid-resistant asthma has been associated with activation of the IL23/Th17 axis, similar to the pathogenesis of atopic dermatitis. Guan et al. [65] used an IL-23p40 peptide-based virus-like particle vaccine and showed that immunization of mice with this vaccine induced long-lived anti-IL-23 antibodies and ameliorated indices of asthma and atopic dermatitis. Epicutaneous sensitization to OVA followed by intranasal challenge led to eosinophilic inflammation in the airways and the skin, which was prevented with the use of the vaccine. The vaccine also decreased the levels of antigen-specific antibodies induced by OVA sensitization.

Fonseca et al. [66] showed that immunotherapy with DNA encoding *Mycobacterium leprae* Hsp65 attenuates airway eosinophilia, pulmonary inflammation and mucus production through an IL-10-dependent mechanism.

Transplantation of autologous hematopoietic stem cells expressing disease-causing antigens induces tolerance. This approach, called molecular chimerism, has been shown to be effective in models of organ transplantation and autoimmune diseases. Baranyi et al. [68] showed that a similar approach inducing microchimerism could induce long-term tolerance to grass pollens. The authors achieved this by transducing bone marrow cells with recombinant viruses that express membrane-anchored Phl p 5 allergen. They then transplanted these modified cells into syngeneic non-myeloablated recipients and showed that microchimerism (less than 1%) was sufficient to induce B cell tolerance, and possibly also T cell tolerance, to the grass pollens.

Mechanisms of atopy and allergic inflammation

Another group of manuscripts studied mechanisms leading to the development of atopy and allergic inflammation. These manuscripts included a review on the factors that mediate allergic sensitization to inhaled allergens in murine models along with several studies on the role of adjuvants or the roles of other molecules that activate the immune system during the development of allergic sensitization.

Mouse models of allergic airway inflammation often use systemic routes of sensitization in the presence of an exogenous adjuvant. More recently, research has focused on models that do not use added adjuvants to induce allergic sensitization, but administer the allergen directly into the airways. These models use a variety of allergens, routes of administration and protocols and

often present conflicting results. Guibas et al. [69] reviewed studies that sensitized animals through direct administration of allergen into the airways and discussed the important factors that may mediate the development of allergic sensitization versus development of tolerance. The authors discuss the role of characteristics of the allergens themselves and characteristics of the animals used in the development of allergic sensitization. They also discuss environmental factors that may influence the animals during the sensitization protocol and the role of anaesthesia. Unfortunately, there has been little effort to directly compare the effects of these factors in individual studies. These types of comparisons are required before we can make definite conclusions on the role of these factors in the development of allergy.

Heydenreigh et al. [70] demonstrated the presence of adjuvant activity on inhaled pollens. In this study, the authors cultured bacteria they isolated from the surface of grass pollens and then studied the adjuvant potential of these bacteria. The authors showed that gram-positive bacteria that are present on timothy grass pollen have the ability to enhance dendritic cell (DC) maturation and inflammatory responses and may help initiate allergic immune responses to the pollen.

Goel et al. [71] showed that a cockroach allergen, Per a 10, has a serine protease-dependent effect on DC maturation. Increased DC maturation enhances the ability of DC treated with Per a 10 to induce Th2 cell differentiation. The serine protease activity of Per a 10 preferentially up-regulates CD86 on DC, compared with protease-inactive Per a 10 molecules, and induces high levels of Th2 cytokines when Per a 10-treated DCs are cocultured with autologous CD4⁺ cells. This study underscores the adjuvant-like effects of the serine protease activity of allergens.

Forbes-Blom et al. [72] showed that staphylococcal-derived superantigens enhance CD4⁺ T cell responses to allergens presented to the skin. This effect is mediated by direct activation of Vb8⁺ T cells in the skin by the superantigen and leads to increased skin inflammation. One administration of the superantigen has a prolonged effect on the immune response to the specific allergen that was encountered at the same time as the superantigen. Therefore, superantigens in the skin may have adjuvant activity facilitating allergic sensitization to food allergens presented through the skin.

Periostin has been identified as an important biomarker of Th2-biased asthma, but its function in asthma has not been identified *in vivo*. Gordon et al. [73] showed that periostin knockout mice exhibited increased AHR and serum IgE levels, but had no differences in markers of remodelling in a model of mucosal sensitization to *Aspergillus fumigatus*. The defect was identified as an inability of periostin deficient mice to

develop regulatory T cells (T_{reg}) due to a lack of TGFb1, which is induced by periostin.

Hematopoietic progenitors are recruited to the lung in allergic diseases with subsequent *in situ* development of inflammatory cells [74]. The exact mechanisms leading to this recruitment are not known. Punia et al. [75] showed that the Th2 cytokines IL-4 and IL-13 prime hematopoietic progenitor cells to respond to the chemotactic effects of SDF-1a because they increase the incorporation of CXCR4, the SDF-1a receptor, into the lipid rafts. This effect of IL-4 and IL-13 is evident in progenitors from both peripheral and cord blood, but it is not evident in eosinophil lineage-committed progenitor cells.

High-mobility group box 1 protein (HMGB1) is an endogenous signal that prolongs eosinophil survival and has chemoattractant properties. Shim et al. [76] showed that HMGB1 levels are increased in the sputum of asthmatics compared with normal controls. To further define the role of HMGB1 in asthma, they then used a murine model. They showed that HMGB1 is increased in the lung of mice sensitized and challenged with OVA and that HMGB1 blockade attenuated all inflammatory changes in this model. HMGB1 blockade also prevented certain aspects of DC activation, indicating that this may be the mechanism of HMGB1 involvement in the development of allergic inflammation.

Many of the studies on the basic mechanisms of allergic airway inflammation are performed using recombinant or purified allergens. The reason for this approach is that such studies can focus on the effect of only one factor in the development of allergy. However, in real life, allergens are inhaled as a part of complex particles or together with other particles that may have immunomodulatory effects. Roschmann et al. [77] studied the biological differences between a complete grass extract and a purified major allergen, Phl p 1, using airway epithelial cells. Two thousand and one hundred and seventy-nine genes showed regulation by both stimuli. Among them, 262 genes were induced more than threefold in airway epithelial cells by grass extract and only 71 genes by the purified allergen. The genes regulated by both stimuli could be separated into 8 clusters with complicated induction patterns. For example, a number of immunologically important genes were up-regulated by the extract, but not by the purified allergen, indicating that other components of the extract may be important for the full development of allergic sensitization. In addition, there were genes that were up-regulated by one of the two stimuli, but down-regulated by the other. Similar differences between grass extract and purified allergen were also found in regard to cytokine release from airway epithelial cells. The differences shown in this study may have widespread relevance in allergic disease. The allergenic

material we inhale is very different from individual allergens, and interactions between various components and our immune system are required for the development of allergy.

Eifan *et al.* [78] showed that allergen challenge in atopic individuals increases the numbers of CD3⁺STA T6⁺ cells in the nose and skin and CD3⁺GATA3⁺ cells in the skin, while a similar effect is not seen in non-atopic individuals. On the other hand, CD3⁺STAT4⁺ cells and CD3⁺T-bet⁺ cells increased in both atopics and non-atopics after PPD challenge. The authors also showed evidence of dysregulation of Th1 transcription factors in atopics, which may be associated with the exaggerated Th2 responses they develop.

Biomarkers of allergic diseases

Over the last few years, there has been a significant increase in the number of studies using cohorts or other patient populations to identify biomarkers that predict the development or severity of atopic diseases. Such biomarkers have the potential to improve our ability to diagnose and treat allergic diseases, but they also offer significant insight into the pathogenesis of these diseases. A number of such studies were published in the journal this year.

A prospective observational birth cohort study was performed in the Netherlands by Belderbos and colleagues [79]. Healthy neonates born after uncomplicated pregnancy of over 37 weeks were recruited, and clinical characteristics and standardized parental questionnaires were collected. Blood was collected at the age of 1 month, and the presence of various blood cells and blood cell subsets was evaluated by flow cytometry. In addition, whole blood was activated with TLR agonists, and cytokines were then measured in the supernatants by ELISA. The primary outcomes were atopic dermatitis (AD) and RSV lower respiratory tract infection (LRTI). The risk of development of the two conditions was not associated in this population. AD was associated with decreased concentrations of basophils and plasmacytoid DC and increased concentrations of NK cells and twofold lower TLR-mediated IL-10 production. The risk of RSV LRTI was not associated with any immune measurements performed in the study. Disrupted post-natal development of the TLR system and dysregulated TLR-mediated cytokine responses in early life may induce persistent dysfunction of innate and adaptive immunity that may lead to the development of atopy.

McLoughlin *et al.* [80] studied the immunomodulatory T cell phenotype and function in cord blood cells and also in peripheral blood mononuclear cells collected at 1 and 2 years of age from a subset of children who participated in the Urban Environment and Childhood Asthma (URECA) study. They found that the characteristics of

immunoregulatory T cells at birth were not associated with the development of allergic sensitization. They also found that the number of immunomodulatory T cells present increased significantly over time. CD4⁺CD25^{bright} and CD4⁺CD25⁺FoxP3⁺ cells increased significantly from birth to 1 year, and CD4⁺CD25^{bright} cells increased again from 1 to 2 years of age. Increases in the numbers of CD4⁺CD25^{bright} cells and their capacity to suppress lymphoproliferative responses at 1 year of age were associated with decreased allergic sensitization at 1 and 2 years of age. This protective effect appeared to be mediated by IL-10 production from CD25⁺ T cells. In contrast, at 2 years of age, there was a positive association of CD4⁺CD25⁺FoxP3⁺ cells with allergic sensitization. The study was not able to identify markers at birth that would predict the development of allergic sensitization, but showed that immunomodulatory T cells present at 1 year of age, which may represent adaptive Tr1 cells, appear to reduce the risk of allergic sensitization. These cells, however, did not predict the risk of wheezing at 1 year, underscoring the fact that most early wheezing is of non-allergic aetiology.

Folsgaard *et al.* [81] assessed the association between chemokines in cord blood and the development of asthma, eczema and allergic rhinitis in the first 6 years of life. They analysed cord blood from asymptomatic at-risk newborn children from the Copenhagen Prospective Study of Asthma in Childhood (COP-SAC₂₀₀₀) birth cohort of mothers with asthma. Cord blood CCL22 levels were significantly associated with total IgE measured at 4 time-points during the first 6 years of life. However, there was no association of CCL22 levels with the development of any particular allergic disease. CXCL10 and CXCL11 were not associated with any outcome analysed in the study. The study suggests that an intrauterine Th2 environment may influence the rates of IgE production later in life. An accompanying editorial [82] discussed the utility of cohorts to understand the importance of immune changes as markers or predictors of the development of allergic disease. The author discussed the major impediments to using these studies to understand the pathophysiology of allergic diseases and concluded that improved means of stratifying subjects into more precisely delineated groups, and the use of more homogeneous patient populations will be a key to enhancing our ability to tease out immune and physiological predictors from these studies.

Savilahti *et al.* [83] studied the association of intestinal defensins with the development of allergic sensitization and atopic dermatitis. For the study, they used a subgroup of infants at high risk of allergy from those who participated in a randomized double-blind placebo-controlled trial of synbiotics versus placebo. At the age of 3 and 6 months, the authors measured fecal

levels of human neutrophil peptide (HNP) 1-3 and b-defensin 2 (HBD2). Follow-up for allergic sensitization and development of allergic diseases lasted 5 years. High HBD2 levels at 6 months of age were associated with increased risk of allergic sensitization at 5 years, while high HNP 1-3 levels were associated with decreased risk of atopic dermatitis. HBD2 also correlated positively with α 1-antitrypsin levels in these infants, but there was a negative correlation between fecal HNP 1-3 and α 1-antitrypsin levels. Increased fecal α 1-antitrypsin indicates higher permeability of mucosa and is, thus, a marker of inflammation. Several studies have shown that the composition of intestinal flora affects the risk of atopy. High HBD2 secretion restricts the growth of bacteria and may have affected the intestinal flora in these subjects and possibly affected the development of allergy. However, the exact mechanisms of this interaction between the presence of defensins and development of allergic diseases or why one defensin is associated with allergic sensitization and another one with the development of atopic dermatitis is not clear. Further studies may shed light on this area.

Lange et al. [84] studied potential interactions between maternal intestinal flora and wheeze in early childhood. The researchers recruited 60 pregnant women, but did not select the participants for the presence of any specific allergic disease such as allergy or asthma. A stool sample was collected from participating women between their 24-week prenatal visit and delivery, and intestinal flora was analysed. The primary outcomes for the infants were wheeze and eczema, and the secondary outcome was atopic wheeze. They found that higher counts of maternal total aerobes and enterococci were associated with increased risk of infant wheeze after adjustment for breastfeeding, day care attendance and maternal atopy. However, no organisms were associated with either eczema or atopic wheeze.

Another study evaluated the effect of parental smoking on cytokine production from peripheral blood of infants at the age of 3 and 12 months [85]. The subjects of this study were randomly chosen from the study population of a randomized double-blind placebo-controlled (DBPC) intervention trial (ISRCTN14869647, www.controlled-trials.com) performed at south-western Finland. Children of smoking parents, in all cases the father was a smoker, had significantly elevated levels of IL-4, IL-5, IFN- γ and TNF at 12 months of age, but not at 3 months of age. The study also showed that parental smoking increased the levels of Th2 cytokines released from peripheral blood mononuclear cells (PBMC) stimulated with concanavalin A.

Hematopoietic progenitors, especially those committed to eosinophil basophil lineage (Eo/B), contribute to allergic inflammation. Weisse et al. [86] studied the presence of Eo/B progenitors in peripheral blood from

60 1-year-old children enrolled in the LINA (Lifestyle and environmental factors and their Influence on New-born Allergy risk) birth cohort. Children with skin manifestations (atopic dermatitis or cradle cap) within the first year of life had higher numbers of circulating IL-3-, IL-5- or GM-CSF-stimulated Eo/B colony-forming units (CFUs) at 1 year. In children with cradle cap, a positive correlation was found between Eo/B CFUs and exposure to tobacco-smoke-related volatile organic compounds.

D'Vaz et al. [87] studied the effects of fish oil supplementation in early infancy on the immune responses of the infant. The study included a subgroup of healthy term infants born to allergic women recruited at 36 weeks of pregnancy in Perth, Western Australia, for a double-blind, randomized controlled study designed to assess the effects of infant fish oil supplementation on immune function and infant allergies. Infants were randomized to receive fish oil or control live oil capsules. Blood was collected at 6 months of age, and the response of PBMCs to allergens, mitogens or TLR activators was studied. Post-natal fish oil supplementation increased infant n-3 polyunsaturated fatty acid (PUFA) levels and was associated with lowered allergen-specific Th2 responses, but elevated polyclonal Th1 responses.

Two studies analysed the effects of probiotics or synbiotics on infant immune function. Probiotics and synbiotics have been proposed to modulate susceptibility to allergic diseases, and there is more information from animal models to support these effects. In infants with atopic dermatitis, a synbiotic mixture did not alter cytokine production by anti-CD3-/anti-CD28-stimulated PBMCs [88]. Administration of the synbiotic mixture also had no effect on the percentage of circulating T_{reg} cells and the levels of cytokines and chemokines in the serum. In another study, daily administration of probiotics in term infants with no allergic disease for 9 months had a modest effect on T cell maturation indices [89].

Mechanisms of immunotherapy

The mechanisms of disease improvement after immunotherapy are still under investigation. A number of studies published in the journal studied these mechanisms.

Bonvalet et al. [90] assessed allergen-specific CD4⁺ T cell responses in PBMCs from 89 grass pollen allergic individuals enrolled in a double-blind placebo-controlled sublingual immunotherapy (SLIT) study conducted in an allergen exposure chamber. SLIT induced a 29.3% improvement in the average rhinoconjunctivitis total symptom score in the active group when compared to the placebo group, but only minor changes in the immune parameters studied. There were also no major changes in the number or polarization of allergen-specific CD4 T

cells following immunotherapy. The study did not identify any immune marker to predict efficacy of immunotherapy.

Thyagarajan et al. [91] studied the effect of oral immunotherapy with peanut on *ex vivo* basophil activation. Immunotherapy induced basophil anergy in response to IgE-mediated stimuli, but did not affect basophil activation through other pathways. Anergy of effector cells may contribute to the efficacy of desensitization, but further work is needed in this area.

The mechanisms of oral tolerance are unclear and may include anergy or apoptosis of antigen-specific T cells, but also may include the generation of antigen-specific T_{reg} cells. Kawakami et al. [92] studied the role of Th17 immunity in the development of oral tolerance. Mice sensitized and challenged with OVA were fed OVA before rechallenge; OVA feeding induced oral tolerance and decreased all measures of airway inflammation and AHR. Transfer of OVA-specific Th17 cells or administration of IL-17A abrogated oral tolerance induction, probably through IL-6 induction in the gut, which suppressed T_{reg} cell expansion. The effect was not seen when mice were administered OVA-specific Th2 cells.

Maazi et al. [93] used a mouse model of OVA-mediated allergic airway inflammation to study the role of T_{reg} in allergen-specific immunotherapy (SIT). They showed that T_{reg} cells play a complicated role that may be different at different phases of immunotherapy. Depletion of $CD4^+CD25^+$ cells before SIT prevented the effects of SIT on AHR, but had no effect on airway inflammation. In contrast, depletion of $CD4^+FOXP3^+$ cells after SIT treatment was performed, partially reversed the effects of SIT on eosinophilic inflammation, but had no effect on AHR. No treatment affected serum antigen-specific IgE levels. The study suggests that different T_{reg} cell types mediate the full effect of SIT on AHR and allergic airway inflammation in murine models.

Rask and colleagues [94] used a mouse model to study the antigenicity and allergenicity of various formulations of grass pollen that can be used for subcutaneous immunotherapy. They immunized mice with these preparations intraperitoneally and measured antigen-specific immunoglobulin responses and also performed *in vitro* T cell proliferation assays. They showed that the magnitude of the allergen-specific IgG antibody responses and the magnitude of *in vivo*-induced T cell stimulation were driven primarily by the aluminium hydroxide (AlOH) concentration used. In contrast, the allergen concentration used was the most important factor for the *in vitro* T cell stimulation results. Using basophil activation as a measure of allergenicity, the authors showed that the potential to elicit IgE-mediated adverse events is linked to the allergen concentration. The authors concluded that by adjusting the allergen/

AlOH ratio, we may be able to optimize immunogenicity, but at the same time decrease immediate hypersensitivity side-effects of the vaccine.

Clinical desensitization to drugs involves exposure to escalating doses of drug over short periods of time. *In vitro*, this method leads to loss of mast cell and basophil responsiveness. MacGlashan [95] studied the loss of syk and FcεRI, two well-described end-points of basophil desensitization, using a model of subthreshold desensitization of basophils *in vitro*. He showed that both critical signalling molecules are lost during subthreshold desensitization and that treatment with IL-3 blunts the loss of syk, but not that of FcεRI.

IgE

A number of manuscripts studied different aspects of IgE biology a subject reviewed by Potaczek and Kabesch [96]. Jarvinen et al. [97] studied the presence of IgE antibodies against human milk in the serum of infants with cow's milk allergy that remained symptomatic after strict maternal milk avoidance. Sixty percent of milk allergic infants had antibodies recognizing human milk epitopes. There was a trend towards IgE antibodies against more human milk epitopes in infants that did not respond to elimination diet. Only infants that did not respond to elimination diet had IgE anti-human milk antibodies that were functional *ex vivo*.

Eckl-Dorna et al. [98] studied the potential of PBMC from allergic patients to produce antigen-specific IgE *ex vivo* and correlated the amount produced from these cells with the antigen-specific IgE present in the serum of the same patients. They showed that a significant amount of IgE in their PBMC cultures was coming from basophils present in the culture. Most of the newly synthesized IgE in these cultures was coming from the $CD138^+$ plasma cells and not from B or B memory cells. Finally, they showed that the *ex vivo* production of IgE from PBMC was extremely low compared with the IgE they could measure in patients' serum. They concluded that most of the antigen-specific IgE in the serum does not originate from cells present in the blood.

There is evidence that the composition and complexity of the allergen-specific IgE repertoire rather than only the specific IgE concentration determines the efficiency of basophil activation, as well as the formation of IgE–allergen–CD23 complexes on B cells and subsequent antigen presentation and T cell activation. Wilmsen et al. [99] studied the complexity of the allergic patient's IgE repertoire and found that it correlates with serum concentration of allergen-specific IgE, a finding discussed in an accompanying editorial by Kleine-Tebbe [100].

IgE, like all other isotypes of antibodies, is present in newborn serum, but the mechanisms for acquisition of

maternal antibody isotypes other than IgG are not known. Paveglio et al. [101] showed that the absorption of allergen-specific IgE by breastfed offspring was dependent on FcRn expression in the offspring. IgG₁ anti-IgE/IgE immune complexes were present in foster mothers and correlated strongly with the levels in the offspring. They also showed that the FcRn was required for neonatal mice to absorb IgG₁ anti-IgE/IgE immune complexes fed to them. This observation raises the possibility that FcRn may also mediate the transplacental transfer of maternal IgE to the fetus.

Interactions between the immune and nervous systems in allergy

Three manuscripts studied interactions between the immune system and the nervous system in asthma.

Ogawa et al. [102] showed that chronic stimulation with house dust mite antigens increases nerve growth factor (NGF) on the bronchial epithelium in murine asthma models. Increased NGF levels lead to substance P production and increased AHR. Blocking NGF in this model prevented AHR, but had no effect on allergic inflammation and subepithelial fibrosis. This observation further supports the notion that AHR and airway inflammation are regulated differently in these murine models.

Wang et al. [103] studied the effects of allergy on the nervous system using a rat model of skin allergic inflammation. The authors showed that allergic inflammation induced overexpression of the 5-HT receptor 3 (5-HT R3) in the trigeminal nerve along with increased mast cell numbers in the inflamed skin area and led to trigeminal hyperalgesia. This effect could be reproduced with 5-HT administration in the skin.

Lastly Potenziari and Undem updated readers on the latest thinking around the pathogenesis of itch, which is such a hallmark of allergic inflammation [104].

Cells involved in allergy

A few studies used *ex vivo* or *in vitro* models with cells implicated in the pathogenesis of allergic diseases to further understand the role of these cells in the development of allergic sensitization and allergic inflammation including a comprehensive review by Gros and Novak on the role of cutaneous dendritic cells in allergic inflammation [105].

Eosinophils are now considered important immunomodulatory cells. Esnault et al. [106] showed that human eosinophils increase IL-17 production by anti-CD3-/anti-CD28-activated CD4⁺ T cells. Levels of IL-1 β in the supernatants from eosinophils correlated with their ability to induce IL-17 from T cells, and neutralization of IL-1 β abrogated the eosinophil effect. Blockade of eosinophil

accumulation in the airways with mepolizumab decreased the presence of IL-17 in the airways of allergen-challenged individuals.

Markwick et al. [107] studied the expression of chemokine receptors on human airway smooth muscle cells. They showed that these cells expressed multiple chemokine receptors, but CCR3 was the most frequently expressed receptor. CCR3 activation induces p44/42 MAPK signalling, but does not affect smooth muscle proliferation, migration or VEGF production from these cells. CCR3 activation, however, protected smooth muscle cells from apoptosis induced by caspase 3-independent pathways.

The cytokine milieu of the lung is a cardinal determinant of the development of allergic inflammation. CCL15, a C-C chemokine that activates CCR1 and CCR3, can recruit eosinophils, monocytes, lymphocytes and DC, but its role in asthma had not been studied. Joubert et al. [108] showed that CCL15 is constitutively expressed by human airway smooth muscle cells and is present at higher levels in biopsies from asthmatics versus biopsies from normal individual. CCL15 expression by smooth muscle cells is up-regulated by TNF- α , and IFN γ synergistically enhances this up-regulation. Although the role of CCL15 in asthma is still not clear, this observation underscores the complexity of the airway inflammatory environment and indicates that multiple interventions may be required for the treatment of allergic airway diseases.

IL-25 is a cytokine associated with Th2 responses, and it is increased in asthmatic airways. The IL-25/IL-17RB pathway may be another therapeutic target in allergic airway inflammation. The IL-25 receptor IL-17RB primarily localizes to endothelial cells in asthmatic airways. Human umbilical vein endothelial cells (HUVEC) express IL-17RB constitutively, and IL-25 induces an IL-17RB-dependent up-regulation of bEGF release by HUVEC [109]. The authors also showed that IL-25 and bEGF were both elevated in asthmatic airways and that the two factors correlated with each other.

Glucocorticoid-induced tumour necrosis factor (TNF) receptor-related ligand (GITRL) and its receptor GITR are important for the cutaneous influx of activated T cells associated with inflammatory skin diseases including atopic dermatitis. Byrne et al. [110] showed that GITRL expression is induced on human keratinocytes by Th2 cytokines and TNF- α . They also showed that skin from acute atopic dermatitis lesions exhibits increased expression of GITRL compared with normal skin, skin from chronic atopic dermatitis lesions or skin from psoriatic plaques.

Higaki et al. [111] used dispersed nasal polyp cells (DNPCs) to study the effects of LPS on chronic rhinosinusitis with polyps. Staphylococcal enterotoxin B (SEB)

induced multiple cytokines from DNPCs, and pre-incubation with LPS inhibited SEB-induced cytokine production, an effect that was COX2/PGE2 dependent. In the presence of inhibitors of the COX2/PGE2 axis, SEB had a pro-inflammatory effect on DNPCs. This observation could indicate that the NSAIDS may be detrimental by increasing the pro-inflammatory effects of bacterial products or other pro-inflammatory molecules on nasal epithelium.

Other allergic diseases

Krishnamurti *et al.* [112] studied the role of neutrophils and platelets in a casein-induced mouse anaphylaxis model. They showed that in addition to the classic cells, like mast cells and basophils, neutrophils and platelets also participate in the manifestations of anaphylaxis.

Epithelial barrier dysfunction is critical for the development of allergy. CD98 is known to play a role in intestinal epithelial permeability. Liu *et al.* [113] studied whether CD98 is also important for the development of respiratory allergy. Nasal epithelium from patients with allergic rhinitis expressed high levels of CD98, and there was a positive correlation between CD98 expression and the presence of SEB. SEB induced CD98 in airway epithelial cell lines and also induced a CD98-dependent increase in monolayer permeability. In a mouse model of allergic rhinitis induced by SEB plus OVA, blockade of CD98 prevented the manifestations of the disease.

Atopic keratoconjunctivitis (AKC) is a T cell-mediated disease, but the majority of subjects with the disease also have an atopic phenotype. Allergic sensitization in these subjects, however, does not correlate with the severity of the disease or the development of symptoms. Nivenius *et al.* [114] studied the potential role of allergic sensitization in AKC using conjunctival provocation tests. The symptomatic response to the allergen challenge was similar between patients with AKC and patients with seasonal allergic conjunctivitis. However, there were differences in the cytokines detected in the tears 48 h after the allergen challenge, a time when all symptoms had resolved. Larger and more detailed studies will be required to better understand the role of these cytokines in eye inflammation in patients with AKC.

Other immune diseases

Tang *et al.* [115] studied acute and post-recovery blood samples from patients with Stevens–Johnson syndrome and toxic epidermal necrolysis who reacted to lamotrigine using a lymphocyte transformation test. In the majority of the cases, the test did not show the presence of reactive cells. There was a trend towards increased

responses after depletion of T_{reg} cells, but T_{reg} cell elimination did not improve the ability of the test to detect reactive cells.

Epidemiology

Even though allergy-related diseases have been a public health problem in the western world for several decades, we still are searching for effective allergy prevention measures. Our lack of success in this area is likely to be explained by the bewildering heterogeneity not only of the diseases themselves, but also of interindividual differences in severity, comorbidity and allergic sensitization. One might ask whether any single patient is really like any other.

To solve this equation of many variables, epidemiological studies on what causes allergy-related diseases have in recent years increasingly included the concept of interaction between environmental exposures in addition to the interaction between genes and environment. The heterogeneity of allergy-related diseases accentuates the importance of proper classification of phenotypes and objective collection of data.

Several epidemiological papers published in 2012 have increased our understanding of risk factors and biomarkers of allergy-related diseases. Trends and patterns of allergic sensitization and development of tolerance have been described. Population-based data on anaphylaxis have hitherto been scarce, but we now have such data. Sleep is important for health in general, and a paper investigating the relationship between the quality of sleep and the severity of rhinitis has recently been published.

Perinatal exposures

2012 also saw the publication of studies on perinatal exposures [116,117]. In the paper by Brew and Marks, 450 children were tested at age 8 years. Children with a birth weight <2.5 kg were excluded, but among the children within the normal weight range, lower birth weight was associated with asthma. The analysis was adjusted for gestational age. Because birth weight and gestational age are correlated, we do not really know what the causal exposure underlying this finding is. One might speculate that alveolarization could play an important role in this context – a theory that most likely will be elucidated in future research. Caesarean section (CS) has been shown to moderately increase the risk of asthma and rhinitis. This has previously been explained in the light of the ‘hygiene hypothesis’ and the importance of exposure to the vaginal flora. In the largest population-based register linkage study so far, including data on more than 80 000 sibling pairs extracted from the Swedish Medical Birth

Register and a national Prescribed Drug Register, an association was found for emergency CS, but not for elective CS. Effects shown in previous studies may have been confounded by factors associated with emergency CS.

Infant feeding

Infant feeding has been a focus of interest during 2012 in Clinical & Experimental Allergy [118–122]. The relationship between breastfeeding and associated risks of allergy-related diseases is controversial. The review by Matheson et al. brought up several critical issues such as reverse causation, that is, disease-related misclassification of exposure. The problem of reverse causation in birth cohorts in relation to feeding has also been brought up by Rosenlund et al. [119]. In many earlier studies, effects of breastfeeding have been seen, particularly on wheeze and asthma during the first years of life; and the paper by Morales and co-workers showed a protective effect of breastfeeding against wheezing and eczema up to 14 months of age. The authors found no indications of reverse causation. During the past couple decades, substantial attention has been focused on the possible effects of intake of antioxidant nutrients on development of allergy-related diseases. Antioxidants seem to protect host cells from damage by reactive oxygen species. Immunomodulatory factors – such as long-chain n-3 PUFA mainly from fish oil – also seem important in this context. Susan Prescott's group from Australia performed a double-blind randomized controlled trial to investigate the effect of fish oil given from birth until 6 months of age in 420 infants with high atopic risk [121]. This supplementation lowered allergen-specific Th 2 responses and elevated polyclonal Th 1 responses. Other studies on fish oil supplementation have so far been somewhat disappointing, because the protective effect against development of allergy-related diseases appears rather short-lived. Therefore, it will be of greatest interest to read future follow-ups when the end-points are disease-related. Low levels of selenium have been postulated to be a risk factor for the development of asthma [123]. However, Thompson and colleagues in a large birth cohort of New Zealand children found little evidence to support this link [124]. Fetal growth has been shown to be an important factor in the occurrence of asthma and atopy although the mechanism remains obscure. This area was reviewed by Tedner and colleagues [125].

Early-life exposure

During 2012, two publications of special interest in relation to exposure early in life emanated from studies of birth cohorts. The study by Herr and co-workers

from France investigated infants from birth to 18 months of age [126]. The drawback of this study is that only 48% of the study base was followed. One of the objectives was to assess wheezing severity in relation to environmental factors. No environmental factors were found to be associated with severity of wheeze. Instead, being sensitized to ≥ 2 inhalant or food allergens (≥ 0.35 kU/L) significantly increased the risk of severe wheeze. In the PASTURE project, headed by Pekkanen and von Mutius, exposure to microbial agents in house dust was studied in a birth cohort of more than 1000 infants in relation to wheeze, eczema and allergic sensitization at 2 years of age [127]. The infants were either children of farmers or control children. The amount of dust containing endotoxin and extracellular polysaccharides was inversely associated with asthma and wheezing, but it was not possible to disentangle the individual effects of these compounds due to high correlation between amount of dust and amount of microbial content. This finding is not to be interpreted to mean that early exposure to microbes has no protective effect against development of allergy-related diseases; rather, it highlights the need for new and better ways to measure exposure to environmental microbes.

Biomarkers

Exhaled nitric oxide (NO) is a well-validated marker of inflammation in the lower and upper airways. Increased exhaled NO levels have previously been reported in subjects without known respiratory symptoms. In an interesting paper, Malinovski et al. have followed 959 randomly selected adolescents [128]. Exhaled NO was measured at baseline, and 4 years later, a follow-up was performed. After exclusion of individuals with asthma, 657 participants remained. What the authors could show was that the higher the exhaled NO levels had been at baseline, the greater was the probability of new-onset rhinitis. This was particularly evident among subjects with allergic symptoms.

For many years, the Isle of Wight birth cohort study has provided us with a large number of interesting scientific papers on causality of allergic disease, disease development and the importance of allergic sensitization in the development of allergy-related diseases. The Isle of Wight cohort has been maintained in an excellent way, and even now, after the 18-year follow-up, 90% of the original cohort remains. Because loss to follow-up in this cohort is very limited, the authors are allowed to generalize their findings. In a paper among the 2012 publications, Roberts and co-workers reported a longitudinal study of allergic sensitization [129]. What they could show was that children who were sensitized at 4 years generally went on to become

sensitized to additional allergens in school age and up to 18 years. Polysensitization was more prevalent among the 18-year-olds than among the 4-year-olds. This changing pattern of allergic sensitization over time needs to be better characterized, and its relevance to clinical disease needs to be established. Most likely, future use of microarray assays to study IgE to allergen components in cohorts – like the Isle of Wight cohort – will provide us with useful data. Even though transition of sensitization to inhalant allergens is unlikely, development of tolerance to some foods is. In a paper by Ahrens et al., the development of tolerance was investigated in 52 children allergic to cow's milk (CM) [130]. These children had a median age of 22 months and were first rechallenged at a median age of 44 months. An initial low IgE to CM of ≤ 5 kU/L significantly correlated with an individual likelihood of outgrowing CM allergy. Testing for IgE to allergen components in CM did not provide any diagnostic benefit in this context. The lack of utility of component-resolved diagnosis in relation to CM allergy is likely due to CM lacking IgE that cross-react with other common allergens in our environment.

Anaphylaxis

During the past few years, severe reactions to food – including anaphylaxis – have come under ever closer scrutiny. Until recently, there had been few population-based studies on this topic, but in 2012, *Clinical & Experimental Allergy* published two such papers: one based on material from a paediatric population in Sweden and the other based on material without age restrictions and done in Spain [131, 132]. In the Swedish study, the incidence of anaphylaxis was 32 per 100 000 person years, and in the Spanish study, the corresponding incidence was 103 per 100 000 person years, with a peak among the age group 0–4 years. This difference in incidence of anaphylaxis is not easily explained, but milder cases of anaphylaxis may have been underestimated in the Swedish or overestimated in the Spanish study. Because the Spanish study also included adults, anaphylaxis to drugs and idiopathic reactions were more common than in the Swedish study. In the Swedish paediatric study, reactions to food were by the far most severe reactions (97%), often leading to a hospital visit. The types of food that elicited reaction differed in the two studies, and this reflected the difference in age. Fish and seafood were by far most common offending foods in the Spanish population (33.3%), whereas peanut, tree nuts, milk and egg were the most common offenders in the Swedish paediatric population. The implications of these studies were further discussed in an editorial by Simon et al. [133].

Quality of life

Impaired sleep affects quality of life, but also physical health. It is thought that allergic rhinitis can affect sleep through both mechanical and inflammatory mechanisms. In a Spanish case study of 2275 adult patients, half had persistent and half had intermittent rhinitis according to ARIA [134]. Treatment did not seem to affect sleep quality in a negative way. Interestingly, those with moderate-to-severe rhinitis (intermittent or persistent) significantly more often reported poor sleep quality and daytime sleepiness than those with mild rhinitis (intermittent or persistent). The interpretation of the results is somewhat hampered by the fact the reference group – those with mild symptoms – only constituted 12.4% of the total patient material.

Clinical allergy

Allergic diseases represent an ideal model for clinical, basic and translational research, due to the fact that the allergic reaction is known in sufficient detail, for historical and cultural reasons. It is also true that more detailed knowledge of the pathogenic mechanisms originates new questions and highlights further grey areas, thus stimulating new research. Clinical research in allergy has, more recently, focused the phenotyping of asthma (that is preliminary to a phenotype-oriented therapy), with the identification of some quite well-defined phenotypes. Another field of interest remains the characterization of allergens; this is in association with the new techniques of molecular allergy diagnosis. Another point of central relevance is the problem of 'severe asthma', which includes its definition, diagnostic criteria and treatment. Finally, there have been numerous developments in the treatment of allergic diseases, including the use of new pharmacologically synthesized drugs, the biological agents and the advancements in allergen-specific immunotherapy. This latter field is expanding also to food allergy, with the new achievements on desensitization to cow's milk and peanut.

Immunotherapy

Allergen-specific immunotherapy (SIT) is a quite distinctive feature of clinical allergy. SIT was empirically introduced more than one century ago, but major and significant developments were achieved only during the last two decades with the use of sublingual immunotherapy (SLIT), allergoids, adjuvants, recombinant allergens and new forms of administration (epicutaneous, intralymphatic) [135, 136]. In addition, desensitization for allergens different from the inhaled ones,

foods (e.g. cow's milk, peach or peanuts) or latex has been attempted [137, 138], with overall positive results. SIT is a biological response modifier that acts in redirecting the Th2 response and involving the activity of T regulatory cells and the production of IgG4 antibodies [139]. Moreover, there have been significant advances in the use of DNA-based SIT, already tested with a bacterial DNA-conjugated allergen. In a recent animal study, it was suggested that heat shock protein 65 DNA (from *Mycobacterium leprae*) can enhance the Th1 response, thus redirecting the response to allergens [140]. This reproduced a previous study in humans, with a ragweed (Amb a 1)-bacterial DNA-conjugated specific immunotherapy [141]. Klimek et al. reported the first study to use a combination of recombinant grass pollen allergens for subcutaneous immunotherapy. In a dose-ranging safety study, they found no adverse events [142]. A potential problem with immunotherapy is sensitizing people to allergens to which they were not previously sensitized. In a definitive study, Baron-Bodo and colleagues demonstrated that this did not occur with sublingual house dust mite immunotherapy [143].

Food allergy

A novel report from a EuroPrevail and UK Food Standards Agency discussed the important issue of risk in relation to food allergy [144]. It was agreed that it was impossible to remove risk of allergic reactions, but that more needed to be done to focus on the issue of risk by interrogating existing data sets to define a tolerable level of risk. In the same issue at the start of the year as these opinions, Tan et al. reviewed the literature on the factors responsible for the large increase in the prevalence of food allergy in recent decades in particular exploring whether epigenetics could provide an explanation [145]. Intramuscular adrenaline is a life-saving treatment in anaphylaxis, but autoinjectors are often not used appropriately. This was highlighted by a study from the United Kingdom by Noimark et al. who surveyed paediatric allergy clinics to ask about adrenaline autoinjector use [146]. They identified 466 patients with autoinjectors who had an episode of anaphylaxis, but only 16% had used their adrenaline pen thirteen of who had needed two doses. The reasons for the low rate of use were explored in the paper. Food challenge is an integral part of the diagnostic work-up of patients with suspected food allergy, but this is not without risk and as a result can be stressful particularly for children and their parents. Knibb et al. explored this issue and found that the procedure did induce significant amounts of stress particularly in the parents, but this was counterbalanced by an improvement in quality of life in the months following the procedure even in

those with a positive challenge [147]. Quality of life in food allergy is of increasing interest to the research community, and the various measures used to assess the quality of life were summarized by Flokstra de Bloc and Dubois [148]. Food protein-induced enterocolitis syndrome is an unusual, but distressing condition. Sopo and colleagues presented data on the clinical features and management of 66 children with this condition [149]. The extent to which the size of the weal or the amount of specific IgE can be used to predict the outcome of a challenge procedure is a popular topic of research. Mehi and colleagues reported on their experience with a large number of children with suspected cow's milk or egg allergy and found that the level of correspondence between specific IgE and skin prick test should be lower than they had anticipated and that neither was especially good at predicting the response to challenge [150]. In contrast, Ahrens et al. did find that the total specific IgE to cow's milk was useful in predicting the likely outcome in cow's milk allergy, but that measuring individual allergens added little new information [130]. The findings of this study were discussed in an interesting editorial by Jarvinen and Celestin [151].

Drug allergy

The prevalence of drug allergy in children is uncertain. Rubio used the large Drug Allergy and Hypersensitivity database consisting of 3275 cases to measure the prevalence of suspected a confirmed drug allergy in children and adults [152]. They found that the prevalence of confirmed drug allergy was lower in children than in adults, but only for maculopapular skin rashes in relation to beta-lactam antibiotics.

Atopic dermatitis and probiotics

Pre- and probiotics continue to be a popular subject for investigation particularly for the prevention of atopic dermatitis, but the findings of various studies remain contradictory [153]. For example, Wickens et al. [122] undertook a study involving intensive treatment with two different probiotics given prenatally to the mother and then to the infant for its first 2 years of life and showed a reduced incidence of eczema and rhinoconjunctivitis at 4 years. Ou and colleagues [154] found in a randomized controlled study that prenatal and postnatal probiotics reduced maternal, but not childhood allergic disease, and Gore et al. who also undertook a randomized control trial of probiotics on the incidence and severity of eczema at 3 years of age [155] found no benefit. The state-of-the-art clinical management of eczema was discussed in an excellent review by de Bruin-Weller et al. [156].

Allergens

Introduction

Allergens from natural sources, such as inhalants, foods and venoms, are the triggers of the allergic reaction when specific IgE directed towards their epitopes is present in human body. Although several additional factors are needed to lead a subject to have symptoms on the exposure to a certain allergen, allergens are the starting structures we need to identify, to know and to use to investigate in detail any IgE-mediated disease. The knowledge on single allergenic compounds, most of them being proteins, is still on the increase, and it does not seem to have reached a plateau after several decades of research. The trend in recent years is well documented in up-to-date Web-based resources (e.g. www.allergome.org), and *Clinical & Experimental Allergy* continues to contribute to the overall knowledge about molecular allergens and their uses publishing a number of highly selected papers. These include papers on allergen characterization, use in allergy diagnosis and patient profiling, the use for epitope mapping and studying underlying immune mechanisms and finally their use for immunotherapy and in animal models.

During the last decades, mostly during the last 20 years, we have seen an astonishing rise of interest on allergenic structure characterization, moving the focus of researchers first, and clinicians later on, from the good old allergenic extracts to allergens as isolated compounds causing allergy. Although allergenic extracts have not yet been abandoned and probably will accompany us for a long period to come in diagnosis and immunotherapy, allergens from natural sources, such as inhalants, foods and venoms, are definitely considered as the real triggers of the allergic reaction when specific IgE, directed towards their epitopes, is present in human and non-human body. To have IgE binding, an allergen is not the only factor required to develop a symptomatic allergic disease on the exposure to allergenic sources, but allergens *per se* are the structures we need to characterize and to investigate IgE-mediated diseases. The knowledge on single allergenic compounds, most of them being proteins, is still on the increase, and it does not seem to have reached a plateau after several decades of research. The Allergome database (www.allergome.org) well documents the trend in recent years. In the 8-year period, between 2005 and 2012, when Allergome started to show online statistics of entered data (<http://www.allergome.org/script/statistic.php>), of the 18 215 selected publications on all Internet-reachable scientific journals, papers increased 57% if dedicated to allergenic extracts (from 1241 to 1948), whereas papers dealing with allergenic molecules

recorded a + 38.5% (from 1201 to 1664), notwithstanding allergenic molecules are not available to everyone for research or any other purpose. The continuous rise of a never-ending interest in both areas of studies on allergenic preparations is well documented by the number of entries for allergenic extracts and for molecules in the same period, being the former 1039 in 2005 and 2265 at the end of 2012 and the latter increasing from 1175 to 2597 in the same time period. Published contributions to such increase is scattered within almost 50 allergy-related journals, but also in non-allergy ones. Selected and highly impacting papers are still published in top allergy journals.

In this review, we will report on how the *Clinical & Experimental Allergy* journal (CEA) and other journals continue to contribute to the overall knowledge around molecular allergens and their use, with a number of highly selected papers. CEA spans its contribution from newly discovered allergens, to the use of allergens in diagnosis and patients' profiling, to their use for epitope mapping, to study the underlying immune mechanisms and finally to their use for allergic disease immunotherapy and in animal models.

Newly discovered allergens

Blank and co-workers described the identification as bee venom allergens of two proteins known and classified as royal jelly proteins [157]. Royal jelly is considered a healthy food supplement, and cases have been reported royal jelly allergy by ingestion, but it was not known that the same proteins are also expressed in bee venom and act as allergens. Beside the very comprehensive work done by the research group in the effort to obtain the full panel of bee venom allergens, their published finding might configure a new clinical and immunological syndrome combining the beginning of an IgE production either by ingestion or by injection of the same molecule and possibly a clinical reactivity by one or both routes of exposure. As for all newly discovered allergens, these major royal jelly proteins deserve further diagnostic and epidemiological studies. Two additional allergens from the Asian wasp *Vespa magnifica* venom officially named Vesp ma 2 and 5 and belonging to the hyaluronidase and the antigen 5 groups, respectively, were described [158]. Two additional potential allergens, phospholipase A1 and melittin, are listed in the Allergome as retrieved from the Uniprot database by a dedicated Allergome *in silico* procedure, thus expanding our knowledge on the given species.

Allergens for allergy diagnosis and patients' profiling

New allergens very often go to expand a panel of known ones within the given source and the known

homologous group of proteins. In the need of profiling patients with such increasing number of molecules, researchers may use either a multitude of assay runs by means of the 'classical' singleplex systems (e.g. Immulite from Siemens or ImmunoCAP from ThermoFisher) or using the modern microarray technology [159]. Using the mite extract and available mite allergens on the singleplex and the multiplex testing systems, Bronnert and co-workers reported in CEA a study on 123 mite allergic subjects [160]. Their study led to a positive conclusion on the comparability of results obtained by any means, and of only a few allergens to be tested, the singleplexing approach can be used as a first-line option. Similarly, Baron-Bodo et al. evaluated, using all the above-mentioned singleplex and multiplex testing for IgE and IgG4, the variations of 509 mite allergic patients' profiles after sublingual immunotherapy (SLIT), using three mite allergens, Der p 1, 2 and 10 [143]. The study excluded neosensitizations after the SLIT course. Last, using another singleplex platform (Advia, Siemens), Barber and co-workers reported on 477 mite allergic patient IgE profiling using mite extracts, including also *Acarus siro*, *Blomia tropicalis*, *Glycyphagus domesticus*, *Lepidoglyphus destructor* and *Tyrophagus putrescentiae*, and a panel of natural and recombinant mite and shrimp allergens (Der p 1, Der f 1, Der p 2, Der f 2, Lep d 2, Blo t 5, Der p 10 and Pen m 1) [161]. Authors used the above approach to profile subjects recruited in 10 different sites of Spain and comparing their geographical findings. It is of note that the three studies mentioned above used a very limited number of allergens compared with those known and reported in the Allergome, for instance the 28 known for *Dermatophagoides farinae* and the 18 for *Dermatophagoides pteronyssinus*. Indeed, more comprehensive studies are needed including as much mite allergens as possible among those available and as nicely carried out by Barber et al., including not only those from house dust mites living in Western countries, to draw final conclusions on all the above aspects. In the view of the authors of this review, the multiplexing approach seems to be technically and economically unavoidable for such future extended studies.

Even more complex than the mite allergens are wheat allergens. Wheat as species (*Triticum aestivum*) plays a role in several allergic diseases, from releasing the grass pollen allergens to the role in food and occupational allergy, by ingestion or inhalation or skin contact with seed (flour) allergens. So far, 60 proteins have been described as allergenic, of which 20 are officially named. Pahr and co-workers reported the use of nine wheat pollen and seed allergens to obtain the IgE profile of patients with respiratory symptoms caused by wheat flour exposure or with pollen allergy [162]. To achieve their goal, they used the dot blot technique for

IgE detection and several other methods for patient and allergen characterization. Further, they explored the presence of homologous allergens in six different cereal species or wheat variety, concluding for the possible role of many cereals to cause symptoms at least in subjects sensitized to 1-Cys-peroxiredoxin, now named Tri a 32. This study on wheat allergens has been accompanied by the study by Takahashi et al. dealing with the identification of additional molecular markers for the wheat-dependent exercise-induced anaphylaxis syndrome (WDEIA), showing the IgE towards the high molecular weight glutenin (Tri a 26) being complementary to the omega-5-gliadin (Tri a 19) [163]. The findings of this paper were discussed in an accompanying editorial [164]. Similarly, but using an experimental microarray, Hofmann et al. showed other wheat gliadins to be relevant to the Tri a 19-negative subjects affected by WDEIA [165]. Overall, the early reports where Tri a 19 was reported to play a unique role in the FoodDEIA syndrome seem now to be challenged by these new findings, much more after those from Romano and co-workers who, by means of the ISAC 89 microarray version, identified IgE to the LTP as the main cause of FDEIA in an Italian cohort [166]. Results obtained by the microarray allergenic molecule-based diagnosis were not reachable before using just the skin testing or the extract-based singleplex testing used by the same group in the past.

Broadening the number of plant-derived LTP and including those from foods and pollen, Pascal and co-workers described the sensitization profiles of 45 patients by a combination of singleplex and multiplex diagnostic approaches [167]. Their study highlighted that a number of risk factors that, when associated with LTP sensitization, might lead to severe reactions. As LTP and thaumatin-like proteins (TLP) are indeed the major focus of Mediterranean research groups, studies have been reported in other journals on an in-depth characterization of broader panels of LTP and TLP from many different sources [168–170]. The use of microarrayed allergens, like in the single molecule *versus* multiple molecule IgE inhibition assay, has been adopted by the above authors to obtain a high number of cross-inhibition results using minimal amount of sera and inhibitors.

Studies on profiling patients with allergens from animal-derived foods have been published by *Clinical & Experimental Allergy* as well as by other journals. Well-established cow's milk and hen's egg allergens have been the main focus of research groups, in all cases trying to understand whether the use of allergenic molecules might be helpful in predicting severity of the reactions or tolerance, either naturally occurring or during oral immunotherapy courses. As previously noted, Ahrens and co-workers evaluated 52 paediatric

patients were affected by milk allergy, trying to find out a prognostic marker of their naturally developed tolerance [130]. Overall, they could identify some IgE level to the main milk allergens, which could help at that regard, but in the authors' opinion, the information collected in such way could not balance the increasing cost. In this regard, we would like to briefly mention the study from Alessandri et al. where a marker of clinical reactivity has been identified in subjects not tolerating a fully matured cow's milk-based cheese [171]. Authors used IgE detection on a molecule-based microarray for both patient's IgE profiling and for a large number of IgE inhibition assays run with dozens of extract preparations. In their studied population, having IgE against β -lactoglobulin (Bos d 5) was reported to be a risk marker of clinical reactivity for children fed with that cheese. The biochemical and immunological study nicely showed how the heat-unstable Bos d 5 is very resistant to the long-lasting enzymatic pre-digestion in the cheese and to the simulated human gut digestion. Still on the cow's milk story, Jarvinen and co-workers published an interesting paper showing that lactating babies have IgE against human milk proteins most of them belonging to the subset of those not gaining any improvement in their allergy by the mother cow's milk strict avoidance diet [172]. Authors cautiously concluded that there is a great need for further studies, but it is intriguing how the IgE autoreactivity story has been reported also at this very young age.

In parallel with the milk allergen studies, Alessandri and co-workers investigated markers for hen's egg reactivity using the ISAC microarray for diagnosing the IgE reactivity to the egg allergens and for having the full allergy profile of their patients [173]. The ovomucoid (Gal d 1) was found to be a risk-associated allergen. In fact, having IgE against ovomucoid caused paediatric patients to react also to boiled egg, making the hypothesis of using it for oral immunotherapy not realistically achievable. Gal d 1-positive subjects were also found to be at risk of developing additional sensitization to allergens from inhalants.

Allergens for epitope mapping and to study underlying immune mechanisms

Two papers were published in *Clinical & Experimental Allergy* dealing with the immune response to grass pollen allergens. The first of the two by Bonvalet and co-workers [42] evaluated the specific response to recombinant Phl p 1 and 5 as a result of sublingual immunotherapy and the second by Roschmann and co-workers [43] evaluating mediator release and airway epithelial cells gene expression using either grass pollen extract or the recombinant Phl p 1 [174, 175].

As a result of the comparison, authors of the second paper conclude that more than one component in the grass pollen extract is important for the immune response, and point to the possible role of either interaction of non-allergenic components with the immune system or even the allergen-to-allergen interaction in the material released in biological fluids on pollen exposure.

B and T epitope mapping using allergenic molecules has been reported in several studies to achieve different aims like in that from Ayuso and co-workers where authors searched for epitope specificities predicting clinical reactivity to shrimps [176]. Four different molecules obtained from *Litopenaeus vannamei* and named Lit v 1, 2, 3 and 4, being a tropomyosin, an arginine kinase, a myosin light chain and a sarcoplasmic calcium-binding protein, respectively, were used. Authors who performed their studies with microarrayed compounds tested not only the intact molecules, but also a battery of 15-mer peptides overlapping 12 amino acids from the four allergens. Main findings lead authors to identify epitopes on studied allergens, which seem to mark the clinical reactivity of allergic patients. Nevertheless, they recall the need for a larger sample size to draw final useful conclusions.

In line with the above study, conclusion is the one from Willumsen and co-workers. The Danish research group, using a well-established Der p 2-based model, described the complexity of epitope IgE recognition as the key point to understand the IgE immune response in allergic diseases not only in a cross-sectional view, but also in the disease progress as described by the allergy march [177]. The strategy used by the authors was to complement Der p 2 allergic sera with a panel of in-house-produced recombinant antibodies specific for different Der p 2 epitopes and having different affinities for the epitopes themselves. The method used is an assay, which evaluated the ability to form complexes between IgE, Der p 2 and CD23 expressed on the surface of CD23⁺ B cells, enabling to assess differences (clonality, affinity, concentration) in the IgE repertoires of Der p 2-allergic individuals.

A contribution to a further understanding of IgE production comes from Eckl-Dorna and co-workers [178]. Using seven recombinant allergenic molecules, Bet v 1, Phl p 1, Phl p 2, Phl p 5, Der p 2, Ole e 1 and Fel d 1, they set an ex vivo model to investigate IgE production in allergic subjects. At the end of their comprehensive approach to the issue, authors concluded that only a minor part of IgE is produced in the peripheral blood by CD138-positive plasma cells. Willing to consider new therapeutic strategies targeting allergen-specific IgE production, there is a need to focus on other sites of IgE production, such as local tissues or the bone marrow.

In the aim to describe cellular mechanisms controlling allergic response, Goel and co-workers chose Per a 10 as a model allergen [179]. Per a 10 is a relatively new cockroach allergen with protease activity that was shown by the authors to bias Th2 responses by up-regulating CD86 and reducing IL-12 production.

Allergens for immunotherapy

It is now more than 20 years that many clinical research groups try to understand whether allergenic extracts could be replaced by allergenic molecules to obtain clinical meaningful results with immunotherapy. *Clinical & Experimental Allergy* reported that the study of Klimek and co-workers where a subcutaneous immunotherapy trial with four grass pollen allergens, namely Phl p 1, 2, 5 (two isoforms) and 6, was performed as the first randomized dose-ranging safety study [142]. Although the primary end-points of the study were safety-related, recording just grade I and II adverse events leading to positive conclusions on safety concerns using the grass allergen cocktail, the secondary end-point was clinical efficacy measured by conjunctival provocation test. Authors reported that a higher dose of allergens was needed in 3 of 4 treated patients to elicit a conjunctival reaction. This result was significantly different from the lack of tolerance improvement recorded in the placebo group.

In the aim of searching for new compounds to be safely and effectively used in immunotherapy, a study from Twaroch and co-workers focused on Alt a 1, the first known allergen from *Alternaria alternata* fungus and the most relevant in terms of prevalence in the allergic population [180]. Sera from eighty Austrian and Italian *Alternaria* allergic subjects were used to show the full biological activity of the wild recombinant Alt a 1 done by measuring its IgE binding and its activity in the basophil activation test. Producing by synthesis four long peptides (32–39 amino acids) spanning the full Alt a 1 length, authors showed the lack of IgE binding, and, coupling the same peptides to KLH, obtained blocking IgG when a rabbit was immunized. This study certainly increases the number of tools available for future immunotherapy against respiratory allergic diseases, mostly because it is the first attempt considering a fungal allergen.

In line with the previous study, Bonura and co-workers [181] using their expertise with the *Parietaria* pollen major allergenic molecule models generated a recombinant Par j 1/Par j 2 mutant hybrid molecule to be used in immunotherapy [181]. Assays performed using ten human sera showed a marked reduction in both IgE binding and the basophil activation, whereas the hybrid induced an identical proliferation of CD3+ cells in all the patients as the wild-type Par j 1 and 2

molecules used as controls. Antibodies obtained by immunizing mice with the new hybrid molecule were capable competing for the human IgE epitopes. As for the Alt a 1 study, this paper adds a potential new tool for a safer future immunotherapy.

Allergens for animal models

The use of allergenic molecules in the development of animal models of allergy is not new as the earliest reports are dated back to the forties when horse and hen's egg albumins were first used for the purpose. It is noteworthy that the increase in the allergen number, as reported in the introduction, has not been followed by a parallel increase in allergen-addressed animal models. Up to now, a food allergen, ovalbumin (Gal d 2), is used for whatsoever animal model, regardless of whether the general immune response is under investigation or any respiratory, skin, eye, gut allergic disease is to be simulated. As from the Allergome entries, ovalbumin accounts nowadays 4244 published papers where the molecule has been used for any purpose. This is a huge number if compared to the other most used allergens such as Der p 1 ($n = 89$) or Bet v 1 ($n = 75$). Starting from the latest scientific evidences, as reported above in the section dedicated to newly discovered allergens, we now know that different allergens bear different capability to cause allergic diseases of different kinds and severity. Publishing studies addressing such aspects should be thus highly encouraged. It is noteworthy that *Clinical & Experimental Allergy* published studies by Krishnamurthy and co-authors on a milk casein (Bos d 8), one from Starkl and co-workers on Ara h 2 from peanuts, both addressing the need to create a food anaphylaxis model, and the one from Baranyi and co-workers where Phl p 5, a respiratory grass pollen allergen, was used as a model for a complex mouse immune system manipulation for future immunotherapy interventions [182–184]. Nevertheless, the journal published ten ovalbumin-based animal model studies in 2012 [140,185–193]. This compared with 526 published in the International scientific literature last year including 63 in the other four major allergy journals.

Reviews on allergens and conclusions

Two important reviews in the allergen field appeared in the journal during the 2012. Prof. Reto Crameri overviewed the topic of the role of specific IgE directed towards epitopes present on human proteins [194]. It is our personal opinion that this aspect of allergen biology is much overlooked. This conflicts with the high number of human allergenic proteins reported in the scientific literature (62 Allergome entries), with a

recent increase caused by the use of biologicals, including enzyme replacement therapies in genetically determined diseases. Cramer's review is thus very welcome as it invites us to break the barriers of classical allergology and look beyond the concept of allergens as foreign compounds from the human body. Second, linked to the need to understand more about the environment-to-human self-allergen boundaries is the review from Virtanen *et al.* [195] where authors make an overview of lipocalins as a model of mammalian allergens, concluding that such proteins exhibiting sequence identities of 40–60% over species might be immunologically at the borderline of self and non-self. This has to be considered when investigations on the allergic response of immune system are carried out considering allergen-driven or non-allergen-driven models.

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Conflict of interests

AM is the creator of the Allergome and the CEO and CSO of Allergy Data Laboratories s.c. MAC declares no conflict of interest.

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