

REVIEW ARTICLE

Update in clinical allergy and immunology

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Abstract

In the recent years, a tremendous body of studies has addressed a broad variety of distinct topics in clinical allergy and immunology. In this update, we discuss selected recent data that provide clinically and pathogenetically relevant insights or identify potential novel targets and strategies for therapy. The role of the microbiome in shaping allergic immune responses and molecular, as well as cellular mechanisms of disease, is discussed separately and in the context of atopic dermatitis, as an allergic model disease. Besides summarizing novel evidence, this update highlights current areas of uncertainties and debates that, as we hope, shall stimulate scientific discussions and research activities in the field.

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Research progress goes ahead fast, and each issue of *Allergy* distributes a dozen of new and interesting papers providing actual research results, opinions and reviews in different fields of allergy and clinical immunology. Over time, it becomes difficult to keep an overview on the novelties and trends given by these articles. Here, we give an update aiming to summarize interesting papers published in *Allergy* over the last years and putting them in context with recently published work in the field. As we were not able to cover all fields, we selected three research subjects: (i) Hygiene hypothesis: the role of the microbiome in allergy; (ii) Pathogenic mechanisms of allergic diseases and their potential therapeutic implications; (iii) Atopic dermatitis (AD): skin barrier and allergic inflammation. In our view, the selected papers provide new insights in clinically and pathogenetically important research as well as novel targets and strategies for therapy that might become starting points for future research.

Environmental biodiversity, microbiome and allergy: immunology taught by germs

The hygiene hypothesis and beyond

Since the landmark publication 1989 by Strachan (1), the hygiene hypothesis has been expanded by a growing body of

evidence associating environmental exposure to microorganisms with lower prevalence of atopic disorders such as asthma and hay fever (2–4). As one such example, the farming environment was shown to be protective for allergies through exposure to different bacterial products, including endotoxin and muramic acid (5–8). Underscoring these observations, Ege et al. (9) recently showed that children growing up on farms were exposed to a greater microbial diversity and had a lower risk of developing asthma.

In line with this epidemiological evidence, a study conducted in the border region of Karelia showed more frequent allergic disorders on the Finnish side as compared to the Russian population (10). Although these areas are geographically adjacent with genetically similar populations, differences in lifestyle and environments were thought to account for the divergent allergy prevalence that was subsequently found to increase a decade later (11–15). Additional investigations in the Karelia region only recently provided a more detailed picture of environmental factors that may have contributed to the higher prevalence of atopic disorders in the Finnish population (16). Several salient and noteworthy findings emerged from this survey: Individuals that lived in a biodiverse environment (forest and agricultural areas) had a greater generic diversity of skin microbiota compared to those residing in urban regions or near water surfaces.

Moreover, environmental biodiversity was inversely related to atopy, and individuals with atopy had a lower diversity, but not abundance, of skin commensal gammaproteobacteria found to correlate with interleukin (IL)-10 expression by peripheral blood mononuclear cells (PBMC) of healthy individuals. This study for the first time provided evidence for the association of atopy with the diversity of macrobiota (flowering plant and land use) and microbiota (skin microbial community). How can a more biodiverse environment with more flowering plant influence our skin microbiota? One possible mechanism previously described is that pollen grains and particles may serve as vehicles for microbes endowed with an adjuvant effect that may influence immune responses through Toll-like receptor (TLR) activation on dendritic cells (17, 18). It is noteworthy that the findings from the Karelia studies have contributed to establishing a successful nationwide Finnish programme for the prevention of allergies (19, 20).

Although the above observational data do not provide mechanisms as to how the environment influences atopy, it is likely that environmental microbiota is a key determinant of commensal skin microbial flora, shaping commensal colonization of vast digestive and respiratory mucosal surface areas (Fig. 1).

For decades, it has been known that the intestinal tract harbours an extensive community of commensal bacteria, and with the advent of non-culture-based identification techniques, such as bacterial 16S DNA sequence analysis, we now know that the intestinal microbiome consists of at least 500–1000 different bacterial species. These bacteria exist in a mutualistic relationship with the host, and dysbiosis of this microbiota and/or dysbalance of their metabolic activity can underlie diseases (21). The microbiota is thought to be seeded during birth with infants born via the natural vaginal route having a flora represented by bacterial species typical of the mothers vaginal or gut microbiota, whilst infants born by caesarean section are more exposed to environmental bacteria and species typically found on skin, for example *Staphylococcus* (22, 23). Following the cessation of milk as the primary

food source and consumption of solid food, the microbiota becomes increasingly diverse (24). Although dietary changes influence the constituents of this microbiota, remarkably, data indicate that the commensal microbiota of individuals is relatively stable. Thus, the microbiota might represent one consistent stimulus that shapes the maturation and functionality of host cells through childhood to adulthood (25).

Within this context, it is tempting to draw parallels with epidemiological data that suggest early life origin of lung diseases. For example, events early in life, such as exposure to a rich microbial environment on farms, is protective against the development of allergic asthma as compared to the less diverse microbial environment of westernized cities (26). Data from *in vivo* models of allergic inflammation add further weight to this concept. Axenic (germ-free) mice that harbour no microbiota exhibit an enhanced propensity towards the development of allergic inflammation in the lung following allergen exposure, suggesting that microbial-mediated education of the immune system is required for later development of balanced immune responses (27, 28). In support of such results, direct exposure of the airways to innocuous bacteria can protect mice against allergic airway inflammation by altering pulmonary macrophage and dendritic cell maturation states such that they no longer support the induction of local allergic responses (29). In a recent study by Olszak et al. (28), it was elegantly shown that there is a developmental window in neonates when exposure to microbes is critical for appropriate immune maturation. Utilizing axenic mice, the authors showed that in the absence of microbial exposure, immune cell development was dysregulated such that there was an accumulation of invariant natural killer T (iNKT) cells in the lung and colonic lamina propria. The consequence of this cellular accumulation was exaggerated allergic airway inflammation following allergen inhalation. One particularly important finding from this study was that axenic mice could only be protected against the exaggerated inflammation when they were recolonized with a microbiome during the neonatal period, not when recolonized as adults (28).

Taken together, these studies provide experimental data supporting a functionally positive effect of microbial exposure, particularly during the neonatal period. Moreover, direct exposure to bacteria in the airways appears to be sufficient for eliciting a protective effect (29); however, certainly further studies are required to delineate the respective roles of the intestinal and respiratory microbiota.

The recent discovery of the lung microbiome, although controversial at first, is now gaining wider support and recognition (30–33). There appears to be a core community represented by, but not limited to, the bacterial phyla Proteobacteria, Bacteroidetes, Firmicutes, Fusobacteria and Actinobacteria. In asthmatic lungs, pathogenic Proteobacteria, such as *Haemophilus spp.*, are over-represented whilst Bacteroidetes phyla are more common in healthy lungs (30). Respiratory researchers worldwide are now including microbiome analysis in their studies, and an 'atlas' of the geographical location of various bacterial species in healthy or diseased lungs is forming; however, there remains a paucity of

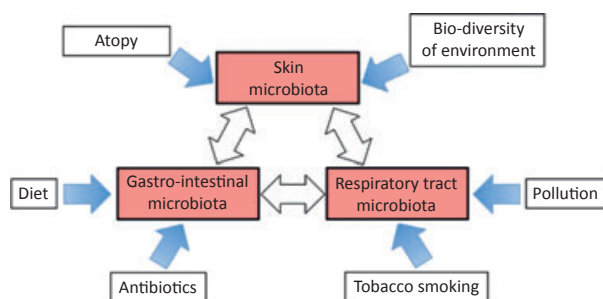


Figure 1 Diagram illustrating the influence of different factors on the organism's microbiota (skin, gastro-intestinal, respiratory tract). Internal host factors (e.g. atopy) and external factors (e.g. bio-diversity of the environment, pollution, smoking, diet and antibiotic use) may shape the organism's microbiota that exerts important immune-modulatory functions.

information concerning the functional relevance of the airway microbiome (34). Comparatively, there is a plethora of functional data from research on host–microbe mutualism in the intestine, where the bacterial burden is orders of magnitude higher (35). Looking out, it is going to be key for the respiratory research field to determine whether analysis of the airway microbiome might add most value within the context of disease biomarkers, or whether indeed the microbiome is relevant for disease development, progression and management. It is indeed conceivable that future novel treatment for allergic disorders may include strategies to shape the microbiome in the organism to enhance conventional approaches with immunotherapy.

Novel insights into pathogenic mechanisms of allergic diseases and their potential therapeutic implications

In the recent years, a considerable body of novel insights has been accumulated on predisposing genetic factors, environmental risk factors, pathogenic mechanisms, subtypes of disease and related phenotype and treatment responses that together highlight the complexity of the pathophysiology of various allergic diseases. Clinicians and researchers in the field are challenged by the circumstance that diseases, such as asthma and atopic dermatitis, often present with a ‘phenotype’ of miscellaneous clinically observable characteristics without direct relationship to an underlying pathophysiology (36, 37). On the other hand, the elucidation of cellular and molecular mechanisms will lead to the identification of novel therapeutic targets and to the definition of specific disease subtypes (endotypes) that may benefit from individualized treatment approaches. In that perspective, here, we highlight some recent evidence from experimental work that may have future diagnostic or therapeutic implications.

Glucocorticoid production in the lung

Steroids are widely used as systemic or topical treatment in allergic diseases, but adverse effects or resistance towards these drugs remain problematic. Endogenous glucocorticoids (GC) represent potent anti-inflammatory mediators synthesized and released in the circulation by the adrenal glands under control of the hypothalamic–pituitary–adrenal (HPA) axis. Although adrenal glands are considered the major source of endogenous GC, extra-adrenal GC production has been reported in the intestine (38), skin and other tissues (39, 40), and more recently in the lungs (6). Interestingly, lung tissue was shown to constitutively express the complete steroidogenic enzyme machinery with only the rate-limiting enzyme P450_{scc} (the Cyp11a1 gene product) being induced by inflammatory stimuli, such as anti-CD3 antibody, lipopolysaccharide (LPS) or tumour necrosis factor (TNF)- α (41). However, increased levels of local corticosterone after immune cell stimulation appear to be predominantly produced by 11 β -hydroxysteroid dehydrogenase type 1 (HSD1)-mediated conversion of inactive serum dehydrocorticosterone. As ovalbumin-induced allergic airway inflammation failed to promote lung GC synthesis,

future studies in human patients may reveal whether defects in lung GC synthesis may contribute to the pathogenesis of asthma. Pharmacological induction of local GC de novo synthesis or of 11 β -HSD1 with subsequent conversion of dehydrocorticosterone may have considerable advantages over current modalities of systemic and topical steroid treatment (40).

PARP-1: linking IL-4 and eosinophil inflammation

A central role in the initiation and perpetuation of Th2-type immune responses is held by IL-4, which promotes the expansion and activation of Th2 lymphocytes, and by IL-5 that is responsible for differentiation, survival and activation of eosinophils (Fig. 2). IL-4 also stimulates the expression of IL-5 in a pathway that sequentially involves Jak1/3 activation, STAT-6 nuclear translocation and transcription of GATA-3 (GATA binding protein-3), a master transcriptional regulator of IL-5 expression. In a recent study, it was shown that PARP-1, the founding member of the family of poly (ADP-ribose) polymerases (PARP), regulates GATA-3 expression by stabilization of STAT-6 and prevention of its calpain-mediated cleavage (42). Indeed, inhibition of PARP-1 either pharmacologically or genetically has been shown to attenuate allergen-induced inflammation, lung injury and airway hyperresponsiveness (AHR) in animal models (3). As PARP-1 acts downstream of IL-4, but upstream of IL-5, it is plausible that patients with eosino-

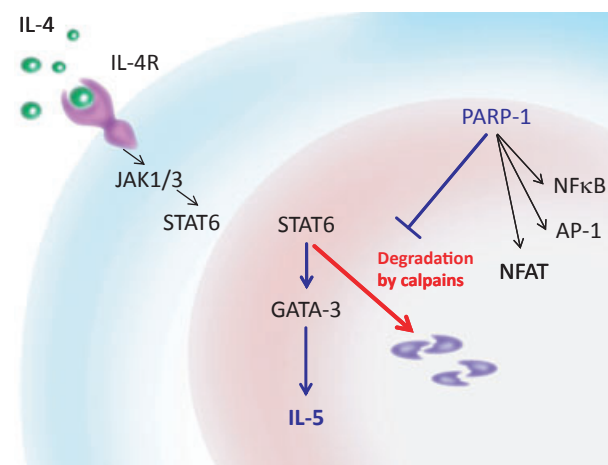


Figure 2 Role of PARP-1 in regulating IL-5 expression and the STAT6 signalling pathway. IL-4 activates the receptor-associated kinases JAK-1 and JAK-3, which leads to tyrosine phosphorylation, dimerization and nuclear translocation of STAT-6 (details not shown). PARP-1 regulates the transcription of pro-inflammatory transcription factors including NF κ B, AP-1 and NFAT. PARP-1 also stabilizes STAT6 by inhibiting its calpain-dependent degradation (red arrow). By this mechanism, PARP-1 presumably supports STAT-6 promoter binding and activation of various proinflammatory genes and pathways, including GATA-3-mediated expression of IL-5 (blue arrow).

phil-predominated inflammation might primarily profit from therapeutic approaches targeting PARP-1. PARP inhibitors are currently tested in clinical trials of cancer and acute life-threatening diseases, such as myocardial or brain ischaemia-reperfusion injury, which will also address existing concerns regarding adverse effects and drug safety, especially because PARP enzymes are involved in DNA strand break repair (43).

Hypoxia drives allergic inflammation

Hypoxia-inducible factor (HIF)-1 as a member of the HIF family of heterodimeric transcription factors mediates alterations in gene expression in multiple tissues in response to hypoxia. A growing body of evidence suggests that HIF-1 plays a role in allergic airway inflammation (44). Heterozygous-null mice partially deficient in HIF-1 α were protected from lung eosinophilia in response to ovalbumin (OVA) challenge in a mouse model (45). In another study, mice conditionally knocked out for HIF-1 β were protected from allergen-driven airway inflammation in response to antigen challenge after OVA sensitization (46). OVA-specific immunoglobulin (Ig)E was also diminished suggesting that HIF-1 β is involved in alum (adjuvants)-driven T helper (Th)2 sensitization. In the same study, experiments using pharmacological inhibitors revealed that the action of HIF-1 β in allergic airway inflammation depends at least partially on vascular endothelial growth factor (VEGF). Notably, levels of both HIF-1 β and VEGF were enhanced in bronchial lavage fluid and lung tissue from patients with asthma and nasal lavage from patients with rhinitis directly after allergen challenge. As tissue oxygenation during inflammation may be lower due to oedema and in increased O₂ consumption by accumulated inflammatory cells, HIF activation might also play a role in other allergic diseases. Future studies are required for the identification of 'footprints of HIF activation' (44), for the dissection of its pathophysiological role in allergic diseases, and its potential as a molecular target of therapeutic intervention, such as with specific small molecule inhibitors of HIF (47).

Pruritus: more than just histamine

Pruritus, defined as an unpleasant sensation provoking the desire to scratch, significantly affects the quality of life of patients with allergic diseases and has been even regarded as the diagnostic hallmark of atopic dermatitis (48). The pathophysiology of pruritus is complex, and a plethora of mediators are thought to participate in immunological and neurophysiological pathways that act at peripheral and/or central (neuro-) anatomical sites (48, 49). Histamine, for which four receptors (H1R, H2R, H3R and H4R) have been identified, is probably the best studied of these mediators. The fact that in most countries, H1-antihistamines are purchased over the counter without prescription as the most frequent form of self-medication for allergic diseases (50) contrasts with the disappointing clinical efficacy observed for drugs targeting H1R and H2R (48, 51, 52). In this regard,

the fourth histamine receptor H4R gained much attention as a potential novel drug target, because this receptor appears to be involved in the pathogenesis and perpetuation of allergic inflammation and pruritus, as well as genetic dysregulation of H4R expression on immune and nonimmune cells in atopic dermatitis (52, 53). Recently, in an animal model of chronic allergic contact dermatitis induced by repeated epicutaneous challenge using 2,4,6-trinitro-1-chlorobenzene (TNCB), the H4R receptor was shown to be implicated in the exacerbation of skin lesions, recruitment of mast cells and eosinophils, promotion of a Th2 cytokine profile and an increase in serum IgE (54). Given the many overlapping and nonoverlapping functions of H1R-H4R in the pathophysiology of allergic inflammation and pruritus, a combination therapy might be more effective. Indeed, a most recent study showed that combined treatment with H1R and H4R antagonists improved picryl chloride (PiCl)-induced dermatitis in NC/Nga mice and attenuated scratching behaviour, having similar therapeutic efficacy to prednisolone (55). However, distinct antihistamine compounds or their combination may affect neurofunctional interferences, as reported for first-generation H1-antihistamines that may impair vigilance, cognitive function, memory and psychomotor performance, and have been associated with reduced work productivity, accidents and other fatal events (50, 56). Therefore, antihistamine combination therapies will have to be carefully elucidated in studies on adverse effects and drug safety.

Interleukin (IL)-31 represents another molecule that besides other physiological functions (i.e. cell proliferation, haematopoiesis) plays a dual role in allergic inflammation and pruritus (57). Evidence derived from experimental genetic and pharmacological models of allergic disease, with assessment of scratching behaviour and immunological parameters, underscores its significant prurigenic and pro-inflammatory role (58, 59). IL-31 was cloned in 2004 by Dillon et al. (58), who found that predominantly Th2-skewed activated T cells produced IL-31 *in vitro* (58). In following studies, skin-infiltrating cutaneous lymphocyte antigen (CLA)-positive T cells and peripheral blood CD45R0⁺ (memory) CLA⁺ T cells were identified as cellular sources of IL-31 (60), and more recently dermal mast cells (61). Whereas IL-31 was found to be virtually absent in nonpruritic psoriatic skin inflammation, it was significantly overexpressed in pruritic atopic dermatitis and at highest levels detected in prurigo nodularis, one of the most pruritic forms of chronic skin inflammation (62).

IL-31 binds to a heterodimeric IL-31 receptor (IL-31R) complex consisting of a gp130-like receptor chain IL-31RA and oncostatin M receptor (58), which eventually leads to downstream activation of Jak/STAT, PI3K/AKT and MAPK signalling pathways (57), in a cell type-dependent manner (63). A tissue distribution analysis of the IL-31 receptor heterodimer revealed that IL-31RA transcripts were most abundantly expressed in dorsal root ganglia, where the cell bodies of cutaneous sensory neurons reside (62). Furthermore, the IL-31R heterodimer is expressed on immune and nonimmune cells, latter including intestinal, airway and skin epithelial cells (57). Therefore, it is thought that IL-31 might induce

pruritus either *directly* by modulating the function of sensory neurons or *indirectly* via the activation of IL-31R-expressing local or infiltrating cells (57).

Interestingly, the expression and secretion of IL-31 has been shown to be enhanced in T cells or PBMCs *in vitro*, if cells are exposed to staphylococcal superantigens staphylococcal enterotoxin B (SEB) and TSST-1 (toxic shock syndrome toxin 1) (62) or to the endogenous antimicrobial peptide LL-37 (64). In analogy, the expression of its heterodimeric receptor IL-31R can be induced or up-regulated in keratinocytes, monocytes, macrophages or dendritic cells by microbial factors (staphylococcal superantigens and exotoxins, Toll-like receptor agonists), as well as endogenous mediators of inflammation, including the cytokine interferon- γ (IFN- γ) (58, 63, 65, 66). As superinfection with staphylococcal bacteria in AD, and generally with microbes in allergic diseases, is common and is associated with disease pathogenesis and aggravation, these findings implicate a role of IL-31 in these processes and highlight its potential as a novel target for therapeutic intervention.

New functional roles of basophils

In addition to their role in IgE-mediated responses and protection against parasites, basophils play a role in initiating Th2 responses via secretion of IL-4 or may function as antigen-presenting cells (APCs) (67). However, the concept that basophils act as APCs for induction of Th2 responses (68) is a matter of debate, such as in the light of the definition of a *bona fide* APC (the expression of MHC-II alone does not necessarily designate functional antigen-presented properties), technical pitfalls and the relevance of specific animal models for human IgE-mediated allergy (69). Recently, Eckl-Dorna et al. (70) reported that human basophils were not able to present the birch pollen allergen Bet v 1 to T lymphocytes, whereas a mixture of basophil-depleted APCs did. In another study by Kitzmüller et al. (71), it was found that human basophils, although they efficiently bound Bet v 1 through IgE/FcεRI complexes on their surface, did not internalize allergen. In this study, only marginal levels of cathepsin S and invariant chain (required for antigen processing and presentation) were detected in human basophils and only upon stimulation with IL-3 and IFN-γ. Kitzmüller et al. (71) further showed that Bet v 1-pulsed basophils failed to induce proliferative and cytokine responses in Bet v 1-specific, HLA-DR-restricted T cell clones. The role of basophils as APC in IgE-mediated allergy in humans remains enigmatic, and future studies will shed light on this still unresolved issue.

Atopic dermatitis: impaired skin barrier and allergic inflammation

Structural and functional defects of the skin barrier

In the last decade, our understanding of the pathogenesis of atopic dermatitis (AD) has profoundly been revised (Fig. 3), proceeded from the discovery of loss-of-function mutations

of epidermal structural proteins resulting in skin barrier dysfunction in association with atopic dermatitis (AD) (72). In multiple studies, the strong association of filaggrin mutations with atopic dermatitis, in particular early onset and persistent AD, the development of asthma, food allergy and rhinitis as well as elevated IgE levels has been demonstrated in different ethnic populations (73–77).

Proper composition and structure of the stratum corneum is the prerequisite for sufficient epidermal barrier function (78). AD patients with filaggrin mutations were shown to have an impaired skin barrier function indicated by a significantly higher transepidermal water loss (TEWL), elevated skin pH levels, altered expression of ceramides in the stratum corneum compared with healthy controls (79). A marked increase of AD prevalence was noticed in carriers of filaggrin mutations above the general increase in the prevalence of AD, asthma and rhinitis suggesting an increased susceptibility to environmental factors (80). In line with this, an association of filaggrin mutation and increased risk of nickel dermatitis as well as increased rate of sensitization to fragrance mix in patients with AD due to extensive exposure have been reported despite an overall lower prevalence of contact sensitization in AD compared with healthy individuals (81–83). The expression of filaggrin and its degradation products is mainly determined by the genotype but is further modulated by skin inflammation resulting in decreased expression of filaggrin and natural moisturizing factor (NMF) in AD skin (84). Filaggrin breakdown products such as urocanic acid (UCA) and pyrrolidone carboxylic acid (PCA) were demonstrated to maintain an acidic pH resulting in a reduced expression of proteins involved in colonization and immune evasion of *Staphylococcus aureus* and thus to inhibit bacterial growth (85). Recently, extracellular vesicles secreted by *Staphylococcus aureus*, which have been detected in skin of patients with AD, were shown to induce AD-like inflammation and causing a Th17-cell response in skin-draining lymph nodes in an animal model

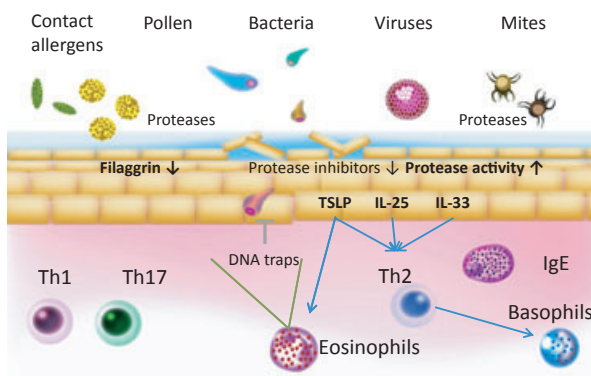


Figure 3 New concepts of pathogenic mechanisms in atopic dermatitis. Environmental factors, filaggrin deficiency, high protease activity contribute to epidermal barrier dysfunction, followed by keratinocyte cytokine production, resulting in T helper 1, 2 and 17 immune responses and activation of innate immune cells.

(86). Thus, *Staphylococcus aureus* might be involved in AD pathogenesis by secreting either extracellular vesicles that contain pathogenic proteins such as alpha-haemolysin and cysteine protease, or enterotoxins acting as superantigens and stimulating IgE responses, or both (87, 88). The lack of antimicrobial peptide expression, for example human beta-defensins and cathelicidine in AD skin, due to inhibitory effects of Th2 cytokines on keratinocytes, was shown to contribute to microbial infections observed in most patients with AD (89). Indeed, patients with severe AD as assessed by severity index, concomitant asthma and rhinitis and spectrum of IgE-sensitizations tend to be more susceptible to eczema herpeticum (90). Decreased serum levels of the cathelicidine LL-37 have been observed in patients with AD compared with controls correlating with decreased vitamin D levels (64).

Danger signals sent by keratinocytes

Thymic stromal lymphopoietin (TSLP) is released by keratinocytes upon the activation of PAR-2 receptors promoting T helper 2-type responses and thus links epidermal barrier disruption and inflammation (91). A dysregulation of proteases and their inhibitors in the skin due to mutations (92) or exposure to exogenous proteases, for example from mites (93) or pollen (94), contributes to defective epithelial barrier, pro-inflammatory cytokine production by keratinocytes and subsequent inflammation (Fig. 3). In addition to its effects on dendritic cells, T and B cells, basophils, mast cells, NKT cells, TSLP was shown to regulate eosinophil chemotaxis (95) and stimulate the formation of eosinophil extracellular DNA traps (96). These extracellular DNA traps generated upon stimulation with *Staphylococcus aureus* or TSLP effectively inhibit bacterial growth (96). Thus, eosinophils are suggested to contribute to host defence in AD in case of pathogenic and/or commensal bacteria entering the skin because of the defective skin barrier and scratching (Fig. 3). An increased production of TSLP in AD skin might promote airway sensitization to house dust mites subsequently resulting in the manifestation of allergic asthma as shown in an animal model (97). In addition to TSLP, other danger cytokines such as IL-25 and IL-33 are expressed in the epidermis of AD lesions and thus might promote eosinophil inflammation (98). Recently, an increased expression of IL-33 and its receptor ST2 in AD skin after allergen or SEB exposure as well as in association with filaggrin deficiency has been reported (99).

Innate immune cells involved in AD

According to their cytokine expression pattern, eosinophils have the potential to be involved in immunoregulation, remodelling and tissue damage in the skin (99). Eosinophils have been observed colocalized with other immune cells such as mast cells (100). Via physical interaction and/or cytokine production, mast cells directly affect eosinophil viability (100). Like eosinophils, mast cells are involved in host defence, for example by expressing dectin-1, a recognition receptor for fungi (101). In mast cells of patients with AD, a

reduced expression of dectin-1 but enhanced release of IL-6 in response to moulds was found compared to healthy controls supporting the view of a disease amplifying effect by moulds in AD (101). A cell type that was brought into focus of allergy research is basophils. Immunohistochemistry using a novel human-specific anti-basogranulin antibody (BB1) revealed basophil infiltration in lesional skin suggesting an active pathogenic role in AD (102).

Translating research into therapy for AD

Restoration of the disrupted skin barrier and reduction in inflammation are the main goals of an effective treatment for AD. A comprehensive characterization of patients with AD and subgroups is regarded as prerequisite for an effective use of new therapies that specifically target certain components of the skin barrier, inflammatory cells and/or their mediators (37). Biologics targeting B and T cells, eosinophils, IgE or TNF- α have been reported to be effective in at least subgroups of patients with AD (103). Currently, a number of emollients that should substitute missing or defective components of the skin barrier, for example ceramides and lipids, have been studied in clinical trials (104–106). The application of a lipid raft-modulating agent, miltefosine, resulted in a sustained improvement in AD and increase of regulatory T cells in the skin (107). In a murine model, dietary sphingolipids were found to increase ceramide synthesis and thus recover damaged skin barrier (108). The intake of n-3 long-chain polyunsaturated fatty acids as well as ruminant fatty acids exerts protective effects on the development of AD in infants (109).

Although the use of protease inhibitor provided promising results in an animal model (110), the first study evaluating the effect of a protease inhibitor in human failed to show an effect in AD (111). Interestingly, besides their immunosuppressive effects, topical corticosteroids and calcineurin inhibitors were shown to normalize epidermal differentiation and expression of filaggrin and loricine in AD skin (112, 113). However, by reducing the expression of antimicrobial peptides, involucrin, small proline-rich proteins and lipid-synthesizing lipids, corticosteroids inhibit the restoration of the skin barrier at the same time (114).

Towards disease-modifying strategies

Currently available therapeutic procedures mainly target symptoms and clinical signs of AD, and information on long-term outcomes or modification of the disease course is limited. Considering the natural course, its early onset paving the way for the atopic march, chronic skin inflammation, increasing allergic sensitization and development of autoimmunity, AD qualifies as a candidate for disease-modifying strategy (115). The effects of a disease-modifying strategy that should act directly on the pathophysiologic process concomitant with an improvement in clinical signs have to be assessed by validated biomarkers (115). So far, various severity scores (116, 117), serum and skin biomarkers (118, 119) are available, although their spectrum and validity does not

seem sufficient to properly follow the progress of disease-modifying strategy.

Conclusion

In this update, we highlight selected experimental data from the recent years that confirm or challenge current or previous views and concepts in the field of allergy and that answer or raise relevant questions to clinicians and scientists. We started this update by revisiting the classical hygiene hypothesis from a current perspective. Indeed, the microbiome might influence the pathogenesis and clinical course of the whole spectrum of allergic disease entities. Next, we summarized an impressive body of recent insights into pathogenic mechanisms and on molecular or cellular players that eventually might be exploited as biomarkers or targets of common or individualized treatment strategies. The outcome of the interplay between allergens, the microbiome and the host depends on genetic and environmental factors; here, novel aspects of this interaction were discussed in the context of AD, which to a certain extent may also be relevant for other allergic diseases. The abundance of novel data is promising, but comes along with the awareness that much effort is still required to reach satisfactory disease-modifying strategies and individualized treatment options.

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Conflict of interest

The authors declare no conflict of interest relevant to this manuscript.

Author contribution

All authors equally contributed to this update in designing and writing the manuscript and literature search.

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