

REVIEW ARTICLE

Mechanisms and potential therapeutic targets in allergic inflammation: recent insights

S. von Gunten¹, F. Cortinas-Elizondo¹, M. Kollarik^{2,3}, C. Beisswenger⁴ & P. M. Lepper⁴

¹Institute of Pharmacology, University of Bern, Bern, Switzerland; ²Department of Medicine, The Johns Hopkins University School of Medicine, Baltimore, MD, USA; ³Department of Pathophysiology, Jessenius Medical School, Martin, Slovakia; ⁴Department of Internal Medicine V, University Hospital of Saarland, Homburg, Germany

To cite this article: von Gunten S, Cortinas-Elizondo F, Kollarik M, Beisswenger C, Lepper PM. Mechanisms and potential therapeutic targets in allergic inflammation: recent insights. *Allergy* 2013; DOI: 10.1111/all.12312.

Keywords

allergy; immunomodulation; inflammatory mechanisms.

Correspondence

Dr. Stephan von Gunten, Institute of Pharmacology, University of Bern, Friedbühlstrasse 49, CH-3010 Bern, Switzerland.

Tel.: +41 (0)31 632 3281

Fax: +41 (0)31 632 4992

E-mail: stephan.vongunten@pki.unibe.ch

Accepted for publication 30 September 2013

DOI:10.1111/all.12312

Edited by: Thomas Bieber

Abstract

Deeper insight into pathogenetic pathways and into the biological effects of immunomodulatory agents will help to optimize or adopt therapeutic strategies for atopic disorders. In this article, we highlight selected findings of potential therapeutic relevance that emerged from recent mechanistic studies with focus on molecular and cellular aspects of allergic inflammation. Furthermore, the often complex mechanisms of action of pleiotropic immunomodulatory agents, such as glucocorticoids, vitamin D, or intravenous immunoglobulin (IVIG), are discussed, as their dissection might reveal targets for novel therapeutics or lead to a more rational use of these compounds. Besides reporting novel evidence, this article points to areas of current debate or uncertainty and aims at stimulating scientific discussion and experimental work.

Clinicians and scientists working in the field of allergy experience a rapidly growing body of original articles on specific aspects of allergic disease. Here, we highlight recent mechanistic insights that reveal potential targets for therapy or may lead to a more rational use of distinct therapeutic agents. Novel therapeutic strategies for atopic diseases may be derived not only from the analysis of molecular and cellular processes in allergic inflammation, but also from a better understanding of the mode of action of therapeutic compounds used in daily practice or tested in clinical trials. While pleiotropic agents, such as glucocorticoids, vitamin D, or intravenous immunoglobulin (IVIG), act in manifold and often complex ways, careful dissection of their molecular mechanisms of action may lead to the identification of potential drug targets or reveal pathogenetic differences between specific patient subgroups (e.g., genetic polymorphisms). For more comprehensive and in-depth information on specific topics, we refer to the abundant body of timely and excellent review articles in the field. In the present article, areas of scientific uncertainty or debate are also discussed, as the identification of today's gaps of knowledge and the awareness of

methodological limitations are mandatory for goal-directed research and the improvement in therapeutic strategies for atopic disorders.

The allergic effector unit (AEU)

Allergic inflammation is a complex multiphase response that is characterized by an early-phase reaction typically initiated upon allergen-mediated activation of mast cells and the consequent recruitment of circulating inflammatory leukocytes, in particular eosinophils, leading to the late-phase reaction. Mast cells and eosinophils are recognized as the dominating cells in allergic inflammation and given that both cells co-exist in the inflamed tissue during the late-phase or chronic stage of allergic inflammation, a cross-talk between them is a likely scenario to take place (1). This key mast cell-eosinophil interplay, that is, both based on secreted mediators and on ligand-receptor interactions (Fig. 1), has been designated as the allergic effector unit (AEU) and may play a central role in maintaining allergic inflammation (2). Physical associations between mast cells and eosinophils were found

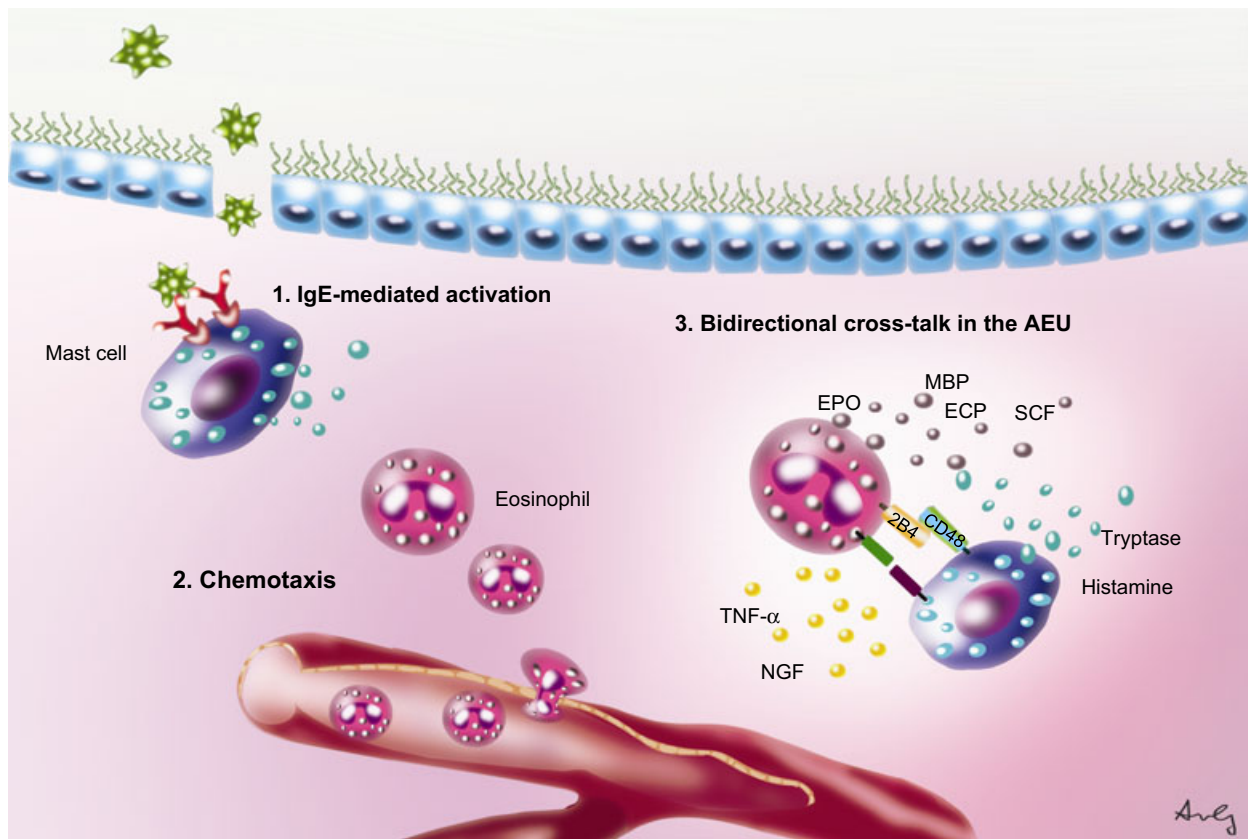


Figure 1 Bidirectional cross-talk in the allergic effector unit (AEU). 1. IgE-mediated activation of mast cells by bound allergen (early phase). 2. Chemotaxis of eosinophils to the site of allergic inflammation. 3. Bidirectional cross-talk and activation between mast

cells and eosinophils as AEU (late phase or chronic stage), involving both released mediators and surface receptors (e.g., 2B4-CD48 interaction). Illustration by Aldona von Gunten.

in inflamed tissue sections, such as human nasal polyps and asthmatic bronchi, as well as mice skin with atopic dermatitis (2). Despite the observation of such conjugates *in situ*, the exact role of mast cell–eosinophil interactions in allergen-induced allergic disorders or atopic dermatitis remains to be explored. Notably, it was reported that both resting and activated mast cells enhance and maintain the survival of eosinophils by soluble mediators, such as granulocyte-macrophage colony-stimulating factor (GM-CSF), but more significantly by direct physical contact via the CD2-like receptors CD48 and 2B4. Remarkably, this increase in eosinophil viability was even seen in dexamethasone-treated eosinophils in coculture with mast cells. Therefore, mast cell-mediated eosinophil survival is likely to contribute to the persistence of late-phase or chronic aspects of disease, such as maintained inflammation, tissue remodeling, and angiogenesis, and may offer a possible explanation of the anti-IL-5 therapy failure to eliminate eosinophils from asthmatic airways (2). In a further study, a bidirectional activation of both cells was reported (3). Eosinophils augmented mast cells' mediator release, as shown in β -hexosaminidase and tryptase release assays, through the CD48-2B4 interaction. Similarly, mast cells also enhanced eosinophils' degranulation as assessed by eosinophil

peroxidase (EPO) secretion; however, this event was independent of CD48-2B4 contact. Additionally, an upregulation in the expression of intercellular adhesion molecule (ICAM)-1 on eosinophils was also seen during long-term full-contact AEU cocultures; as well as an increased phosphorylation of activatory signaling molecules, and a high release of the pro-inflammatory cytokine tumor necrosis factor (TNF)- α , likely contributing to the Th2 polarization in allergy (3). Therefore, an outstanding, yet nonsymmetrical function of the CD48-2B4 interaction is shown to be key within the AEU cross-talk (3). Most recently, it was shown that eosinophil major basic protein (MBP)1 induces activation of fibroblast-derived membrane (FBM)-primed mast cells (4). MBP1-induced mast cell activation was partially mediated by MBP1- β 1 interaction on the mast cell surface and involved signaling by *src*-kinase Hck. Leukotrienes released by mast cells (5) and eosinophils (6) might also contribute to the AEU bidirectional activation (3), as both cell types express leukotriene receptors (7). Moreover, these lipid mediators could enhance the persistence of allergic responses, as they are known to trigger airway inflammation, bronchoconstriction, and tissue remodeling in asthma (8). Altogether, these findings point toward a mast cell–eosinophil AEU that enhances activation in both cells

via soluble or physical factors and thus may contribute to the persistence of the allergic inflammatory reaction (3).

Basophils as amplifiers of allergic inflammation

Recent development of analytical tools (e.g., specific identification markers, basophil-deficient animal models, *in vitro* differentiation of mouse basophils) has considerably augmented the number of studies on basophils and revealed some unacknowledged roles for the basophils (9). For example, apart from their involvement in IgE-mediated allergic reaction and their protective role against parasites, it has been suggested that basophils may be acting as antigen-presenting cells (APCs) and induce or amplify Th2 responses (10, 11), although these aspects of basophil biology still remain a matter of debate (12–15). While evidence derived from animal models has revealed novel mechanistic aspects, the role of basophils in human allergen-induced disorders or in atopic dermatitis remains to be further elucidated.

Basophils are often recruited to the affected tissue in allergic disorders such as asthma, rhinitis, and atopic dermatitis (9, 16). Basophil recruitment and tissue accumulation might involve mast cell-derived prostaglandin D2 (PGD2), as it may act as a chemotactic factor for prostaglandin D2 receptor CRTH2-expressing cells, including basophils, Th2 lymphocytes, and eosinophils (11). It has recently been suggested that basophils may contribute to exacerbation of airway inflammation by viral infection, as treatment with double-stranded (ds) RNA poly(A:U) increased basophil functions by inducing Th2-type cytokine and histamine production and increased peripheral basophil recruitment *in vivo* (17). Basophil-mediated aggravation of inflammation and tissue remodeling might involve the release of TNF- α following IgE-dependent activation and subsequent stimulation of monocytes to produce the protease matrix metalloproteinase-9 (MMP-9) (18).

Strategies aimed at preventing cell recruitment and specific network interactions are a promising area for the development of more effective therapeutics. For example, OC000459, a CRTH2-antagonist, has been shown to exert beneficial clinical outcomes in patients with allergic rhinoconjunctivitis (19) and eosinophil esophagitis (20), likely by preventing the activation and recruitment of CRTH2-positive inflammatory cells.

Local synthesis of glucocorticoids in peripheral tissues

Although the potent anti-inflammatory effects of local or systemic glucocorticoid (GC) therapy are well appreciated for the local or systemic treatment for allergic diseases, corticosteroid resistance and adverse effects of concurrent or past GC application remain unresolved issues (21, 22). The molecular mechanisms of steroid resistance are complex and include genetic variability, defective glucocorticoid receptor (GR) binding and nuclear translocation, increased expression of inhibitory GR β , transcription factor activation, abnormal histone acetylation, or immune mechanisms, such as increased numbers of Th17 cells that might orchestrate neutrophilic

inflammation (21). To address current challenges of GC therapy, the molecular mechanisms of GC biology need to be further elucidated, which might lead to the rational design of novel therapeutics, such as selective glucocorticoid receptor modulators (SGRMs) (23, 24).

While endogenous circulating glucocorticoids are mainly produced by the adrenal gland under control of the hypothalamic–pituitary–adrenal (HPA) axis, accumulating evidence suggests that extra-adrenal production of glucosteroids in tissues might regulate local immune responses (25–27). Notably, glucosteroids have also been reported to be locally produced by several organs affected by allergic diseases, including skin, intestine, and the lung (25, 26, 28). As a stress response or following immune cell stimulation active GC can be either locally produced *de novo* from cholesterol, or by conversion of circulatory GC by the enzyme 11 β -hydroxysteroid dehydrogenase type 1 (11 β -HSD1) in a tissue-dependent manner (25–27). Although the complete GC-synthesizing enzymatic machinery is expressed in the murine lung, increased levels of corticosterone after immune cell stimulation are primarily produced via 11 β -HSD1-mediated conversion of inactive serum dehydro-corticosterone (28). While immune cell activation by anti-CD3 antibody, lipopolysaccharide (LPS), or TNF- α induced corticosterone synthesis, ovalbumin-induced allergic airway inflammation failed to promote lung GC synthesis (28). In analogy, another study showed absence of local steroid production in Th2 cytokine-mediated colitis, whereas local GC concentration was substantially increased in Th1-type colitis (29), suggesting that at least in these experimental models, Th1- but not Th2-type cytokines are able to induce local GC synthesis. Other factors may also induce or suppress local GC synthesis. For instance, 11 β -HSD2 is a different enzyme that solely inactivates endogenous GC and might counterbalance the local activity of 11 β -HSD1 (25). However, pharmacological induction of local endogenous GC synthesis (Fig. 2) may have several advantages compared with current therapy with exogenous GC, especially in terms of bioavailability and adverse effects (27).

Vitamin D

Recent awareness on the pleiotropic effects of vitamin D, including immunomodulatory functions, has stimulated a rapid increase in the number of publications on vitamin D and atopic diseases (30). Vitamin D receptors (VDRs) are nuclear receptors expressed in essentially every human tissue that, following ligand binding and dimerization with the retinoid X receptor (RXR), modulate the transcription of a broad variety of genes including immunomodulatory genes, or junction genes responsible for the integrity of the physical epithelial barrier (30, 31). Diverse effects of vitamin D on cells of innate and adaptive immunity have been reported including pro-allergic T helper type 2 (Th2) polarization and cytokine production (IL-4, IL-5, and IL-10), but conversely also anti-allergic effects such as inhibition of IgE production by B cells, inhibition of dendritic cell (DC) maturation and migration, or conversion of CD4⁺ T cells to T regulatory cells (Tregs) (reviewed in (31)). In light of these studies, it is

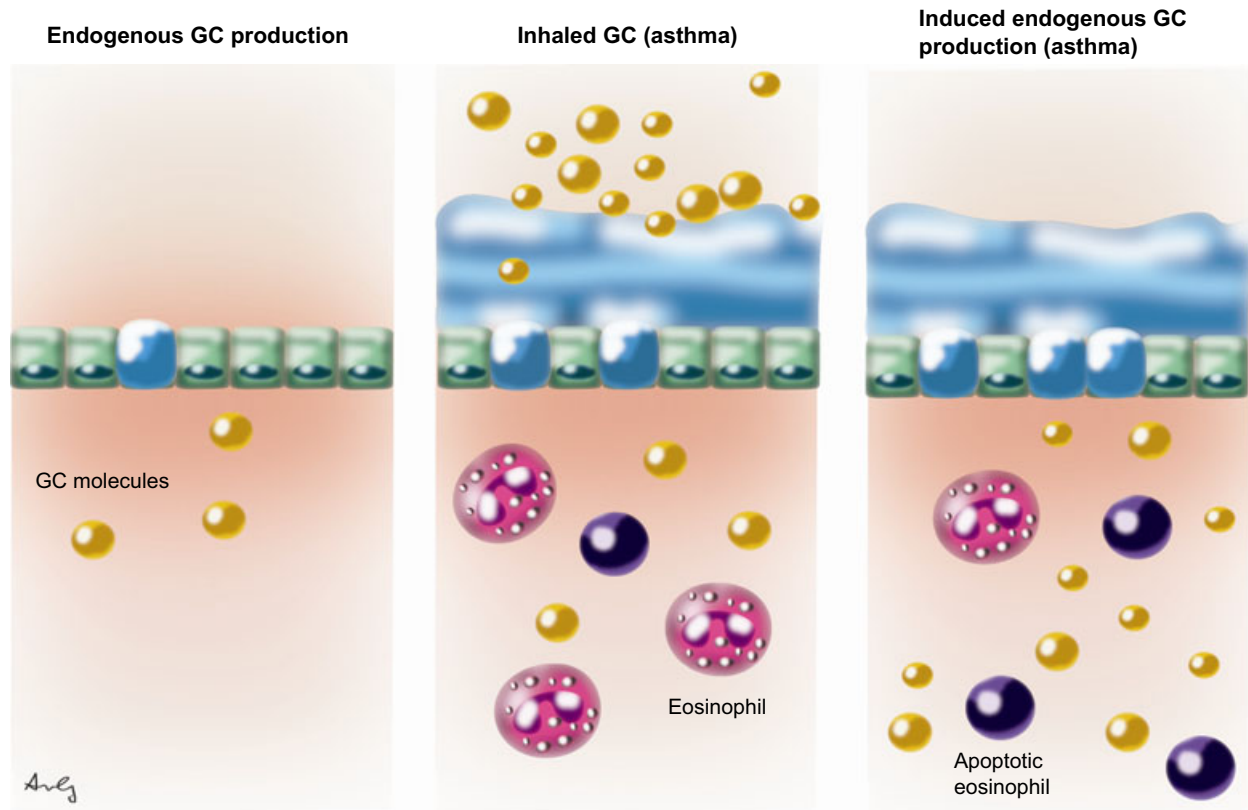


Figure 2 Induction of endogenous glucocorticoid (GC) production as a potential therapeutic approach in asthma. Left panel: Local production of GC in lung tissue. It remains to be shown whether failure of endogenous tissue production of GC contributes to the pathogenesis of asthma. Middle panel: Eosinophil apoptosis

induced by inhaled GC is shown, exemplary for the manifold action of GC. Right panel: Enhanced local production of endogenous GC in the airways as a potential therapeutic strategy in asthma. Illustration by Aldona von Gunten.

conceivable that differences in vitamin D levels and metabolism, or aberrant responses of immune cells to vitamin D (32), affect allergic immune reactions. Several studies have linked genetic differences of enzymes of vitamin D metabolism to variability in serum vitamin D concentrations and to individual genetic susceptibility for atopic disorders. For instance, single nucleotide polymorphisms (SNPs) of CYP24A1, involved in clearance of 25(OH)D and other vitamin D metabolites, have been linked to differences in total immunoglobulin E (IgE) levels (33), and early assessment of its genetic variant, together with the simultaneous consideration of the vitamin D status, might serve as a means to identify newborns at risk of developing allergen sensitization and subsequent allergy (34). Besides the individual genotype linked to vitamin D metabolism, a plethora of other genetic or environmental factors such as ethnicity, gender, immunophenotype, socioeconomic status, and seasonal exposure to sunlight might influence vitamin D bioavailability and its role in allergy (30, 35, 36). Elevated IgE production has been observed in humans with both extremely low or high vitamin D concentrations, suggesting that unbalanced vitamin D levels deviating from a certain threshold could lead to aberrant

immune responses (37). Current definitions of vitamin D deficiency is based on serum vitamin D levels determined as biomarker of bone health, which might be different from requirements to achieve or maintain immune homeostasis. Results from a metabolomic analysis of exhaled breath condensate (EBC), an approach called breathomics, suggested a lack of an active metabolite of vitamin D2 of children with severe asthma in the lung (38). This study indicates that the determination of the tissue-tropic vitamin D status of the organ involved could be a valuable approach to study the role of vitamin D in allergic disease and might eventually better reflect disease-relevant processes than the assessment of systemic vitamin D levels. Whether vitamin D deficiency or its excess predispose or prevent allergy is controversially discussed, as is the related question about the benefit of vitamin D supplementation (30, 31, 39, 40). The need for well-designed prospective studies has been emphasized given the current controversy on the role of vitamin D in allergy (30, 31). Future research might show to which extent immunomodulatory effects of active vitamin D metabolites, or newly developed synthetic VDR agonists with less calcemic side-effects, are influenced by different concentrations of these

compounds (41). Furthermore, the requirement for mechanistic studies has emerged to take into account genetic variability of components of vitamin D pathways in different patient subpopulations.

PARP-1

Recent evidence suggests that the nuclear protein poly(ADP-ribose) polymerase-1 (PARP-1) acts as a key player in allergic inflammation (12, 42, 43). While its role in DNA damage repair has long been appreciated, more recent studies highlighted the role of PARP-1 in gene regulation through a variety of mechanisms, acting as a coregulator for DNA-binding transcription factors, a regulator of DNA methylation, a modulator of chromatin, or as scaffold protein for the transcriptional machinery (42). PARP-1 contains three functional domains (Fig. 3): one amino-terminal DNA-binding domain containing zinc fingers, a C-terminal catalytic domain that exerts its polymerase activity by sequential transfer of adenosine diphosphate (ADP)-ribose subunits from NAD^+ to protein acceptors, and a central automodification domain that acts itself as acceptor of ADP-ribose moieties, but also allows for protein–protein interactions involved in PARP-1 biology (42, 44). PARP-1 mediates stimulation or repression of gene transcription in a gene- and cell-type-specific manner and has been identified as a coactivator of master transcription factors of inflammation NF- κ B, AP-1, and NFAT that also play a role in allergic inflammation (42, 43). Recently, Datta et al. reported that PARP-1 stabilizes signal transducer and activator of transcription-6 (STAT-6), a transcription factor involved in IL-4 receptor signaling, by preventing its calpain-

dependent degradation (45). They found that PARP-1 gene deletion in mice was associated with reduced GATA-3 mRNA and protein expression, and GATA-3 binding to the IL-5 gene promoter was largely absent in IL-4-treated PARP-1 $^{-/-}$ splenocytes. Given the role of IL-5 in differentiation, survival, and priming, PARP-1-mediated stabilization of STAT-6 acting downstream of IL-4 but upstream of IL-5 might be relevant for diseases associated with hypereosinophilia. Provided the potential of targeting PARP-1 as an anti-inflammatory therapeutic strategy for allergic diseases, PARP-1 inhibitors that are currently being evaluated in clinical trials come into focus. While these agents are primarily being tested for oncological indications (44), results from these studies will eventually guide the clinical expansion of specific PARP inhibitors into nononcological diseases (46). Due to the role of PARP-1 in DNA repair, careful investigation of long-term therapy with these agents is warranted in terms of the safety profile (44, 46). In phase I and II studies, the oral PARP inhibitor olaparib was well tolerated and common adverse effects were mild, including fatigue, nausea, and vomiting (46). Further elucidation of PARP-1-related pathways in allergic diseases is required and may have implications for the rational design of PARP-1 inhibitor for nononcologic indications or lead to the identification of novel targets for therapeutic intervention.

Anti-inflammatory effects of intravenous immunoglobulin (IVIG)

The anti-inflammatory effects of IVIG preparations, if administered as a high-dose therapy, are increasingly being appreciated for the treatment of autoimmune and inflammatory disorders (47). Although IVIG has been used successfully for the treatment of severe steroid-dependent asthma, conflicting reports on its efficacy in atopic disease highlight the need for further trials with randomized and controlled study design (48). In animal models of allergic disease, beneficial effects of administration of therapeutic human IVIG have been observed (49, 50) and involved induction of Treg cells mediated by tolerogenic DCs generated after IVIG exposure (50). Furthermore, a better understanding of its mode of action might lead to the identification of disease-specific, active compounds in IVIG and lead to the use of more efficient immunoglobulin fractions (51) or novel therapeutics for atopic disorders. IVIG, consisting predominantly of pooled polyclonal IgG from thousands of donors, is a pleiotropic agent, and many nonmutually exclusive, Fab- and Fc-mediated, mechanisms have been suggested for its anti-inflammatory biological activity, including direct effects on leukocytes (inhibition, cell death regulation), interactions with immunomodulatory proteins (cytokines, chemokines, receptors, adhesion molecules), enhanced antimicrobial defense, or anti-idiotypic binding to pathogenic antibodies (47, 51, 52). Studies in conventional animal models using human IVIG must be critically interpreted in terms of potential xenogeneic loss- or gain-of-function effects and species related-differences of immune effector molecules (e.g., complement, cytokines, receptors) or leukocyte subsets, and

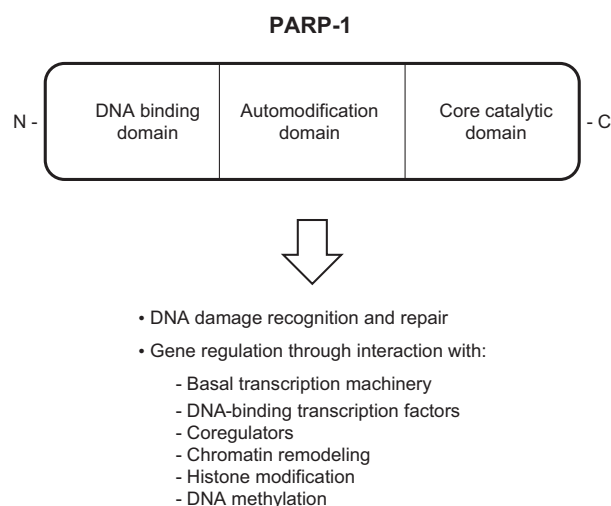


Figure 3 Structure and functions of poly(ADP-ribose) polymerase-1 (PARP-1). PARP-1 is a nuclear protein that plays key roles in a variety of nuclear processes including DNA damage repair and gene regulation. These functions are mediated by three domains (DNA-binding domain, automodification domain, and catalytic domain) and are involved in multiple biological processes including inflammation (see text).

findings emerging from such studies require verification in a human system (53–55).

We have previously shown that IVIG regulates the survival of inflammatory eosinophils following priming by cytokines, while resting eosinophils are comparably resistant to IVIG-mediated death (56). Similarly, eosinophils from patients with hypereosinophilic syndrome (HES) that have been exposed to cytokines *in vivo* are more susceptible to IVIG-mediated death than quiescent cells from healthy donors (56). Functional depletion experiments revealed that this cytokine-dependent death was associated with naturally occurring antibodies to Siglec-8, a receptor known to mediate cytokine-dependent eosinophil death (57). Similarly, IVIG-mediated cell death of inflammatory, but not of unstimulated neutrophils, seems to be Siglec-9 dependent (58). These findings suggest that IVIG-mediated activity remains targeted to granulocytes at the site of inflammation, while circulatory unstimulated neutrophils are prevented from cell death. However, a drop in circulating granulocyte levels following IVIG infusion is observed by clinicians, but is a relatively rare event that might occur in patients with systemically elevated cytokine levels (55). The finding that antibody titers of specific antibodies to immunoregulatory molecules have functional effects (47, 51, 52) suggests that these antibodies bind with high affinity to their targets, but might also explain the requirement of high IVIG concentrations used for anti-inflammatory therapy, in the range of serum IgG levels of 2500–3500 mg per deciliter (47). It has been postulated that IVIG that contains dimeric or multimeric IgG could be more anti-inflammatory (59). Indeed, we found that the dimeric fraction of IVIG contains specific antibodies to Fas and Siglec-9 bound as anti-idiotypic complexes (60). However, dimeric IgG is not stable and is thought to dissociate following injection of IVIG due to changes in temperature, concentration (dilution), and pH (52), resulting in the release of specific antibodies from anti-idiotypic complexes (60). As the extent of dimer formation correlates with the donor pool size (52), it is currently unclear whether anti-idiotypic antibodies to Siglec-9 or Fas have a regulatory function within the anti-idiotypic network of healthy individuals or patients.

While Fab-mediated anti-inflammatory effects of IVIG have been observed in a broad range of both animal models (51) and human systems, the role of Fc-mediated effects is controversially discussed (47, 53). Based on a mouse model of rheumatoid arthritis, it has been postulated that the anti-inflammatory activity of IVIG results from differential sialylation of the Fc core polysaccharide (61). However, recently the groups of D. R. Branch and T. Rispens independently showed in other murine models of immune thrombocytopenia that IVIG-mediated beneficial effects were independent of sialylation of the Fc regions of IVIG (62, 63). Furthermore, in a human whole-blood inflammation assay, the anti-inflammatory activity of IVIG was associated with Fab and not Fc sialylation (64). The mechanisms of action of IVIG in the various murine models may not be consistent, and many of the disease-specific mechanisms must be validated in humans, as animal models may offer only limited insight into human disease (47). In fact, a less simplistic view of its mechanisms

might lead to the appreciation of the magnitude of immunomodulatory effects that this pluripotent agent has on specific pathogenetic pathways in different autoimmune and allergic disorders. Further elucidation of the pleiotropic effects of IVIG may lead to a more rational use of IVIG or specific IVIG subfractions and eventually pave the way to the discovery of novel therapeutic strategies for allergic diseases.

Toll-like receptor (TLR) signaling in asthma

Asthma, which affects approximately one person in 10 in the United States or approximately one in 6 in the UK or New Zealand (65), significantly affects quality of life. A recent global comprehensive survey undertaken by the International Study of Asthma and Allergies in Childhood (ISAAC) study group revealed that the prevalence for asthma, rhinoconjunctivitis, and eczema among teenagers of 13–14 years was as high as 14.1%, 14.6%, and 7.3%, respectively (66). Allergic asthma is a complex disease characterized primarily by eosinophilic pulmonary inflammation, reversible airway obstruction, and mucus production (67). In recent years, the growing understanding of basic innate immune mechanisms has led to a more thorough understanding of atopy and allergy. The so-called hygiene hypothesis proposes that microbial exposure during childhood is protective against the development of atopic disease.

Exposure to indoor allergens is known as a risk factor, but asthma is also associated with high household levels of total and particularly gram-negative bacteria (68), most likely as bacterial products act as adjuvants. It seems to be clear that both environmental and genetic factors contribute to the development of allergic diseases. Studies on the impact of genetic polymorphisms on allergic diseases (69–71) and the importance of gene–environment interactions (72, 73) directed research toward innate immune receptors.

Toll-like receptors (TLRs) are an extensively studied group of cell surface receptors. In every complex species, TLRs are induced in response to challenge by pathogen-associated molecular patterns (PAMPs), as for example LPS or peptidoglycans from gram-negative or gram-positive bacteria, respectively (74). However, these receptors might also play a relevant role in the development of atopy and allergic diseases, and to date, single nucleotide polymorphisms in every TLR gene have been linked to asthma and less so to atopic eczema and atopic sensitization, as summarized elsewhere (75).

First evidence for the involvement of innate immune receptors in allergic disease came from studies on CD14, an innate immunity molecule, that together with TLR4 is primarily involved in LPS recognition. However, in contrast to TLRs, CD14 lacks a transmembrane domain and is therefore not directly involved in the initiation of signaling. A French initiative found gene–environment interactions between 10 polymorphisms in candidate genes such as CD14 in adults that were living in rural areas during childhood (76). The results are bolstered by another study that suggests that peripheral blood mononuclear cells (PBMCs) from asthmatic children with the TT genotype at position –159 of the CD14

gene synthesize more IgE upon LPS stimulation than those with the CC genotype (70).

Patients with asthma were shown to have high serum levels of IL-29 (77). CD14-positive cells have been shown to release IL-29 upon IL-4 stimulation and thus contribute to the pathogenesis of allergic inflammation via modulating immune cells' function to release proinflammatory cytokines (77). Additionally, Bratke et al. found that plasmacytoid DCs, which matured under a Th2 dominant environment, showed a significant increase in IL-4 expression following stimulation of TLR7 (78). Whether this would in turn lead to an IL-29 release was not addressed in the latter study.

In addition to allergic asthma being associated with a Th2-oriented response, also Th17 cells seem to play a role in the pathogenesis of asthma. It has been shown that the TLR3 agonist polyinosinic-polycytidylic acid (poly(I:C)) upregulated the *in vitro* IL-17A production of a natural killer T (NKT) cell subset, without modifying type 1 and type 2 cytokines (79). These effects were attributable to IL-1 β and IL-23 release from DCs. Interestingly, TLR7 agonists inhibited the IL-17A production by poly(I:C). This was associated with the increased production of IL-17A-inhibiting cytokines and the dampening of IL-1 β and IL-23 by DCs (79). Results from Choi et al. point toward the same direction (80).

Only recently, Wilson and colleagues showed that the bacterial protein flagellin, a ligand to TLR5, promotes asthma-like responses to house dust extracts and ovalbumin in mice. This response was markedly reduced in *TLR5*^{-/-} mice, but not in wild-type or *TLR4*^{-/-} mice, suggesting that flagellin is a major component of house dust extracts for priming asthma-like responses to inhaled antigens (81).

Another innate immune mechanism that might be involved in the pathogenesis of asthma is the inflammasome. It is activated by endogenous danger signals such as adenosine triphosphate (ATP) or uric acid crystals released from dying cells (82). A critical role of the NLRP3 inflammasome was recently highlighted in experimental allergic asthma induced in the presence of aluminum hydroxide (alum) adjuvant (83). In a study, using an adjuvant-free mouse model of allergic asthma, a critical role of the NLRP3 inflammasome and IL-1 signaling in driving lung inflammation was shown as well (84). The authors of this study demonstrated that NLRP3 inflammasome activation, occurring during allergic lung inflammation, contributes to DC functions and the induction of Th2 response. NLRP3 disruption strongly affected IL-1 signaling, which is crucial for asthma pathogenesis (84). These data suggest that the NLRP3/IL1 pathway might emerge as a novel therapeutic target in allergy.

Millien and colleagues provide sound evidence for the theory that allergic airway inflammation represents an antifungal defensive strategy that is driven by fibrinogen cleavage and TLR4 activation (85). The authors demonstrate that TLR4 is activated by airway proteinase activity to initiate both allergic airway disease and antifungal immunity. *TLR4*^{-/-} mice did not mount a robust allergic airway disease when challenged by proteinase, viable fungi or other triggers but exhibited normal Th2 immunity. These outcomes were induced by proteinase cleavage of fibrinogen, yielding

fibrinogen cleavage products that acted as TLR4 ligands on airway epithelial cells and macrophages (85). It can be assumed that the described pathway might represent a novel therapeutic target in allergic disease.

Extracellular nucleotides

Recently, extracellular nucleotides have gained considerable attention as inflammatory mediators in allergic disorders. Following allergen challenge, high levels of extracellular ATP accumulate in the airways of asthma patients or sensitized mice, as this product is released upon cellular damage or by activated leukocytes (86, 87). ATP and other nucleotides act as agonists of type 2 purinergic receptors, and this binding has been reported to be crucial for the initiation and maintenance of allergic airway inflammation, given that the P2Y₂ receptor appears to be involved in asthmatic airway inflammation by mediating ATP-triggered migration of DCs and eosinophils (88). Likewise, an increase in ATP hydrolysis or a deficiency of the P2Y₂ receptor attenuate the recruitment or activation of these cells, indicating a central role of purinergic signaling in the pathogenesis and maintenance of allergic inflammation (86, 88).

Purinergic signaling is terminated by receptor desensitization (89), and/or scavenging of extracellular nucleotides by ecto-nucleoside triphosphate diphosphohydrolases (NTPDases) that mediate their hydrolysis. ATP is catabolized into adenosine through a two-step enzymatic process mediated by NTPDase1/CD39 and CD73 (5'-ectonucleotidase) (90). CD39 expression is induced by proinflammatory cytokines, oxidative stress, and hypoxia through several transcription factors, such as Sp1, Stat3, and zinc finger protein growth factor independent-1 (91). CD39 mediates the hydrolysis of nucleotides in a Ca²⁺- and Mg²⁺- dependent manner and therefore plays a central role in regulating the effect of ATP-mediated inflammation in the airways (92). Notably, it has been reported that *Cd39* deficiency in murine experimental models is associated with a decrease in allergic airway inflammation, likely as a consequence of an ATP-mediated desensitization effect of P2YR in *Cd39*^{-/-} DCs that affects their migration and Th2 priming capacity (90). Similarly, mice deficient of CD73 exhibit attenuated hyperresponsiveness and less eosinophil and mast cell infiltration in allergic inflammation (93). Conversely, it was recently shown that inhibition of CD39 resulted in high levels of extracellular nucleotides, therefore confirming the central role of this ectonucleotidase in regulating the amount of nucleotides produced upon antigenic challenge. However, the high quantities of extracellular nucleotides resulted in P2Y receptor activation, which indirectly triggers thromboxane 2 (TXA₂) release, and allergic bronchospasm (87). Additionally, it was observed that the P2Y₄ and P2Y₆ receptors were upregulated upon sensitization, suggesting a potential pathogenic role of these purinergic receptors (87). Taken together, recent studies point toward the therapeutic potential of approaches targeting purinergic signaling that might involve strategies to block P2 receptors and/or to enhance the catabolism of extracellular nucleotides by ectonucleotidases.

Pruritus

Pruritus (itch) is defined as an unpleasant sensation provoking the desire to scratch and constitutes an essential feature of atopic dermatitis (94). Chronic itch can be caused many different etiologies and is a burdensome condition that often substantially affects the quality of life. The itch in allergic and other inflammatory diseases of the skin is categorized as pruriceptive. This denotes the fact that the itch originates from the activation of primary sensory nerve terminals located in the skin and in some mucosal areas (95); in contrast, neuropathic and neurogenic itch originate from damaged nerves and from inappropriate central nervous system activation, respectively. The treatment of pruritus in allergic and other conditions is still not satisfactory, and it is a serious unmet clinical need (94).

Current evidence favors the concept that the itch sensation is mediated by specific itch pathways, that is, specialized primary sensory neurons connected to dedicated second-order neurons in the spinal cord. Although the itch pathways have not been conclusively elucidated, a great progress has been made in this area in recent years [reviewed in (95)]. The studies in humans and nonhuman primates indicate that at the level of primary sensory neurons, there are at least two itch pathways – (1) histamine-sensitive C-fibers unresponsive to heat and mechanical stimulation that mediate the histamine-induced itch (96, 97), and (2) histamine-insensitive mechanically sensitive C-fibers (98, 99). There is also evidence suggesting that at least two central pathways in the spinal cord are involved in the itch signal transmission. Importantly, a recent paper provided a strong evidence that the itch pathways mediating responses to a wide range of pruritogens (classed as inducing histamine- and non-histamine-related itch) utilize the neuropeptide natriuretic polypeptide b (Nppb) for neurotransmission (100). Nppb appears to act as an excitatory neurotransmitter in the first synapse between the primary sensory neurons and the spinal cord second-order neurons that express Nppb receptor (Npra), upstream of another important itch neurotransmitter gastrin-releasing peptide (GRP). These findings reveal a molecular convergence of itch pathways and provide a novel rational target for the development of therapies to alleviate chronic itch.

A number of substances produced in inflammation are capable of stimulating the itch C-fibers and cause pruriceptive itch in humans and animal models. In addition to histamine, these substances include serotonin, leukotrienes, proteases, substance P, IL-31, TLR7 agonists, lysophosphatidic acid, autotaxin, and others. Importantly, in a manner analogous to sensitization of pain-mediating nociceptive pathways (e.g., hyperalgesia and allodynia), the itch pathways can be also sensitized. For example, compared with healthy skin, the atopic dermatitis patients have a tendency to itch upon minimal stimuli because of reduced itch threshold and perceive a prolonged itch duration (94). Consistently, the dose of histamine required to evoke itch in lesional skin of patients with atopic dermatitis is lower than in normal healthy skin (101). The mechanisms of the itch pathways sensitizations have not been clarified; it is often speculated that

they are similar to those in pain pathways and include central and/or peripheral sensitization.

Cold contact urticaria (CCU) is a common subtype of physical urticaria characterized by itchy wheal and flare responses due to the release of histamine and other proinflammatory mediators after exposure to cold (102). This has been attributed to the high propensity of mast cells of patients with CCU to degranulate in response to cold exposure. The evidence for mast cell degranulation has been established by increased serum histamine levels and the localized release of tryptase in postchallenge biopsies (103). In extreme situations, such as swimming in colder water, this condition can be life-threatening because of a more generalized mast cells degranulation and anaphylactic reaction (102). While the mechanisms triggering the mast cell degranulation are largely unknown (104), an interesting clinical experiment indicated that the mast cell degranulation may be secondary to nerve activation (105).

Symptomatic treatment of choice is the use of nonsedating antihistamines that also effectively treat the pruritus. A recent study addressed the question whether up-dosing with new H₁-antihistamine bilastine results in improved effectiveness in cold contact urticaria (106). Overall, the study concluded that the fourfold up-dosing increased efficacy of bilastine and supports urticaria treatment guidelines. However, the study also noted that maximal effect on itch was already achieved with the standard dose. The median pruritus score (on the scale 0–3, with 75% confidence limits) for the placebo treatment was 2.0 (1.25–3.0), and 3 of 20 patients reported no itch. Following treatment with the standard bilastine dose of 20 mg daily, the pruritus score was 0 (0–1) ($P = 0.001$ vs placebo) with 13 of the 20 patients reporting no itch. There was no difference between the effect of 20 mg and increased doses of 40 mg and 80 mg. These data are consistent with the conclusion that much of the itch in cold contact urticaria is mediated by H₁-receptor (presumably on the histamine-sensitive C-fiber itch pathway). That the fourfold increase in the H₁-antihistamine dosing did not eliminate itch in some patients demonstrates a minor contribution of an H₁-independent mechanism to itch in contact cold urticaria. Given that mast cells contain also pruritogens other than histamine (e.g., proteases), it is interesting that in CCU characterized by a mast cell degranulation, the itch is nearly entirely H₁-mediated. It suggests that either the mast cells degranulate in a manner that leads predominately to histamine release or the mast cells that degranulate are predominately located in the vicinity of histamine-sensitive itch nerve terminals in the skin.

The histamine H₄ receptor (H₄R) plays important roles in the activation of mast cells, eosinophils, monocytes, DCs, and T cells. The experiments utilizing the H₄R-deficient mice (107) or H₄R antagonists (108) indicated that H₄R plays roles in pruritus and acute inflammation. A recent study explored the possibility that the combination of a H₁R antagonist and H₄R antagonist attenuates chronic dermatitis in a model of chronic allergic dermatitis established in NC/Nga mice (109). The authors found that the combination of H₁R antagonist olopatadine and H₄R antagonist

JNJ777120 improved scratching behavior and was more effective than each of the antagonists individually. The effect of antihistamines on itch in this study can be attributed to both the effect on inflammation (the treatment reduced the tissue mast cells, cytokines, and chemokines) and the direct effect on the itch-mediating pathways. Histological analysis demonstrated that 40% of spinal (dorsal root ganglia, DRG) small diameter C-fiber neurons (the population that also includes itch C-fibers) express the histamine H4 receptor (110). Interestingly, the H1R and H4R antagonists also partially reduced the level of NGF in inflamed skin. Inasmuch as NGF is known to contribute to sensory hypersensitivity in C-fibers, reducing the NGF levels is predicted to reduce the C-fiber hypersensitivity and may thus contribute to reduced scratch behavior in this model.

Conclusions

The network of cells and mediators involved in the pathogenesis and maintenance of allergic inflammation is complex. Being aware that it is not possible to cover all important evidence in the field, here we highlight select advances in deciphering this network with potential implications for novel therapeutic strategies. Besides novel pathogenetic insights

derived from basic and translational research, a better understanding of the diverse pathways engaged by pleiotropic immunomodulatory molecules and further analysis of genetic differences will lead to the identification of novel therapeutic targets and a more rational and individualized use of therapeutics in allergic disease.

Acknowledgments

SVG is supported by the Swiss National Science Foundation (SNSF) grant No. 310030_135734 and the Bulgarian-Swiss Research Programme (BSRP) No. IZEBZ0_142967. M.K. is supported by 2012/33-UKMA-10 and VEGA 1/0037/11. The authors thank Aldona von Gunten for the illustrations in Figs 1 and 2.

Author contributions

All of the authors wrote sections of the article, drafted revisions, and approved the final draft.

Conflicts of interest

The authors declare that they have no conflicts of interest.

References

- Minai-Fleminger Y, Elishmereni M, Vita F, Rosa Soranzo M, Mankuta D, Zabucchi G et al. Ultrastructural evidence for human mast cell-eosinophil interactions in vitro. *Cell Tissue Res* 2010;**341**:405–415.
- Elishmereni M, Alenius HT, Bradding P, Mizrahi S, Shikotra A, Minai-Fleminger Y et al. Physical interactions between mast cells and eosinophils: a novel mechanism enhancing eosinophil survival in vitro. *Allergy* 2011;**66**:376–385.
- Elishmereni M, Bachelet I, Nissim Ben-Efraim AH, Mankuta D, Levi-Schaffer F. Interacting mast cells and eosinophils acquire an enhanced activation state in vitro. *Allergy* 2013;**68**:171–179.
- Ben Zimra M, Bachelet I, Seaf M, Gleich GJ, Levi-Schaffer F. Eosinophil major basic protein activates human cord blood mast cells primed with fibroblast membranes by integrin- β 1. *Allergy* 2013;**68**:1259–1268.
- Bossi F, Frossi B, Radillo O, Cugno M, Tedeschi A, Riboldi P et al. Mast cells are critically involved in serum-mediated vascular leakage in chronic urticaria beyond high-affinity IgE receptor stimulation. *Allergy* 2011;**66**:1538–1545.
- Sun J, Dahlén B, Agerberth B, Haeggström JZ. The antimicrobial peptide LL-37 induces synthesis and release of cysteinyl leukotrienes from human eosinophils – implications for asthma. *Allergy* 2013;**68**:304–311.
- Galli SJ, Tsai M, Piliponsky AM. The development of allergic inflammation. *Nature* 2008;**454**:445–454.
- Pezato R, Świerczyńska-Krępa M, Niżankowska-Mogilnicka E, Derycke L, Bachert C, Pérez-Novo CA. Role of imbalance of eicosanoid pathways and staphylococcal superantigens in chronic rhinosinuitis. *Allergy* 2012;**67**:1347–1356.
- Karasuyama H, Obata K, Wada T, Tsujimura Y, Mukai K. Newly appreciated roles for basophils in allergy and protective immunity. *Allergy* 2011;**66**:1133–1141.
- Wynn TA. Basophils trump dendritic cells as APCs for TH2 responses. *Nat Immunol* 2009;**10**:679–681.
- Wakahara K, Van VQ, Baba N, Bégin P, Rubio M, Delespesse G et al. Basophils are recruited to inflamed lungs and exacerbate memory Th2 responses in mice and humans. *Allergy* 2013;**68**:180–189.
- von Gunten S, Marsland BJ, von Garnier C, Simon D. Update in clinical allergy and immunology. *Allergy* 2012;**67**:1491–1500.
- Knol EF, Gibbs BF. Basophils and antigen presentation: of mice and not men? *Allergy* 2012;**67**:579–580.
- Eckl-Dorna J, Ellinger A, Blatt K, Ghanim V, Steiner I, Pavelka M et al. Basophils are not the key antigen-presenting cells in allergic patients. *Allergy* 2012;**67**:601–608.
- Kitzmüller C, Nagl B, Deifl S, Walterskirchen C, Jahn-Schmid B, Zlabinger GJ et al. Human blood basophils do not act as antigen-presenting cells for the major birch pollen allergen Bet v 1. *Allergy* 2012;**67**:593–600.
- Ito Y, Satoh T, Takayama K, Miyagishi C, Walls AF, Yokozeki H. Basophil recruitment and activation in inflammatory skin diseases. *Allergy* 2011;**66**:1107–1113.
- Ramadan A, Van Pham L, Machavoine F, Dietrich C, Alkan M, Karasuyama H et al. Activation of basophils by the double-stranded RNA poly(A:U) exacerbates allergic inflammation. *Allergy* 2013;**68**:732–738.
- Falkenroth S, Poulsen LK, Bindslev-Jensen C, Woetmann A, Odum N, Poulsen BC et al. IgE-mediated basophil tumour necrosis factor alpha induces matrix metalloproteinase-9 from monocytes. *Allergy* 2013;**68**:614–620.
- Horak F, Ziegelmayer P, Ziegelmayer R, Lemell P, Collins LP, Hunter MG et al. The CRTH2 antagonist OC000459 reduces nasal and ocular symptoms in allergic subjects exposed to grass pollen, a randomised, placebo-controlled, double-blind trial. *Allergy* 2012;**67**:1572–1579.
- Straumann A, Hoesli S, Bussmann C, Stuck M, Perkins M, Collins LP et al. Anti-eosinophil activity and clinical efficacy of the CRTH2 antagonist OC000459 in eosinophilic esophagitis. *Allergy* 2013;**68**:375–385.
- Barnes PJ. Corticosteroid resistance in patients with asthma and chronic

- obstructive pulmonary disease. *J Allergy Clin Immunol* 2013;**131**:636–645.
22. Seguro LPC, Rosario C, Shoenfeld Y. Long-term complications of past glucocorticoid use. *Autoimmun Rev* 2013;**12**:629–632.
 23. Nixon M, Andrew R, Chapman KE. It takes two to tango: dimerisation of glucocorticoid receptor and its anti-inflammatory functions. *Steroids* 2013;**78**:59–68.
 24. Vandevyver S, Dejager L, Tuckermann J, Libert C. New insights into the anti-inflammatory mechanisms of glucocorticoids: an emerging role for glucocorticoid-receptor-mediated transactivation. *Endocrinology* 2013;**154**:993–1007.
 25. Hardy RS, Raza K, Cooper MS. Endogenous glucocorticoids in inflammation: contributions of systemic and local responses. *Swiss Med Wkly* 2012;**142**:w13650.
 26. Taves MD, Gomez-Sanchez CE, Soma KK. Extra-adrenal glucocorticoids and mineralocorticoids: evidence for local synthesis, regulation, and function. *Am J Physiol Endocrinol Metab* 2011;**301**:E11–E24.
 27. von Gunten S, Kaufmann T. Glucocorticoids 'on air'. *Allergy* 2012;**67**:144–146.
 28. Hostettler N, Bianchi P, Gennari-Moser C, Kassahn D, Schoonjans K, Corazza N et al. Local glucocorticoid production in the mouse lung is induced by immune cell stimulation. *Allergy* 2012;**67**:227–234.
 29. Noti M, Corazza N, Mueller C, Berger B, Brunner T. TNF suppresses acute intestinal inflammation by inducing local glucocorticoid synthesis. *J Exp Med* 2010;**207**:1057–1066.
 30. Bozzetto S, Carraro S, Giordano G, Boner A, Baraldi E. Asthma, allergy and respiratory infections: the vitamin D hypothesis. *Allergy* 2012;**67**:10–17.
 31. Benson AA, Toh JA, Vernon N, Jariwala SP. The role of vitamin D in the immunopathogenesis of allergic skin diseases. *Allergy* 2012;**67**:296–301.
 32. Kanda N, Hau CS, Tada Y, Sato S, Watanabe S. Decreased serum LL-37 and vitamin D3 levels in atopic dermatitis: relationship between IL-31 and oncostatin M. *Allergy* 2012;**67**:804–812.
 33. Vimalaswaran KS, Cavadino A, Hyppönen E. Evidence for a genetic interaction in allergy-related responsiveness to vitamin D deficiency. *Allergy* 2012;**67**:1033–1040.
 34. Liu X, Wang G, Hong X, Wang D, Tsai HJ, Zhang S et al. Gene–vitamin D interactions on food sensitization: a prospective birth cohort study. *Allergy* 2011;**66**:1442–1448.
 35. Keet CA, Matsui EC, Savage JH, Neuman-Sunshine DL, Skripak J, Peng RD et al. Potential mechanisms for the association between fall birth and food allergy. *Allergy* 2012;**67**:775–782.
 36. Devereux G, Wilson A, Avenell A, McNeill G, Fraser WD. A case-control study of vitamin D status and asthma in adults. *Allergy* 2010;**65**:666–667.
 37. Hyppönen E, Berry DJ, Wjst M, Power C. Serum 25-hydroxyvitamin D and IgE – a significant but nonlinear relationship. *Allergy* 2009;**64**:613–620.
 38. Carraro S, Giordano G, Reniero F, Carpi D, Stocchero M, Sterk PJ et al. Asthma severity in childhood and metabolomic profiling of breath condensate. *Allergy* 2013;**68**:110–117.
 39. Weisse K, Winkler S, Hirche F, Herberth G, Hinz D, Bauer M et al. Maternal and newborn vitamin D status and its impact on food allergy development in the German LINA cohort study. *Allergy* 2013;**68**:220–228.
 40. Heimbeck I, Wjst M, Apfelbacher CJ. Low vitamin D serum level is inversely associated with eczema in children and adolescents in Germany. *Allergy* 2013;**68**:906–910.
 41. Hartmann B, Heine G, Babina M, Steinmeyer A, Zügel U, Radbruch A et al. Targeting the vitamin D receptor inhibits the B cell-dependent allergic immune response. *Allergy* 2011;**66**:540–548.
 42. Kraus WL, Hottiger MO. PARP-1 and gene regulation: progress and puzzles. *Mol Aspects Med* 2013, doi: 10.1016/j.mam.2013.01.005.
 43. Szabó É, Kovács I, Grune T, Haczku A, Virág L. PARP-1: a new player in the asthma field? *Allergy* 2011;**66**:811–814.
 44. Rouleau M, Patel A, Hendzel MJ, Kaufmann SH, Poirier GG. PARP inhibition: PARP1 and beyond. *Nat Rev Cancer* 2010;**10**:293–301.
 45. Datta R, Naura AS, Zerfaoui M, Errami Y, Oumouna M, Kim H et al. PARP-1 deficiency blocks IL-5 expression through calpain-dependent degradation of STAT-6 in a murine asthma model. *Allergy* 2011;**66**:853–861.
 46. Curtin NJ, Szabo C. Therapeutic applications of PARP inhibitors: anticancer therapy and beyond. *Mol Aspects Med* 2013, doi: 10.1016/j.mam.2013.01.006.
 47. Gelfand EW. Intravenous immune globulin in autoimmune and inflammatory diseases. *N Engl J Med* 2012;**367**:2015–2025.
 48. Orange J, Hossny E, Weiler C, Ballou M, Berger M, Bonilla F et al. Use of intravenous immunoglobulin in human disease: a review of evidence by members of the Primary Immunodeficiency Committee of the American Academy of Allergy, Asthma and Immunology. *J Allergy Clin Immunol* 2006;**117**:S525–S553.
 49. Kaufman GN, Massoud AH, Audousseau S, Banville-Langelier AA, Wang Y, Guay J et al. Intravenous immunoglobulin attenuates airway hyperresponsiveness in a murine model of allergic asthma. *Clin Exp Allergy* 2011;**41**:718–728.
 50. Massoud AH, Guay J, Shalaby KH, Bjur E, Ablona A, Chan D et al. Intravenous immunoglobulin attenuates airway inflammation through induction of forkhead box protein 3–positive regulatory T cells. *J Allergy Clin Immunol* 2012;**129**:e1653.
 51. Svetlicky N, Ortega-Hernandez O-D, Moulton L, Guillemin L, Thiesen H-J, Altman A et al. The advantage of specific intravenous immunoglobulin (sIVIG) on regular IVIG: experience of the last decade. *J Clin Immunol* 2013;**33**:S27–S32.
 52. Durandy A, Kaveri S, Kuijpers T, Basta M, Miescher S, Ravetch JV et al. Intravenous immunoglobulins—understanding properties and mechanisms. *Clin Exp Immunol* 2009;**158**(Suppl 1):2–13.
 53. Miescher SM, Käsermann F. The future of immunoglobulin therapy: an overview of the 2nd international workshop on natural antibodies in health and disease. *Autoimmun Rev* 2013;**12**:639–642.
 54. Trinath J, Hegde P, Balaji KN, Kaveri SV, Bayry J. Intravenous immunoglobulin-mediated regulation of Notch ligands on human dendritic cells. *J Allergy Clin Immunol* 2013;**131**:e1251.
 55. von Gunten S, Simon HU. Cell death modulation by intravenous immunoglobulin. *J Clin Immunol* 2010;**30**(Suppl 1):S24–S30.
 56. von Gunten S, Vogel M, Schaub A, Stadler BM, Miescher S, Crocker PR et al. Intravenous immunoglobulin preparations contain anti-Siglec-8 autoantibodies. *J Allergy Clin Immunol* 2007;**119**:1005–1011.
 57. Nutku E, Aizawa H, Hudson SA, Bochner BS. Ligation of Siglec-8: a selective mechanism for induction of human eosinophil apoptosis. *Blood* 2003;**101**:5014–5020.
 58. von Gunten S, Schaub A, Vogel M, Stadler BM, Miescher S, Simon HU. Immunological and functional evidence for anti-Siglec-9 autoantibodies in intravenous immunoglobulin (IVIg) preparations. *Blood* 2006;**108**:4255–4259.
 59. Teeling JL, Jansen-Hendriks T, Kuijpers TW, de Haas M, van de Winkel JGJ, Hack CE et al. Therapeutic efficacy of intravenous immunoglobulin preparations depends on the immunoglobulin G dimers: studies in experimental immune thrombocytopenia. *Blood* 2001;**98**:1095–1099.
 60. Schaub A, von Gunten S, Vogel M, Wymann S, Rügsegger M, Stadler BM et al. Dimeric IVIG contains natural anti-Siglec-9 autoantibodies and their anti-idiotypes. *Allergy* 2011;**66**:1030–1037.
 61. Kaneko Y, Nimmerjahn F, Ravetch JV. Anti-inflammatory activity of immunoglobulin G resulting from Fc sialylation. *Science* 2006;**313**:670–673.

62. Leontyev D, Katsman Y, Ma X-Z, Miescher S, Käsermann F, Branch DR. Sialylation-independent mechanism involved in the amelioration of murine immune thrombocytopenia using intravenous gammaglobulin. *Transfusion* 2012;**52**:1799–1805.
63. Guhr T, Bloem J, Derksen NI, Wührer M, Koenderman AH, Aalberse RC et al. Enrichment of sialylated IgG by lectin fractionation does not enhance the efficacy of immunoglobulin G in a murine model of immune thrombocytopenia. *PLoS ONE* 2011;**6**:e21246.
64. Käsermann F, Boerema D, Rüeggsegger M, Hofmann A, Wymann S, Zuercher A et al. Analysis and functional consequences of increased Fab-sialylation of intravenous immunoglobulin (IVIG) after lectin fractionation. *PLoS ONE* 2012;**7**:e37243.
65. Masoli M, Fabian D, Holt S, Beasley R. The global burden of asthma: executive summary of the GINA Dissemination Committee report. *Allergy* 2004;**59**:469–478.
66. Mallol J, Crane J, von Mutius E, Odhiambo J, Keil U, Stewart A et al. The International Study of Asthma and Allergies in Childhood (ISAAC) Phase three: a global synthesis. *Allergol Immunopathol (Madr)* 2013;**41**:73–85.
67. Busse WW, Lemanske RF Jr. Asthma. *N Engl J Med* 2001;**344**:350–362.
68. Ross MA, Curtis L, Scheff PA, Hryhorczuk DO, Ramakrishnan V, Wadden RA et al. Association of asthma symptoms and severity with indoor bioaerosols. *Allergy* 2000;**55**:705–711.
69. Niebuhr M, Langnickel J, Draing C, Renz H, Kapp A, Werfel T. Dysregulation of toll-like receptor-2 (TLR-2)-induced effects in monocytes from patients with atopic dermatitis: impact of the TLR-2 R753Q polymorphism. *Allergy* 2008;**63**:728–734.
70. Sackesen C, Birben E, Soyer OU, Sahiner UM, Yavuz TS, Civelek E et al. The effect of CD14 C159T polymorphism on in vitro IgE synthesis and cytokine production by PBMC from children with asthma. *Allergy* 2011;**66**:48–57.
71. Vercelli D. Discovering susceptibility genes for asthma and allergy. *Nat Rev Immunol* 2008;**8**:169–182.
72. Arbour NC, Lorenz E, Schutte BC, Zabner J, Kline JN, Jones M et al. TLR4 mutations are associated with endotoxin hyporesponsiveness in humans. *Nat Genet* 2000;**25**:187–191.
73. Eder W, Klimecki W, Yu L, von Mutius E, Riedler J, Braun-Fahrlander C et al. Toll-like receptor 2 as a major gene for asthma in children of European farmers. *J Allergy Clin Immunol* 2004;**113**:482–488.
74. Akira S, Takeda K, Kaisho T. Toll-like receptors: critical proteins linking innate and acquired immunity. *Nat Immunol* 2001;**2**:675–680.
75. Tesse R, Pandey RC, Kabesch M. Genetic variations in toll-like receptor pathway genes influence asthma and atopy. *Allergy* 2011;**66**:307–316.
76. Smit LA, Siroux V, Bouzigon E, Oryszczyn MP, Lathrop M, Demenais F et al. CD14 and toll-like receptor gene polymorphisms, country living, and asthma in adults. *Am J Respir Crit Care Med* 2009;**179**:363–368.
77. He S, Li T, Chen H, Ma W, Yao Q, Yang H et al. CD14+ cell-derived IL-29 modulates proinflammatory cytokine production in patients with allergic airway inflammation. *Allergy* 2011;**66**:238–246.
78. Bratke K, Klein C, Kuepper M, Lommatzsch M, Virchow JC. Differential development of plasmacytoid dendritic cells in Th1- and Th2-like cytokine milieu. *Allergy* 2011;**66**:386–395.
79. Vultaggio A, Nencini F, Pratesi S, Petroni G, Romagnani S, Maggi E. Poly(I:C) promotes the production of IL-17A by murine CD1d-driven invariant NKT cells in airway inflammation. *Allergy* 2012;**67**:1223–1232.
80. Choi JP, Kim YS, Kim OY, Kim YM, Jeon SG, Roh TY et al. TNF-alpha is a key mediator in the development of Th2 cell response to inhaled allergens induced by a viral PAMP double-stranded RNA. *Allergy* 2012;**67**:1138–1148.
81. Wilson RH, Maruoka S, Whitehead GS, Foley JF, Flake GP, Sever ML et al. The Toll-like receptor 5 ligand flagellin promotes asthma by priming allergic responses to indoor allergens. *Nat Med* 2012;**18**:1705–1710.
82. Bianchi ME. DAMPs, PAMPs and alarmins: all we need to know about danger. *J Leukoc Biol* 2007;**81**:1–5.
83. Eisenbarth SC, Colegio OR, O'Connor W, Sutterwala FS, Flavell RA. Crucial role for the Nalp3 inflammasome in the immunostimulatory properties of aluminium adjuvants. *Nature* 2008;**453**:1122–1126.
84. Besnard AG, Guillou N, Tschopp J, Erard F, Couillin I, Iwakura Y et al. NLRP3 inflammasome is required in murine asthma in the absence of aluminum adjuvant. *Allergy* 2011;**66**:1047–1057.
85. Millien VO, Lu W, Shaw J, Yuan X, Mak G, Roberts L et al. Cleavage of fibrinogen by proteinases elicits allergic responses through Toll-like receptor 4. *Science* 2013;**341**:792–796.
86. Idzko M, Hammad H, van Nimwegen M, Kool M, Willart MAM, Muskens F et al. Extracellular ATP triggers and maintains asthmatic airway inflammation by activating dendritic cells. *Nat Med* 2007;**13**:913–919.
87. Chávez J, Vargas MH, Rebollar-Ayala DC, Díaz-Hernández V, Cruz-Valderrama JE, Flores-Soto E et al. Inhibition of extracellular nucleotides hydrolysis intensifies the allergic bronchospasm. A novel protective role of ectonucleotidases. *Allergy* 2013;**68**:462–471.
88. Müller T, Robaye B, Vieira RP, Ferrari D, Grimm M, Jakob T et al. The purinergic receptor P2Y2 receptor mediates chemotaxis of dendritic cells and eosinophils in allergic lung inflammation. *Allergy* 2010;**65**:1545–1553.
89. Enjyoji K, Sevigny J, Lin Y, Frenette PS, Christie PD, Esch JSA et al. Targeted disruption of cd39/ATP diphosphohydrolase results in disordered hemostasis and thromboregulation. *Nat Med* 1999;**5**:1010–1017.
90. Idzko M, K Ayata C, Müller T, Dürk T, Grimm M, Baudiß K et al. Attenuated allergic airway inflammation in Cd39 null mice. *Allergy* 2013;**68**:472–480.
91. Antoniolli L, Pacher P, Vizi ES, Haskó G. CD39 and CD73 in immunity and inflammation. *Trends Mol Med* 2013;**19**:355–367.
92. Vitiello L, Gorini S, Rosano G, la Sala A. Immunoregulation through extracellular nucleotides. *Blood* 2012;**120**:511–518.
93. Schreiber R, Castrop H, Kunzelmann K. Allergen-induced airway hyperresponsiveness is absent in ecto-5'-nucleotidase (CD73)-deficient mice. *Pflügers Arch* 2008;**457**:431–440.
94. Buddenkotte J, Steinhoff M. Pathophysiology and therapy of pruritus in allergic and atopic diseases. *Allergy* 2010;**65**:805–821.
95. Potenziari C, Udem BJ. Basic mechanisms of itch. *Clin Exp Allergy* 2012;**42**:8–19.
96. Schmelz M, Schmidt R, Bickel A, Handwerker HO, Torebjork HE. Specific C-receptors for itch in human skin. *J Neurosci* 1997;**17**:8003–8008.
97. Schmelz M, Schmidt R, Weidner C, Hilliges M, Torebjork HE, Handwerker HO. Chemical response pattern of different classes of C-nociceptors to pruritogens and algogens. *J Neurophysiol* 2003;**89**:2441–2448.
98. Johanek LM, Meyer RA, Friedman RM, Greenquist KW, Shim B, Borzan J et al. A role for polymodal C-fiber afferents in non-histaminergic itch. *J Neurosci* 2008;**28**:7659–7669.
99. Namer B, Carr R, Johanek LM, Schmelz M, Handwerker HO, Ringkamp M. Separate peripheral pathways for pruritus in man. *J Neurophysiol* 2008;**100**:2062–2069.
100. Mishra SK, Hoon MA. The cells and circuitry for itch responses in mice. *Science* 2013;**340**:968–971.
101. Ikoma A, Rukwied R, Stander S, Steinhoff M, Miyachi Y, Schmelz M. Neuronal sensitization for histamine-induced itch in

- lesional skin of patients with atopic dermatitis. *Arch Dermatol* 2003;**139**:1455–1458.
102. Krause K, Zuberbier T, Maurer M. Modern approaches to the diagnosis and treatment of cold contact urticaria. *Curr Allergy Asthma Rep* 2010;**10**:243–249.
 103. Kaplan AP, Garofalo J, Sigler R, Hauber T. Idiopathic cold urticaria: in vitro demonstration of histamine release upon challenge of skin biopsies. *N Engl J Med* 1981;**305**:1074–1077.
 104. Siebenhaar F, Weller K, Mlynek A, Magerl M, Altrichter S, Vieira Dos Santos R et al. Acquired cold urticaria: clinical picture and update on diagnosis and treatment. *Clin Exp Dermatol* 2007;**32**:241–245.
 105. Husz S, Toth-Kasa I, Obal F, Jancso G. A possible pathomechanism of the idiopathic cold contact urticaria. *Acta Physiol Hung* 1991;**77**:209–215.
 106. Krause K, Spohr A, Zuberbier T, Church MK, Maurer M. Up-dosing with bilastine results in improved effectiveness in cold contact urticaria. *Allergy* 2013;**68**:921–928.
 107. Dunford PJ, Williams KN, Desai PJ, Karlsson L, McQueen D, Thurmond RL. Histamine H4 receptor antagonists are superior to traditional antihistamines in the attenuation of experimental pruritus. *J Allergy Clin Immunol* 2007;**119**:176–183.
 108. Seike M, Furuya K, Omura M, Hamada-Watanabe K, Matsushita A, Ohtsu H. Histamine H4 receptor antagonist ameliorates chronic allergic contact dermatitis induced by repeated challenge. *Allergy* 2010;**65**:319–326.
 109. Ohsawa Y, Hirasawa N. The antagonism of histamine H1 and H4 receptors ameliorates chronic allergic dermatitis via anti-pruritic and anti-inflammatory effects in NC/Nga mice. *Allergy* 2012;**67**:1014–1022.
 110. Strakhova MI, Nikkel AL, Manelli AM, Hsieh GC, Esbenshade TA, Brioni JD et al. Localization of histamine H4 receptors in the central nervous system of human and rat. *Brain Res* 2009;**1250**:41–48.