

SPECIAL ARTICLE

Developments in the field of allergy in 2008 through the eyes of *Clinical & Experimental Allergy*

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Summary

In 2008, many thousands of articles were published on the subject of allergic disease with over 200 reviews, editorials and original papers in *Clinical & Experimental Allergy* alone. These represent a considerable amount of data and even the most avid reader could only hope to assimilate a small fraction of this knowledge. There is therefore a pressing need for the key messages that emerge from a journal such as *Clinical & Experimental Allergy* to be summarized by experts in the field in a form that highlights the significance of the developments and sets them in the context of important findings in the field published in other journals. This also has the advantage of making connections between new data in conditions such as asthma, where articles often appear in different sections of the journal. As can be seen from this review, the body of work is diverse both in terms of the disease of interest and the discipline that has been used to investigate it. However, taken as a whole, we hope that the reader will gain a flavour of where the field is mature, where there remain controversies and where the cutting edge is leading.

Asthma

Stress may worsen asthma, but the mechanisms linking stress to asthma are not well understood. Joachim et al. [1] reported that the percentage of TNF α -producing T cells was increased in the peripheral blood of asthmatic subjects compared with healthy controls. Stress perception in asthma correlated with percentages of TNF α -producing T cells and serum brain-derived neurotrophic factor (BDNF) levels, while forced expiratory volume in 1 s (FEV₁) (% predicted) was negatively correlated to BDNF. This suggests that stress might destabilize asthma by inducing a pro-inflammatory cytokine profile. If true, stress management might provide a supplement therapy for asthma.

Numerous genes have been linked to asthma, although many associations have not been replicated in further cohorts. Several further gene polymorphism associations have been reported recently in single cohorts of asthmatics including the thromboxane A₂ receptor, TNF α , IL-13, cyclooxygenase 2, CASP10 and angiotensin-converting enzyme [2–6]. In a screening and validation asthma cohort,

a polymorphism in the NPPA gene coding for an atrial natriuretic peptide was found to associate with disease [7].

Ion channels are attractive targets for the modulation of airway inflammatory and structural cell function in asthma. Agrawal et al. [8] showed that the 'anti-allergic' drug suplatast increases Cl-channel activity in human eosinophils. However, opening of these channels have been implicated in the enhancement of many cell functions such as migration, and so whether this is relevant to the anti-inflammatory activity of suplatast is unclear. The transient receptor potential (TRP) family of cation channels is likely to be important for many airway processes. TRPC6^{-/-} mice exhibited reduced eosinophilic airway inflammation in a mouse asthma model, but increased airway hyperresponsiveness, perhaps due to compensatory up-regulation of TRPC3 [9].

Infiltration of the airway smooth muscle (ASM) bundles by activated mast cells is a key phenotypic feature of asthma and likely to be central to the pathogenesis [10, 11]. Several mediators have significant effects on ASM contractility and proliferation (reviewed in [12],

and co-culture of human lung mast cells with human ASM cells leads to enhanced mast cell mediator release [13] and enhanced ASM contractility [14]. Interestingly, mast cell tryptase also leads to β_2 -adrenoceptor desensitization via protease-activated receptor 2, providing a potential mechanism for poor bronchodilator reversibility in some asthmatic subjects [15]. The ability of leukotriene D₄ to increase epithelial TGF- β_1 secretion, which in turn induces ASM proliferation, has been proposed as a mechanism behind ASM hyperplasia in asthma [16]. However, several studies have failed to find evidence of ASM proliferation *in situ* in asthmatic airways, suggesting other mechanisms such as recruitment of ASM progenitors might be more important.

The Th2 hypothesis is still favoured by many as an explanation for the immunopathology of asthma, although this is probably a great oversimplification of an extremely complicated and heterogeneous disease [17, 18]. Several further studies in ovalbumin (OVA) mouse models of asthma have shown that diverse factors can skew the immune response from Th2 to Th1 and attenuate the pathological changes, including vaccination with a live strain of *Francisella tularensis* [19], inhibition of poly(ADP-ribose) polymerase by thieno[2,3-*c*]isoquinolin-5-one (TIQ-A) [20] and co-administration of LPS [21].

Viral infection, particularly with rhinovirus, plays an important role in the development of asthma exacerbations. Viruses including RSV have also been proposed to promote the development of asthma. In support of this, in a mouse model, RSV infection primed the lung of mice that were not previously systemically sensitized to develop airway remodelling in response to allergen upon sole exposure via the airways [22].

The increased susceptibility of asthmatics to rhinovirus infection has recently been related to deficient IFN- λ 1 (IL-29) and IFN- λ 2/3 (IL-28) production by bronchial epithelial cells and macrophages [23]. However, when looking at induced sputum, it was found that asthmatic adults have increased sputum IL-28 mRNA but similar IL-29 mRNA expression when compared with healthy subjects. Sputum IL-29 mRNA (but not IL-28) correlated negatively with asthma symptoms in steroid-naïve patients and was significantly higher in steroid-treated than in steroid-naïve patients. These results suggest that asthmatic subjects have substantial type III IFN- λ mRNA levels in the airways but does not exclude the possibility of a deficient response to rhinoviral infection.

Genistein is a non-selective tyrosine kinase inhibitor present in soy. It was able to inhibit the release of LTC₄ by human eosinophils and blocked the activity of p38 MAP kinase [24]. After dietary supplementation, eosinophil LTC₄ production was also reduced. Clinical studies of soy isoflavone supplementation in asthma are warranted.

An indirect bronchial challenge with mannitol and AMP more closely reflects the presence of airway inflam-

mation than a direct challenge with methacholine. However, in steroid-naïve patients, although the severity of mannitol reactivity correlated better than methacholine with percentage sputum eosinophils, in patients with sputum eosinophils > 1%, positive responses to methacholine were more commonly seen [25].

The role of air pollution in the causation and propagation of asthma remains controversial. Nevertheless, in a mouse model, prior exposure to SO₂ as well as OVA aggravated the airway eosinophilia and subepithelial fibrosis [26].

Obesity is a risk factor for asthma and defines a distinct clinical phenotype [27]. Garcia-Marcos and colleagues identified obesity as a risk factor for non-allergic asthma in schoolchildren [28, 29]. Using factor analysis, Pillai and colleagues identified five features that appear to represent important aspects of the disease. These were FEV₁, specific allergen sensitization, self-reported allergies, symptoms of rhinitis and symptoms of asthma. The authors suggest that these may be useful for identifying disease phenotypes in future epidemiological and genetic analyses.

Occupational asthma is a significant problem, and a unique naturally occurring model for understanding asthma pathogenesis. Methylene diphenyl diisocyanate (MDI), widely used in many industries, is a well-recognized inducer of asthma, but the immune-mediated pathways believed to be central to the disease have been difficult to define. Further work has identified the combination of lower respiratory tract symptoms and MDI-albumin conjugate-specific IgG as a strong predictor of MDI asthma [30]. Baker's asthma is another common cause of occupational asthma. In a mouse model, bakery flour dust was strongly pro-inflammatory (neutrophil infiltration) in an IgE-independent manner, but also enhanced OVA-dependent airway inflammation [31]. However, in patients with wheat food allergy and IgE sensitization, inhalation of flour did induce bronchoconstriction [32]. In a mouse model of toluene-2,4-diisocyanate (TDI) asthma, the anti-angiogenic peptide sequence Asp-Tyr-Leu-Lys (DYLK) attenuated remodelling and inflammatory changes in the airways [33].

Inhaled corticosteroids are very safe, but nevertheless there is some systemic absorption, and there have been concerns about systemic side-effects at high doses. A meta-analysis found a small but significant 12% increase in the risk of non-vertebral fracture for every 1000 mg/day increase in the dose of beclomethasone dipropionate or equivalent [34].

The association between house dust endotoxin and asthma is unclear. Gehring et al. [35] found that endotoxin levels in living room floor dust were inversely associated with both a history of asthma and current wheeze in children, suggesting a protective effect. There is still uncertainty as to whether helminthic infections protect

against asthma and allergy. In Bangladesh, the rates of infection with *Ascaris* were similar between current wheezers and never wheezers, but *Ascaris*-specific IgE was significantly elevated in the serum of current wheezers [36]. Concentrations of *Ascaris*-specific IgE were relatively low in both groups compared with the total IgE concentrations, which were also higher in current wheezers compared with never wheezers. Whether the *Ascaris*-specific IgE is biologically relevant or simply reflects an increased propensity to generate IgE in current wheezers is not known.

Antibiotics have been proposed as a risk factor for asthma. However, Wickens and colleagues' birth cohort study suggested that the effect of antibiotics on respiratory disease may be due to confounding by chest infections at an early age when asthma may be indistinguishable from infection [37]. The role of diet in asthma is also controversial. A cross-sectional study in children suggested that intake of n-3 and n-6 polyunsaturated fatty acids may be associated with an increased prevalence of wheeze [38].

Smoking damages the airways and also attenuates the efficacy of inhaled corticosteroids. Perhaps not surprisingly, the airway mucosa of young smoking steroid-naïve asthmatics demonstrated squamous cell metaplasia, and increased expression of subepithelial neutrophil elastase and IFN- γ and intra-epithelial IL-8 [39].

Anti-IgE therapy is effective for some patients with asthma. In a murine model, B cell-targeted therapy was significantly more effective than anti-IgE at attenuating airway inflammation and hyperresponsiveness [40]. In another mouse asthma model, the selective phosphodiesterase-4 inhibitor roflumilast was less efficacious at inhibiting cytokine expression than dexamethasone [41].

Two studies suggested that there is a small but significantly increased risk of asthma following a Caesarean section [42, 43], but this does not seem to be sufficient to account for the increase in asthma prevalence seen in recent years.

Rhinitis

The publication of detailed British Society for Allergy and Clinical Immunology (BSACI) guidelines setting best clinical practice for rhinitis and chronic rhinosinusitis was an important achievement in 2008 [44, 45]. The importance of the continued dissemination of guidelines was highlighted in relation to the ARIA document, where a significant percentage of clinicians failed to refer to such guidance [46]. Several articles also provided important epidemiological data on allergic rhinitis (AR), for example, demonstrating the frequent coexistence with asthma in children [47]. The relationship of obesity with severe asthma but not rhinitis indicates that factors other than airway inflammation contribute to asthma phenotypes [28]. Alcohol consumption in women was associated with an increased risk of perennial allergic rhinitis (PAR) but

not seasonal allergic rhinitis [48]. This adds to the current debate on the relationship between alcohol and airway symptoms [49]. The PAR group may represent a significant proportion of non-AR individuals, but nevertheless this work lends direction towards development of future risk-modification strategies.

Several articles provided insights into disease mechanisms. The demonstration of up-regulation of the transcription factor NF- κ B in nasal polyps while AP-1 levels remain unchanged compared with normal tissue [50] highlights the concept of disease-specific immune signatures in nasal polyposis. The mechanisms that underlie aspirin exacerbation of rhinosinusitis are important and work into aspirin-sensitive asthma may offer insights. In addition to the paper by Shi et al. [5], which demonstrated that asthma severity may be related to particular haplotypes in the COX2 gene promoter, the key role that 15-lipoxygenase plays in the generation of 15-HETE in response to aspirin exposure in an *in vitro* system was investigated [51]. Interestingly, activation of only the prostaglandin (PG) EP2 and EP3 receptors strikingly inhibited aspirin-induced 15-HETE generation, highlighting the important role that PG signalling plays in modulating the balance of arachidonic acid metabolism. Such studies highlight important target-specific therapeutic areas to develop as clinical intervention studies. The mechanisms that lead to viral exacerbation of rhinitis and asthma remain uncertain. It was demonstrated in an *in vitro* rhinovirus infection model, using the BEAS-2B epithelial cell line and PBMCs from atopic asthmatics, that a Th2-skewed inflammatory environment can attenuate the epithelial inflammatory response to virus infection with associated increased viral replication [52]. The demonstration that rhinovirus infection preceding or concurrent with house dust mite (HDM) Der p 1 exposure in a similar cell system is associated with synergistic IL-8 release [53] adds to our understanding of the mechanisms of virus exacerbation of airway inflammation and neutrophilic inflammation. The implication is that the allergic upper airway response to viral infection is similarly impaired and, together with synergism with allergen exposure, explains the severity of viral exacerbation of disease observed in patients with allergic rhinosinusitis.

In cedar pollen-induced rhinitis, it was shown that preventing IgE binding to its high-affinity receptor and thus preventing the activation of the Th2 inflammatory cascade with anti-IgE therapy (omalizumab) is associated with greater clinical efficacy than using an agent that inhibits selective Th2 inflammatory effects (suplatast tosilate). It is further proof of the concept that blocking the initiation of IgE cross-linking-mediated signalling cascade in airway inflammation leads to improved clinical outcomes [54], i.e. the earlier the intervention occurs in the inflammatory cascade, the more likely it is to be effective. H₁ receptor blockade is a universal strategy to

attenuate symptoms of rhinitis but has little effect on obstruction compared with intranasal (IN) corticosteroids. H_3 receptor stimulation leads to inhibition of histamine synthesis and release and possibly improvement in nasal airway patency. The demonstration of H_3 receptor expression in nasal mucosa neuronal and glandular structures in symptomatic AR and a functional role in mucus secretion suggests that an alternate histamine receptor modulation therapeutic approach in AR should now be explored [55]. The duration of immune tolerance and the mechanisms that sustain such changes are important. The demonstration that specific immunotherapy (SIT)-induced allergen-specific blocking IgG can alter IgE-allergen binding, basophil histamine release and facilitated antigen presentation suggests that induction of such a blocking antibody can be used as a successful marker of a successful SIT response [56]. It was also demonstrated that the clinical improvements 3–5 years post-SCIT observed in clinical practice can be associated with persistently attenuated Th2 inflammation as evidenced by a reduction in nasal eosinophils along with cells expressing eotaxin and RANTES in season [57]. It is important to evaluate the expression of IL-10, IL-12 and TGF- β_1 in these patients to further define the mechanism of sustained tolerance. The persistence of memory (m) T cells is fundamental to future allergen responses but the exact life span and mechanisms of such persistence remain unknown. An interesting demonstration that mT cell number persistence in dust-mite-sensitive individuals is static, but cedar pollen-specific mT cell numbers closely followed the pollen pattern, suggests that it is continuous antigen exposure that drives memory-clone size persistence [58].

There is increasing recognition that mucosal sites confer superior efficacy for the induction of immunological tolerance. For individuals with multiple sensitizations, polyvalent vaccine delivery via the mucosa may therefore be particularly relevant. An interesting report is the demonstration in a mouse model of allergen challenge that the delivery of a polyvalent vaccine (to birch and timothy grass) via the nasal mucosa confers greater induction of tolerance than delivery through the systemic route [59]. The development of recombinant hypoallergenic immunotherapy vaccines may reduce the risk of IgE-mediated side-effects, particularly anaphylaxis. One concern, however, is of the reduced clinical efficacy of such an approach. This was demonstrated for recombinant Bet v 1 derivatives in volunteers with pollen-induced allergy [60]. Strategies that can enhance vaccine efficacy are therefore required. Toll-like receptor (TLR) ligands can induce regulatory dendritic cell (DC) populations, leading to subsequent induction of T regulatory cells, particularly in mucosal locations. One strategy would be to develop recombinant hypoallergenic vaccines incorporated with TLR-2 agonists. Lombardi et al. [61] provide an encouraging study showing how specific TLR-2 agonists may serve to enhance the immunomodula-

tory effects of SLIT using both an *in vitro* approach and a mouse model of OVA allergen challenge. The study provides encouraging data showing enhanced induction of immunological markers of tolerance.

Mechanisms

Questions regarding the role of microbes in allergic disease continue to perplex and challenge the scientific community. Studies on how gut commensals may impact the development of allergic disease in early life remain in the spotlight. Several articles in the Journal reported on the immunomodulatory effects associated with the use of probiotics as a primary intervention; however, the results are difficult to reconcile. From the T cell perspective, one study reported a decrease in cytokines induced *in vitro* by allergen in cultures from infants aged 6 months who had received *Lactobacillus acidophilus* from birth [62]. These changes were also associated with increased expression of regulatory cell markers and TGF- β . Similarly, increased IFN- γ was observed in cord blood plasma from women who received supplementation with different bacterial species a few weeks before delivery [63]. Conversely, other studies reported no change in lactobacillus GG (LGG)-stimulated cytokine release from cord blood cells obtained from women who received LGG during pregnancy [64, 65]. Conflicting results were also obtained for other immune parameters. For example, while no association was reported between enhanced IgA production and treatment at 6 months of age by Martino and colleagues, other studies reported higher plasma total IgA in treated infants of this age, or else increased IgA in the breast milk of mothers who had received supplementation [62, 63, 66]. However, increased levels of 'pro-inflammatory' markers such as total IgE and C-reactive protein were also reported in association with increased IgA [66]. In relation to the effect on allergic outcomes, there were two conflicting reports on the risk for developing allergic disease [62, 66]. When considered collectively, these discrepant results may be explained by a variety of factors including the different treatment regimens and experimental systems used to evaluate immune parameters. These studies, coupled with reports of clinical improvement when probiotic bacteria are used as a secondary preventive strategy [67], indicate that further research in this area is warranted.

If we consider the influence of microbial exposures on the development of allergic disease, the assumption has been that early 'favourable' exposures, such as high-dose exposure to environmental bacteria or colonization with specific bacterial species at mucosal sites, can confer protection. The mechanisms involved are highly complex and relate to the type of microbial exposure and elements of the host that cooperate to induce protective responses. The general consensus is that these types of responses likely involve Th1 or regulatory T cell components.

A variety of studies in the Journal reported on immune mechanisms that may contribute to the protective or the non-protective role of microbes in the development of allergic disease. Two studies specifically alluded to decreased anti-bacterial responses in early life that may be attributed to the allergic status of the mother or else preferential exposure to Gram-negative bacteria in the environment [68, 69]. Consistent with a protective role for innate responses in early life, different TLR agonists were reported to attenuate the development of allergic disease or else enhance tolerance induction in mouse models of asthma [21, 61].

The epithelium is at the interface between the environment and the host. As such, it provides a conduit for bridging the innate and adaptive immune responses. However, it is clear that the epithelium contributes to the pathogenesis of allergic disease through complex pathways, many of which still remain to be elucidated. Several reports in the Journal provided new insight into diverse pathways mediated by airway epithelial cells. A role for epithelial cells in bronchial smooth muscle cell hyperplasia was reported through a leukotriene D₄-induced pathway [70]. Modulation of mucus production via a 'decoy receptor' (IL-13R α 2) and induction of goblet cell hyperplasia via the epidermal growth factor receptor were also reported to be mediated by the epithelium [71, 72]. Other studies focused on its regulatory role in tissue remodelling through effects on induction of pro-fibrotic gene expression in lung fibroblasts, inhibition of fibroblast proliferation and abnormal repair processes [73–75].

The epithelium continues to be a focus for studying how viral infections may exacerbate the asthmatic response. Some have suggested that the atopic airway has increased susceptibility to infection with rhinovirus (RV). In keeping with this, one study reported that an atopic environment predisposed to increased viral proliferation and augmented cell damage, which was associated with decreased anti-viral cytokines such as IL-6, IL-8 and RANTES [52]. By contrast, a different study suggested that concomitant exposure to allergen synergized in inducing IL-8 secretion from bronchial epithelial cells [53]. Each of these studies, which used the same cell line (BEAS-2B), highlights how studying the epithelium in the context of atopic status, along with *both* microbes and allergens, will be important for delineating the nature of altered anti-viral responses in the asthmatic lung.

Many recent studies on eczema have focused on how defects in the skin barrier may contribute to onset of the 'atopic march'. However, several reports in the Journal shifted attention to the role of the nervous system in this disease. The association between elevated maternal nerve growth factor (NGF) levels and atopic dermatitis among a Taiwanese birth cohort points to a role for neuro-mediators in this disease [76]. Consistent with this viewpoint, increased levels of transcription of the low-affinity pan-

neurotrophin receptor, p75NTR, in lesional mast cells suggested that increased responsiveness of mast cells to neurotrophins could contribute to inflammatory responses in the skin [77]. Similarly, neurotrophin receptor expression and activity was the highest in peripheral blood eosinophils from eczema patients, as compared with patients with AR or non-atopic controls [78]. Dendritic cells are recognized as pivotal players in the initiation of eczema owing to their close proximity to the host/environment interface, and their capacity to induce Th2 responses. Interestingly, Noga and colleagues reported that these cells produce both NGF and BDNF. Moreover, the production of BDNF was enhanced by LPS or allergen [79]. How this may relate to atopic status or induction of Th2 responses remains to be answered. Nevertheless, these findings point to important neuroimmune interactions that may not only be important for initiation of atopic dermatitis, but could also be relevant to the subsequent chronic inflammatory cascade.

Mouse models continue to be developed that could provide new insights into the immune mechanisms that underlie allergic disease. A detailed description of these is beyond the scope of this article. However, these models encompassed a variety of conditions beyond allergic asthma including food allergy, oral allergy syndrome, baker's asthma and models of cedar pollen dermatitis and 'intrinsic' (IgE-independent) atopic dermatitis [31, 80–83]. Mouse models were used to test more unconventional fields of study including sex-related differences in immune cell function and the effects of dietary unsaturated fats on airway inflammation [84, 85]. The trend is to develop much-needed mouse models that more closely mimic the human condition, by using relevant antigens and modes of antigen administration designed to recapitulate immune events in humans.

Mouse models continue to provide an important approach for testing potential therapeutics, either used to vaccinate against allergic disease or to treat once sensitization has been induced. Novel studies reported in the Journal included reports on the suppressive effects of glutamine on the development of asthma and on the effects of a fermented soy product on established peanut hypersensitivity [86, 87]. In relation to T cell-targeted therapies, two reports described the protective effects of vaccinating with allergen-derived immunodominant peptides via the IN route, with one study finding that IN administration was more effective than the systemic route [59, 88]. By contrast, a separate study reported that inhibiting B cells by administering a soluble receptor for B cell survival factors after allergen sensitization, but before airway challenge, prevented the development of airway hyperresponsiveness. Notably, this was more effective than anti-IgE mAb [40, 89].

Suppressing Th2 responses that drive allergic inflammation remains a cornerstone of therapy. Consistent with this, Pereira-Santos et al. [89] reported expansion of cells

with a phenotype consistent with regulatory T cells, during venom immunotherapy. Because there remains no reliable regulatory T cell marker in humans, in the future, it will be important to identify alternate markers of protective T cell responses if we are to determine whether new treatments for allergic disease could be beneficial. With this in mind, one study reported up-regulation of the inhibitory molecule, B and T lymphocyte attenuator, following allergen-specific immunotherapy. This was associated with decreased IL-5 production after allergen stimulation [90]. In addition to reports of T cell suppression, one study added to the evidence of a role for diminished IgE-facilitated binding of allergen to antigen-presenting cells (FAP) during immunotherapy [56]. While the previous studies focused on the protective effects of allergen immunotherapy, studies in a mouse model reported impaired allergen-specific immunotherapy and augmentation of IgE responses and IL-4 secretion by T cells during allergen sensitization in mice who received anti-histamines [91]. This report raises important questions regarding the use of anti-histamines in patients receiving immunotherapy. Lastly Bateman et al. [92] report the identification of an immunodominant epitope for the T cell recognition of Fel d 1. This type of study is essential for the development of peptide immunotherapy.

Epidemiology

A hot topic in the international literature in 2008 has been the effect of early exposure to antibiotics and antipyretics on subsequent childhood asthma and allergic disease. A cause-effect relation cannot be assessed easily, because respiratory tract infections, associated with increased risk of childhood asthma, are often treated with antibiotics and antipyretics. Articles published in *Clinical & Experimental Allergy* in 2008 have reported on findings and helped in unravelling the associations.

As discussed above, Wickens et al. [37] observed, in a birth cohort from New Zealand, significant associations between antibiotic exposure before 3 months and asthma development up to 15 months [OR 2.32 (95% CI 1.45–3.69)]. With adjustment for chest infections, however, this association decreased to near significance, indicating that the effect of antibiotics on asthma may be due to confounding by chest infection at an early stage. Likewise, Kusel et al. [93] discovered, in a carefully monitored Australian birth cohort, that 54% of children received at least one course of antibiotics in their first year of life and that antibiotic use was associated with asthma [OR 2.3 (95% CI 1.2–4.5)], but this association was reduced after propensity score adjustment. Reports from the ISAAC study indicated increased risk of childhood asthma in children treated with paracetamol [94], although the cross-sectional design does not allow for assessment of the cause-effect relation. Shaheen et al. [95] have pre-

viously proposed that paracetamol exposure in late gestation may cause asthma in the offspring, but the question remains as to whether antibiotic intake actually precedes symptoms of asthma, or are prescribed in response to symptoms of wheezy bronchitis in infants. Reverse causation and confounding-by-indication are thoroughly explained and discussed in an editorial, and call for a more hypothesis-driven research [96]. Perturbations in the gastrointestinal microflora due to antibiotic use in 'industrialized' countries may disrupt the normal microbiota-mediated mechanisms of immunological tolerance in the gastrointestinal mucosa [97] and may be an interesting field of research.

Possibly altered early development of intestinal flora as an explanation for the association between birth by Caesarean section and risk of subsequent asthma in the child is discussed in an editorial [98] and two meta-analyses [42, 43]. Thavagnanam and colleagues found a 20% increase in the subsequent risk of asthma in children who had been delivered by a Caesarean section, OR 1.20 (95% CI 1.14–12.6), and Bager and colleagues observed an increased risk of asthma food allergy, AR and hospitalization for asthma but not for inhalant atopy or atopic dermatitis. There are many explanations for the associations and several potential confounders such as prematurity, intra-uterine growth retardation and maternal smoking, and although it is unlikely that the use of Caesarean section in the last decades could explain the world-wide increase in the prevalence of asthma, the findings are very interesting and call for further studies on the mechanisms.

Early exposures to dogs and endotoxin are discussed in some detail in a few papers published in *Clinical & Experimental Allergy* in 2008. The COAST study followed 275 children over time and observed that dog exposure at birth was associated with decreased atopic dermatitis and wheezing at age 3, which was not explained by exposure to endotoxin, ergosterol or muramic acid measured in dust [99]. In another US study, maternal exposure to indoor dogs or cats during pregnancy was associated with lower cord blood IgE levels in their offspring ($n = 1049$ infants) [100], and in an ISAAC Phase 2 study, an inverse association was found between endotoxin levels in living room floor dust and asthma in children [35]. Anne Wright reviews very elegantly potential pathways to allergic disease in an editorial, in which the two primary hypotheses to explain the increase in asthma and allergies are discussed [101]. On the one hand, there is the 'allergen exposure hypothesis', which postulates that tighter homes and more time spent indoors have increased exposure to HDM and other allergens strongly associated with asthma. On the other hand, there is the 'hygiene hypothesis', which focuses on the possible protective effects of early microbial exposure [102]. Furthermore, Rigotti et al. [103] propose, in a comment to the COAST study, that the protective effect of dog exposure on asthma outcomes

may be mediated through other mechanisms such as psychological factors and physical activity. Large epidemiological studies often have the proper methodology to assess the effect of early exposures on subsequent outcomes in childhood and young adulthood, as they build on carefully formulated questionnaires and objective measurements, as well as suitable analyses and interpretations. It is, however, important to recognize their shortcomings and limitations, one of which is that mechanisms for the associations seen are sometimes overlooked. In the rich literature on the association between early exposure to pets or endotoxin, it is very important to complete the picture by identifying the microbial constituents to which a protective effect can be attributed, and to determine more precisely the relation of immune system alterations to asthma-related outcomes at different ages. This year's articles on the subject do add considerably to the picture.

Self-reported hypersensitivity symptoms following the intake of alcoholic drinks are common. In a Danish community study, these symptoms were markedly more prevalent in persons with AR and asthma than those without [104, 105]. Furthermore, alcohol consumption was found to be associated with an increased risk of developing perennial, but not seasonal, AR in a population of young adult women [48, 49].

Finally, there are a few studies that discuss aspects of the allergic march [106–109] and others that discuss BCG vaccination and tuberculin reactivity as inversely associated with eczema [110, 111] and asthma [38]. The hypothesis we find most intriguing, however, is the paper that proposes that climate change might induce negative effects on respiratory allergic diseases [112]. In particular, the increased length and severity of the pollen season, the higher occurrence of heavy precipitation events and the increasing frequency of urban air pollution episodes suggest that environmental risk factors will have a stronger effect in the following decades. How this will affect the publication of articles on epidemiology in *Clinical & Experimental Allergy* remains to be seen.

Clinical allergy

Drug allergy

Adverse drug reactions (ADR) were defined by the World Health Organization as a noxious and unintended response to a drug that occurs at a dose normally applied in humans. Drug allergy refers to an ADR with an underlying immunological mechanism and is not related to the pharmacodynamics of the drug (for a review, see [113]). Drug allergy reactions are neither dose dependent nor predictable, and affect only predisposed patients.

Allergic drug reactions are classified, according to the time interval between the onset of the symptoms and the drug intake/administration, as immediate, accelerated

or delayed. Immediate reactions correspond to those occurring within 1 h after drug intake, with anaphylaxis and urticaria being the typical manifestations. Delayed reactions appear more than 24 h after drug intake/administration and have different clinical manifestations, ranging from delayed urticaria and maculopapular exanthema to more severe (potentially life-threatening) reactions, such as the Stevens–Johnson syndrome and toxic epidermal necrolysis. Accelerated reactions occur between 1 and 24 h after drug intake/administration, with urticaria being the typical clinical manifestation, and may overlap with immediate and delayed reactions. Drug allergy reactions are mediated predominantly by IgE antibodies or T cells. The pathomechanism of IgE-mediated (mostly immediate) reactions is well investigated, but the mechanisms of T cell-mediated (generally delayed) drug allergy remain considerably elusive (for a review, see [114]).

Epidemiological data on drug allergy are scarce and difficult to interpret, as in the majority of surveys ADR have been assessed in general, ignoring the underlying pathophysiological mechanisms and simply relying on the clinical history without any further diagnostic investigation. In the study by Rebelo Gomes et al. [115], the prevalence of self-reported ADR and drug allergy in children was 10.2% and 6%, respectively. Most of the alleged allergic reactions were non-immediate cutaneous events attributable to β -lactam antibiotics. However, 94% of the children who were evaluated with laboratory investigation, skin tests and drug provocation finally tolerated the initially suspected culprit, thereby re-emphasizing the issue of overdiagnosis and the importance of additional testing [116, 117].

Although the diagnostic management of drug allergy has significantly improved over the past few years, the correct diagnosis of drug allergy remains complex. In general, diagnostic work-up starts with *in vitro* tests (available and validated for only a limited number of drugs) and/or skin tests. However, the results of these test results might not always be predictive of the clinical situation and additional drug provocation tests (DPT) are needed to establish a correct diagnosis. In their study, Bousquet and colleagues confirmed the observations made by Torres and colleagues that in the diagnosis of allergy for β -lactam antibiotics in patients with negative skin tests, DPT are absolutely mandatory [118, 119]. Actually, from the data obtained by Bousquet and colleagues, it emerges that 79 out of 257 (30.7%) patients with a generally immediate allergy for β -lactams demonstrated negative skin prick and intra-dermal tests and had their diagnosis confirmed by DPT. Unfortunately, DPT poses different ethical (dangerous endeavour) and practical limitations that hamper its mainstream application and practically restrict its use to specialized centres where they can be performed under rigorous surveillance conditions [120]. Therefore, *in vitro* tests are of tremendous interest in order to identify the offending agent(s) and to

reduce or avoid challenges, especially in patients who have suffered from a severe reaction or who were exposed to multiple allergens.

In the early 1990s, the discovery of the basophil activation marker CD63 led to the development of a flow cytometric technique to analyse allergen-specific *in vitro* activation of basophils [121]. During recent years, flow-assisted analysis of *in vitro* activated basophils has proven to be a reliable method to diagnose allergy from proteinaceous allergens such as aeroallergens (e.g. HDM, pollen, epithelia), natural rubber latex, hymenoptera venom but also from chemicals such as drugs or related compounds (e.g. curarizing neuromuscular blocking agents, β -lactam antibiotics) with a minimal amount of blood (for a review, see [122]). Abuaf et al. [123] compared two basophil activation markers CD63 and CD203c in the diagnosis of amoxicillin allergy and found the latter to be more sensitive to diagnose anaphylaxis from this drug. However, despite this elegant study, it remains uncertain as to which activation marker is the most reliable for flow-assisted allergy from drug allergy. Obviously, the basophil activation test is only applicable in those cases where the final effector function relies upon activation of mast cells and/or basophils. The fact that the BAT does not discriminate between IgE dependent (bridging of adjacent allergen-specific membrane-bound IgE molecules) and other mechanisms of cell activation (complement, short-sensitizing IgG or even non-immunologic direct histamine release) should not be considered as a drawback.

The correct diagnosis of delayed, generally T cell-dependent, drug hypersensitivities is an even greater challenge, in part because of the very heterogeneous clinical manifestations such as mild exanthema or delayed urticaria, life-threatening and potentially life-threatening conditions such as the Stevens-Johnson syndrome or toxic epidermal necrolysis (TEN) or extra-cutaneous manifestations such as hepatitis or nephritis. Delayed T cell-dependent hypersensitivity reactions are at present subdivided into different subtypes. Discrimination between these subtypes rests on the differences in cytokine production, inclusion of the Th1-Th2 paradigm and the specific cytotoxic activity of CD4⁺ and CD8⁺ T cells. At present, the pathomechanisms of these delayed hypersensitivity reactions remain to be elucidated and demonstration of drug-specific T cell activation is restricted to lymphocyte transformation tests (LTT) that have also been proposed for diagnostic purposes. However, the LTT is a quite laborious and (too) insensitive tool. Therefore, most physicians rely on patch tests and/or a late-reading intra-dermal test or DPTs to document their clinical suspicion. Unfortunately, as recently demonstrated by Padial et al. [124], skin tests seem to be too insensitive to diagnose delayed reactions to β -lactams and additional DPT are necessary to establish a correct diagnosis. However, DPT provocation tests are restricted to patients with mild cutaneous reactions such as a maculopapular or urticarial

exanthema. In conclusion, although considerable progress has been made in the diagnosis of drug allergy, several issues remain to be resolved. For several drugs, the diagnostic value of the available tests remains to be established. In particular, the correct diagnosis of delayed drug allergies remains a major challenge.

Food allergy

Östblom et al. [108] describe the changing pattern of reported reactions to food and sensitization to food allergens in a large cohort of 3104 children; the blood sample was available in 1857. Food hypersensitivity was based on parental reported data; another clinical end-point was doctor-diagnosed food hypersensitivity. Sensitization was defined as IgE level to a mixture of milk, egg, codfish, peanut, soybean and wheat above 0.35 kUA/L. Symptomatic data were available at ages 1, 2, 4 and 8 years, while blood samples at ages 4 and 8 were studied. Doctor-diagnosed food hypersensitivity increased from 3.1% to 7.6% from age 1 to 8. While hypersensitivity of milk and egg decreased, hypersensitivity to tree nuts and peanuts increased. Seventy percent of those with persistent food hypersensitivity were sensitized to food at age 4 and/or 8, while only 15% of those never having had food hypersensitivity were sensitized. Persistent and late-onset (symptoms after age 4) food hypersensitivity markedly increased the risk for asthma, AR and eczema at age 8 compared with those never having had food hypersensitivity. The authors speculated that the increase in nut hypersensitivity is due to increasing prevalence of sensitization to grass and birch.

The study of Matricardi et al. [107] confirmed the strong correlation between of IgE – antibodies to certain foods and aeroallergens in a large German multi-centre allergy study. They compared specific IgE levels of 273 children at ages 2, 5, 7 and 10 years. The percentage of children sensitized to cow's milk and egg (> 1 kU/L) was the highest already at age 2, declining thereafter. In contrast, the percentage of children with increased levels of soy and wheat IgE antibodies increased. Analysis of complete sets of sera showed that 97% of those with soy or wheat antibodies at some time also had antibodies to grass or birch. The same high percentage showed antibodies to soy or wheat at the same age or later than to grass or birch. Quantitatively, a strong correlation existed between the levels of antibodies to wheat and soy. The authors speculate that primary sensitization to soy and wheat seldom takes place, but the bulk of sensitization to them is linked to sensitization to grass and birch. Both studies infer that cow's milk and hen's egg are primary food sensitizing agents with declining prevalence with age, but the increasing rate of sensitization to grass and birch by cross-reactivity leads to sensitization to soy and wheat in late childhood.

Vlieg-Boerstra et al. [125] analysed their paediatric material for a history of anaphylactic reactions. They recorded 21 cases among 441 children with positive double-blind placebo-controlled food challenges. These 21 children, with a median age of 6.1 years, were invited to renewed DBPCFC to assess possible tolerance development. The median time that lapsed after the most recent anaphylactic reaction was 4.3 years. In six patients, a negative DBPCFC resulted: in three out of nine with cow's milk allergy (CMA), in one out of one with hen's egg allergy and in one out of eight with nut allergy. Clearly, remission of even severe symptoms takes place with time. However, if the specific IgE exceeded the established cut-off limits predicting a positive challenge outcome, all challenges in this study were also positive and therefore should have been postponed. Cautious challenges as conducted in the study did not cause serious symptoms and should be undertaken at times. The age of patients and known natural cause of allergy to the specific food should determine the intervals for the challenges.

In a murine model of peanut allergy, Zhang et al. [87] showed that the rate and severity of anaphylactic reactions may be reduced by pre-treatment by a probiotic fermentation product of defatted soybeans or by the same product, where bacteria were killed by irradiation. The symptom score was reduced and the rectal temperature was closer to normal after giving the products; the effect was dose dependent. The levels of peanut-specific IgE were reduced in the treated groups, and stimulation of their splenocytes showed less secretion of IL-4, IL-5 and IL-13 and higher secretion of IFN- γ . The levels of TGF- β and IL-10 were not significantly changed. They infer that down-regulation of Th2 responses is induced by the products.

The tolerization by early, primary food allergens is also reported in the report by Lam et al. [126]. They describe 33 patients with CMA detected over a period of 14 years among adult patients attending an outpatient clinic; they represent only a few percent of all patients with food allergy. In 10 patients, a DBPCFC test was performed, and in 18 specific IgE antibodies were measured in the blood and a skin prick test (SPT) was performed. The majority of patients (19/30) had acquired CMA after age 16. The most frequently reported symptoms were oropharyngeal (26/30); eight had experienced a documented anaphylactic shock. Majority had experienced accidental ingestion of cow's milk. Of the 18 tested, 14 had positive SPT to cow's milk; the most common protein fractions elicited were β -lactoglobulin (11/18), α -lactalbumin (9/18) and casein (3/6). The same proportion of patients (14/18) had increased levels of specific IgE to cow's milk, and the same fractions as for SPT were the most common fractions, although they did not find correlations between SPTs and specific IgE levels for whole cow's milk or its fractions. The eliciting dose for subjective symptoms was low, starting at 0.3 mg. Clearly,

these patients should be identified as the symptoms are severe even with a low dose of cow's milk.

Another type of reactivity to cow's milk is described in the study of Liden et al. [127]. They conducted a rectal challenge with a suspension of cow's milk proteins in 21 patients with Sjögren's syndrome. Both gas and material absorbed in cellulose were obtained 15 h later. In 8/21, the level of NO or myeloperoxidase was above the two standard deviations of 18 healthy controls. They had previously found similar increases of NO and myeloperoxidase in patients with coeliac disease when rectally challenged with gluten. The local challenge with soy protein did not induce increases in the levels of NO or myeloperoxidase. More commonly, patients suspecting an adverse reaction with food and particularly with cow's milk showed these reactions; still, the low number of patients did not allow definitive conclusions.

Gaudin et al. [128] reported on the reactivity of IgE antibodies in the samples from 40 cow's milk-allergic patients with various milk fractions using the microarray method. The recognition intensity was higher for raw milk than for ultra-high-temperature-treated milk and lyophilized skim milk. Of the protein fractions, casein and lactoferrin elicited the strongest average responses; they were also the most frequently recognized fractions. The microarray system used, however, did not recognize β -lactoglobulin or α -lactalbumin; they were recognized by an ELISA system in a high percentage of samples. They observed a specific reaction against human milk in <10%. They speculated that this may be due to cross-reactivity between human and bovine caseins.

The reactivity of samples with cow's milk-specific IgE with human milk proteins was studied by Schulmeister et al. [129] by immunoblotting and immunoblot inhibition experiments. Out of 20 samples with specific IgE to CM from 4.23 to >100 kUA/L, IgE in 17 reacted with breast milk. Sera recognized more commonly different antigens in both milks. IgE reactivity to breast milk was weaker than that to cow's milk antigens. None of the samples from non-allergic persons reacted with breast milk fractions. They show evidence that the reactivity is due to allereactivity. After giving mother's, cow's or goat milk, the reactivity of their milk with positive sera was not changed, suggesting that the reactive fractions are not ingested proteins. Inhibition experiments suggested that some of the IgE-reactive human milk antigens showed no cross-reaction with antigens in cow's milk. On the other hand, cross-reactivity between human and bovine α -lactalbumin was evidenced. The clinical importance of IgE reactivity to human milk was suggested by positive SPT in two patients.

Skin allergy

Atopic dermatitis continues to challenge clinicians and researchers. The importance of barrier function integrity

in atopic eczema has been highlighted by the finding of loss-of-function mutations in the gene encoding filaggrin, which, in turn, influence epidermal permeability, increased transepidermal water loss and percutaneous absorption of allergens and microbes. Filaggrin mutations have been associated with early onset of disease, asthma and ichthyosis and may be found in healthy controls. Dr Ogg presents a thoughtful review of the interaction between barrier function, T cells, microbes and allergens in the complex multifactorial model of disease that is emerging [130]. Because lesional skin expresses lower levels of filaggrin than non-lesional skin in the same individuals, it seems that its expression is tightly regulated and it appears that the Th2 cytokines, IL-4 and IL-13, contribute to this regulation. Th2 cytokines also reduce the expression of antimicrobial peptides in the skin, increase the expression of potential *Staphylococcus aureus* adhesion molecules and reduce the superantigen-induced death of reactive T cells. There is evidence that HLA-expressing keratinocytes may become targets for T cell-mediated lysis. T cell-induced keratinocyte apoptosis is thought to contribute to spongiosis, which is a key histological feature of eczema. *S. aureus* on the skin of atopic dermatitis is significantly more likely to express superantigen and the levels have been shown to correlate with disease severity. Leung et al. [131] have examined the *S. aureus* burden in the homes of patients with atopic dermatitis with different levels of severity. *S. aureus* DNA was extracted from dust samples collected from the bed, the floor next to the bed and the home vacuum cleaner bag, and analysed for the *femB* gene, an *S. aureus*-specific genomic marker. Atopic dermatitis severity was calculated using the eczema area and severity index (EASI). Participants were grouped by relative severity as having mild, moderate, severe or no atopic dermatitis. Most participants with atopic dermatitis had asthma, pets and carpeted bedrooms. Bed dust yielded the highest *S. aureus* concentrations when compared with bedroom floor and vacuum filter bags. Participants with severe atopic dermatitis had significantly more *S. aureus* DNA in bed dust than those with moderate, mild and no atopic dermatitis. The concentration of *S. aureus* DNA in bedding dust correlated strongly with the EASI scores. The authors conclude that higher levels of *S. aureus* in the home and especially the bedroom may contribute to disease severity and persistence in atopic dermatitis. Another interpretation of the data, though, could be that severe atopic dermatitis is more likely to be colonized by *S. aureus* because the skin has been excoriated, leaving open wounds that are prone to *S. aureus* infection.

Chronic urticaria is another disease that remains tantalizingly elusive for clinicians to understand because its activity often appears to be multifactorial even when an underlying autoimmune or infectious cause can be identified in patients with spontaneous disease. Understanding

the role of dietary pseudoallergens as a cause or an aggravating factor in chronic urticaria has a long history dating back over 50 years. Dietary exclusion as a management strategy for chronic urticaria has often polarized believers and non-believers with little firm evidence to rely on. Bunselmeyer et al. [132] have attempted to address this using a low-pseudoallergen diet in addition to pharmacological treatments, followed by a stepwise re-introduction of foods in groups (the incremental build-up food challenge or IBUF) for responders who were then followed up by telephone interview after 3–24 months. Their findings are limited by the lack of a control group or blinding and the small number of patients whose urticaria cleared completely during the 5-week low-pseudoallergen diet (there were only 18 full responders) but several useful findings have emerged from this systematic study of a large population of patients with ordinary chronic urticaria (153 patients were recruited) that may guide clinical practice. Although 68% of the 104 patients who completed the initial diet reported a partial (51%) or a complete (17%) response, only 8% of the total number of patients recruited showed a full response, followed by a recurrence of symptoms during the IBUF, suggesting that pseudoallergen ingestion is a rare cause of chronic spontaneous urticaria but may be an aggravating factor in a reasonable proportion. An estimate of this is hard to extrapolate from the study because of its uncontrolled design and the fairly high drop-out rate. Of particular interest, though, is the small number of patients whose urticaria did clear completely and subsequently underwent a non-blinded re-challenge with designated pseudoallergy food groupings. In contrast to the widespread belief that it is mainly food additives that cause dietary exacerbations of chronic urticaria, the authors found that most of the patients developed urticaria at step II of the re-challenge schedule when they re-introduced foods containing biogenic amines (e.g. well-matured cheeses, tuna, mackerel, avocado, banana and walnut) or step III foods containing natural phenolic substances, flavours and salicylates (e.g. tomatoes, other fruits and vegetables, other nuts, herbs and herbal teas). Exacerbations of urticaria after re-introduction of foods containing additives or alcohol were uncommon. The results of re-challenge of partial responders to the full diet were inconclusive because of the background of spontaneous urticaria. It seems reasonable to offer well-motivated patients a low-pseudoallergen diet on the basis of these findings with the proviso that only a small minority can expect to clear and be able to stop their H₁ antihistamines and to offer an incremental build-up for complete responders based on the IBUF groupings, rather than random selection by the patients. Guideline recommendations are supposed to be evidence based but are often guided more by clinical experience than hard facts because well-controlled studies of diagnostic procedures and

management of urticaria are still scarce. Thomas et al. [133] audited the investigations performed by paediatric allergists in London for chronic urticaria in childhood against the recent recommendations of the BSACI [134]. They found that the majority of children presenting with urticaria underwent at least one investigation and, not surprisingly, this was usually an allergy investigation (SPT or specific IgE test) but the clinical relevance of sensitization in the 41% of children who were sensitized could not be established. The low positive rate for 'additional investigations' mainly on routine blood testing (5.2%) reflects the BSACI guidance (and that of other recently published guidelines) that 'if the clinical history and examination are typical of chronic idiopathic urticaria, then further laboratory investigations are rarely useful'. The audit did not look at emerging tests for functional autoantibodies and this guidance, while true for non-specialist clinics at the present time, will need to be revisited in the future when diagnostic tests for autoimmune urticaria become more widely used in clinical practice.

Allergens

In general, studies of both the structure and the function of allergens using state-of-the-art technologies contribute to a better understanding of allergenicity, including aspects related to cross-reactivity, allergen nomenclature and the identification of antigenic determinants. These novel developments may significantly support the design and production of allergy vaccines as well as standardization of allergen extracts for the diagnosis and specific-immunotherapy of allergy. In this respect, the review by Mari [135] 'When does a protein become an allergen?' discussed comprehensively the usefulness of novel biotechnology tools such as proteomics and molecular biology approaches, information technology tools and microtechnology tools. A similar issue is addressed by Radauer et al. [136], who introduce the AllFam database for classifying allergens into protein families and to elucidate the common structural and biochemical features of allergens. These *in silico* analyses confirmed that allergens are distributed among a small number of protein families and share a limited range of biologic functions. Especially, in the case of allergen with protease activity, Sokol et al. [137] demonstrated that the protease activity of allergens is required for the induction of TH2 and IgE responses. These authors found that basophils act as the sensors of protease allergens and possibly helminths, suggesting a putative new therapeutic pathway for the treatment of allergic diseases. Many food allergens have been characterized very extensively at a molecular level. This knowledge, in combination with an increasing understanding of humoral and cellular immune responses resulting in allergy or tolerance, may lead to novel therapeutic approaches in the future.

Regarding food allergy, Palacin et al. [138] studied the relevance of major kiwifruit allergens in a large kiwi-sensitized and -allergic population. The authors characterized three major allergens, namely, actidin Act d 1, glycosylated thaumatin-like Act d 2 and a novel glycoprotein Act d 3.02 of green kiwifruit (*Actinidia deliciosa*). In addition, the authors describe the association of determined severe symptoms after kiwi ingestion with high IgE levels to Act d 1 and Act d 3. The question as to why the patients with food allergy symptoms show no IgE binding to kiwifruit extracts in the IgE test after eating kiwifruit was addressed by Oberhuber et al. [139]. The authors characterized recombinant Bet v 1-homologous allergens from green (*Actinidia deliciosa*, Act d 8) and gold (*Actinidia chinensis*, Act c 8) kiwifruit. Act d 8 and Act c 8 were suggested as relevant allergens in kiwi fruits for patients sensitized to tree pollen and kiwifruit and may explain pollen-related kiwifruit allergy at the molecular level.

Milk allergy is one of the common human major health concerns world-wide, especially in children. Milk proteins are very heterogeneous, with few common structural or functional features. A component-resolved diagnosis is difficult because just small amounts of sera are available from children. To overcome this problem and to facilitate a sensitive and specific diagnosis, Gaudin et al. [128] developed a protein microassay method to assess the binding of milk allergens to specific IgE antibodies in sera from cow's milk-allergic patients. This assay allowed the detection of IgE $> 2 \times 10^4$ IU per spot. The sensitivity of this method seems to be comparable to ELISA, and is characterized by a low consumption of serum.

Allergic reactions to tree nuts belong to the major allergies against plant-derived foods. In this respect, Willison et al. [140] investigated the cross-reactivity of cashew nuts and pistachio allergens. The authors characterized recombinant pistachio allergen Pis v 3, a 7S vicilin-like protein. Pis v 3 showed a high degree of amino acid sequence similarity to Ana o 1, a vicilin-like allergen from cashew. This information and similar IgE-binding patterns of patient's sera and mouse IgG mAbs may indicate that antibody recognition of these proteins is focused on the most conserved regions of the proteins. In this way, the authors could demonstrate that the cashew and pistachio vicilin homologues are highly cross-reactive.

Jensen et al. [141] investigated the cross-reactivity between peanut and legume allergens. They used extracts of seeds and sprouts from peanut and miscellaneous legume species and compared their allergenicity by several *in vitro* and *in vivo* tests. Cross-reacting allergens were found in various legumes and in case of lupine these allergens may have clinical relevance in some patients.

One of the major initiators of cross-sensitivity between pollen allergy and plant food allergy is the pan-allergen profilin. Asero et al. [142] investigated a large number of consecutive patients with pollen allergy from Italy.

The authors demonstrated the prevalence of hypersensitivity of profilin in pollen-allergic patients and its clinical relevance as a food allergen. This study underlines the clinical importance of being able to diagnose hypersensitivity to a single food allergen instead of mixtures by SPT, which is a useful diagnostic tool for the clinical allergist.

Inhalation allergy to various kinds of pollen is common world-wide. Luengo et al. [143] demonstrated the allergen content, cross-reactivity and clinical relevance of *Senecio jacobea* pollen. This pollen has been identified as an allergenic source in a study with 473 consecutive patients. *S. jacobea* allergens showed strong cross-reactivity to *Artemisia vulgaris* and *Parcetaria judacia* pollen. The authors characterized two major allergens from this pollen: an MDH homologue and a pectate lyase homologue with molecular weights around 40 kDa and 39–41 kDa, respectively. Allergy to ragweed pollen (*Ambrosia artemisiifolia*) belongs to the clinically most important pollen allergies especially in the United States. To improve ragweed allergen standardization and to develop new diagnostics as well as novel vaccines, Wopfner et al. [144] compared the IgE responses of 89 sera from ragweed-allergic patients to recombinant Amb a 1 and natural Amb a 1. Binding studies of mAb raised against natural and recombinant proteins were performed. Although a minor IgE reactivity was detected by recombinant protein as compared with natural protein, a good correlation could be observed between IgE reactivity of natural and recombinant Amb a 1. Other potential inhalation allergen sources are pollen from beech and oak. Their cross-reactivity to birch pollen allergens was investigated by Egger et al. [145] using 49 patient sera from Vienna and Basel. IgE reactivity to birch pollen was stronger than to beech or oak pollen allergens. Birch and beech pollen allergens showed similar IgE profiles due to cross-reactivity. Moreover, the authors identified a new IgE reactive protein as a Phl p 4-like allergen in oak pollen.

Bellinghausen et al. [146] investigated whether the hexameric structure of Per a 3, the major allergen from the American cockroach (*Periplaneta americana*), influences its allergenicity and immunogenicity. The authors demonstrated that the Per a 3 monomer induces a lower Th2 response than Th1 compared with the hexamer. Therefore, the monomeric variant may be a putative candidate for SIT. However, the relevance of these *in vitro* data has to be confirmed by further *in vivo* studies.

Allergen immunotherapy is performed with allergenic extracts prepared from a wide variety of sources. Irañeta et al. [147] report that the contribution of proteolytic enzymes especially in fungal and whole-body insect allergenic extracts induces degradation during storage of allergen mixtures in the absence of glycerol. Concerning immunotherapy and diagnosis with natural allergen extracts, Focke et al. [148] studied the quality and quantity of allergen context in four commercial timothy grass pollen extracts. The authors found a high variation in the

total protein content and major allergens and observed degradation of defined allergens and absence of several important allergens in certain extracts. To overcome the problems with the natural grass pollen extracts, the authors discussed the replacement of natural grass pollen allergen either with mixtures of recombinant allergens or the use of recombinant hybrid allergens containing relevant IgE epitopes.

Hoffmann-Sommergruber et al. [149] discussed the problematic issues of food allergy diagnostics with allergen extracts. The authors suggest replacing food extracts with highly purified allergen preparations. This research group used a novel approach by creating a complex allergen library (EuroPrevall Food Allergen Library) comprising the most important natural and recombinant allergens from major allergenic foods including a set of harmonized quality criteria. The availability of such data and information will improve comparability exercises utilizing purified allergens in the near future.

Especially, in the last few years, an important focus for food manufacturers and regulators responsible for food safety was the allergy assessment of genetically modified crops. In principle, introducing a new food into the market poses the risk of introducing a new allergen. In this regard, Goodman et al. [150] summarized the current guidelines for the allergenicity determination of new crop varieties in a rational way and provided a critical discussion of the usefulness of various tests applied to GMO foods.

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