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Atopic dermatitis: the skin barrier and beyond

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What's already known about this topic?

- Atopic dermatitis is a common and highly debilitating chronic inflammatory skin disorder.
- Immune dysregulation plays a key role, but recent evidence has shifted the focus to skin barrier disruption as the key precursor.
- Loss-of-function mutations in the *FLG* gene are the strongest known genetic risk factor, but there are numerous environmental and immunological factors that influence disease manifestation and course.
- The current therapeutic armamentarium is limited to simple lipid-based barrier-enhancing emollients, topical anti-inflammatory agents, and systemic immunosuppressive therapies.

What does this study add?

- We review how defects in structural epidermal proteins and environmental factors converge to impair skin barrier function, resulting in increased susceptibility to atopic dermatitis.
- We explore the impact of the defective skin barrier on immune responses and the skin microbiome, highlighting how the complex interplay between the skin barrier and immune activation determines the response to environmental factors such as allergens and microbes.
- We outline emerging strategies for treating and preventing atopic dermatitis.

Summary

Atopic dermatitis is the commonest chronic inflammatory skin disorder, affecting up to 20% of children and 10% of adults in industrialised countries. This highly debilitating condition poses a considerable burden to both the individual and society at large.

The pathophysiology of atopic dermatitis is complex, encompassing both genetic and environmental risk factors. Dysregulation of innate and adaptive immunity plays a key role; however, recent epidemiological, genetic and molecular research has focused interest on skin barrier dysfunction as a common precursor and pathological feature. Current understanding of the aetiology of atopic dermatitis highlights disruption of the epidermal barrier leading to increased permeability of the epidermis, pathological inflammation in the skin, and percutaneous sensitisation to allergens. Thus, most novel treatment strategies seek to target specific aspects of the skin barrier or cutaneous inflammation. Several studies have also shown promise in preventing atopic dermatitis, such as the early use of emollients in high-risk infants. This may have broader implications in terms of halting the progression to atopic comorbidities including food allergy, hay fever, and asthma.

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Introduction

Atopic dermatitis (AD, syn. atopic eczema) affects approximately 20% of children and 10% of adults in developed countries[1], with an impact on quality of life comparable to epilepsy or type 1 diabetes[2]. Thus, AD represents a considerable burden both to the individual and to society, with annual costs exceeding £800 million in the UK (including direct and indirect costs, adjusted for inflation[3]), and approximately US$5 billion in the USA[4].

AD remains a clinical diagnosis, although several diagnostic criteria have been developed[5,6]. The disease often presents as pruritic, ill-defined, dry and excoriated patches that can affect any part of the body, but typically show age-related distribution and morphology[7]. Underlying pathological changes include cutaneous infiltration with immune cells, in particular T-lymphocytes, and the development of epidermal oedema ('spongiosis'). AD is also associated with a susceptibility to cutaneous infections, notably by bacteria such as *Staphylococcus aureus* (*S. aureus*), as well as certain viruses including herpes simplex[7]. Furthermore, children with AD have a higher prevalence of extracutaneous infections, including streptococcal pharyngitis, respiratory infections, recurrent otitis and urinary tract infections[8], with recent reports even suggesting a relationship between AD and infective endocarditis[9].

The complex pathophysiology of AD encompasses genetic risk factors, environmental triggers, and dysregulation of innate and adaptive immunity. The traditional paradigm of AD espouses a T_H2-skewed immune system with exaggerated IgE responses to allergens. However, recent genetic, epidemiological and molecular advances have highlighted skin barrier dysfunction as a common precursor and pathological feature. Genome-wide association studies (GWAS) have identified >30 susceptibility loci – of which the strongest are null (loss-of-function) mutations in the gene encoding the epidermal protein filaggrin, which is essential for skin barrier function[10] (Figure 1). Current pathophysiological concepts of AD highlight disruption of the epidermal barrier leading to increased permeability of the epidermis, pathological inflammation, and percutaneous allergic sensitisation[7]. Most novel treatment strategies thus target either the skin barrier or cutaneous inflammation. Furthermore, recent studies aiming to address the skin barrier defect have shown promise in preventing AD, such as early emollient use in high-risk infants[11,12]. This may have broader implications in terms of halting progression of the so-called 'atopic march', thereby preventing development of associated atopic comorbidities, such as asthma.

The following article aims to summarise our current understanding of AD pathogenesis, and how this is informing development of novel strategies for treatment and prevention. This

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narrative review was supported by a systematic search, using key words including 'eczema', 'atopic dermatitis', 'pathogenesis', 'skin barrier', 'immunology', 'treatment' and 'prevention'.

Skin barrier dysfunction

The skin provides a physical barrier against dehydration and environmental challenges. A major constituent of this is the stratum corneum, where keratinocytes undergo apoptosis into flattened 'squames', embedded in a matrix of specialised lipids[13]. Intracellularly, keratin filaments are tightly cross-linked by filaggrin molecules, and filaggrin degradation products are an important source of natural moisturising factor. The latter regulates not only stratum corneum hydration, but also its pH[14,15]. This is key, as acidification by filaggrin metabolites helps maintain skin barrier integrity[16-18].

In 2006, the first loss-of-function mutations in the filaggrin gene (*FLG*) were identified as the cause of the commonest monogenic genodermatosis ichthyosis vulgaris[19]. In parallel, the same group showed that these mutations are the major genetic predisposing factor for AD[20]; this lent support to the 'outside-in' hypothesis[21,22] that functional disruption of the epidermal barrier is the primary pathogenic process in AD, thus facilitating recognition of environmental allergens, irritants and microbes, and initiation an inflammatory cascade.

Filaggrin deficiency impacts on numerous pathways key to skin barrier function, including disruption of keratinocyte differentiation, impaired corneocyte integrity and cohesion[23], impaired tight-junction formation[23], decreased water retention[14], altered lipid formation[24], and enhanced susceptibility to cutaneous infection[23,25,26]. Furthermore, reduced levels of natural moisturising factor increase the normally acidic stratum corneum pH with an allele dose-dependent effect[23], whereby homozygous *FLG* mutation carriers have the highest pH levels. This activates pH-sensitive serine proteases, resulting in premature degradation of corneodesmosomes and activation of IL-1α and IL-1β[27,28]. The acidic mantle is also a prerequisite for the formation of ceramides – the most important lipid class found in the stratum corneum intercellular lipid matrix – thereby directly influencing skin barrier function. Filaggrin deficiency has also been shown to impair the lipid profile and alter acidification pathways in *in vitro* skin models; investigators observed a 2-fold increase in free fatty acids in *FLG*$^{-/-}$ constructs, leading to less ordered intercellular lipid lamellae and higher permeability[24].

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FLG null mutations remain the strongest known genetic risk factor for AD; all constitute nonsense mutations, out-of-frame insertions or deletions that lead to loss or reduction of filaggrin synthesis. Indeed, the presence of a single *FLG* mutation increases AD risk approximately 3-fold compared to the general population[29]. Furthermore, *FLG* mutations predispose to earlier disease onset, prolonged duration and increased severity[30]. The prevalence of *FLG* mutations varies greatly among different populations and is most common in Northern Europeans, with a prevalence of approximately 10% and dominated by a few genotypes such as R501X and 2282del4[29]. There are ethnic differences in the association of *FLG* mutations with AD; in Asian populations, FLG P478S and C3321delA – variants not commonly found in European populations – are associated with increased risk of AD, the atopic march and recurrent skin infections[31-37]. By contrast, in African American children loss-of–function mutations in *FLG2*, but not *FLG*, are associated with increased AD risk[38]. Interestingly, it has also been shown that AD in Asian patients has some features of chronic plaque psoriasis, including increased epidermal hyperplasia, parakeratosis, higher T_H17 activation, and a strong T_H2 component[39]. However, the precise influence of different *FLG* mutations on AD immunophenotype in different ethnicities is still poorly understood.

While reported frequencies of *FLG* mutations range from 18-48% in AD patients, the population-attributable risk remains only 15%, with penetrance around 40%[40]. This implies that *FLG* mutations are neither necessary nor sufficient to cause AD, and that other factors play a role. For instance, T_H2-mediated cutaneous inflammation can result in reduced filaggrin expression in lesional skin – even in patients not carrying a *FLG* mutation[41]. *FLG* intragenic copy number (typically 10-12 repeats) is an independent risk factor; an increased copy number is protective, with each additional *FLG* repeat equating to a 12% risk reduction for AD[42]. Furthermore, the *FLG* gene is only one of at least 45 genes within the epidermal differentiation complex (EDC) on chromosome 1q21, many of which may also contribute to pathogenesis. One such gene encodes hornerin, which is also important for keratinocyte differentiation. Levels of hornerin and other filaggrin-like proteins are decreased in the skin of AD patients[43].

Deep to the cornified envelope, tight junctions in the granular layer form an additional component of the skin barrier, limiting penetration by larger molecules such as allergens and pathogenic microbes, facilitating paracellular passage of soluble mediators and regulating transepidermal water loss (TEWL). Tight junctions are composed of transmembrane proteins such as claudin-1, which is reduced in the skin of AD patients, thus enhancing viral penetration and susceptibility to eczema herpeticum[44]. SNPs in the CLDN1 gene have been associated with AD in two North American cohorts[45].

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The defects in structural epidermal proteins outlined above collectively contribute to a dysfunctional cutaneous barrier that underlies the AD phenotype of dry, inflamed skin, and also serves as a site for allergic sensitisation to antigens. Indeed, *FLG* mutations have been reported to be associated with atopic asthma, allergic rhinitis, nickel allergy, and food allergy[46], suggesting that *FLG* mutation–associated stratum corneum barrier defects lead to increased percutaneous allergen exposure. Apart from one recent and relatively small study[47], *FLG* mutations have not been associated with asthma without AD, and most of the identified asthma-associated genes are not associated with AD, suggesting that atopic asthma is a distinct sub-entity[47]. Since filaggrin is not expressed in the upper airways, systemic sensitisation may be attributable to percutaneous antigen exposure via filaggrin-deficient skin. In line with this hypothesis, percutaneous sensitisation induces allergic responses to allergens in mice challenged via the upper airway[48].

There has been particular interest in transcutaneous sensitisation to food allergens as part of the emerging concept of the dual allergen exposure hypothesis[49], which states that exposure to food antigen via an impaired skin barrier leads to sensitisation[50], whereas oral exposure via the gut leads to tolerance. Infants with peanut allergy are more likely to have had severe AD before 6 months of age, and to have been treated with topical peanut oil[51]. Another study found a 4-fold increase in peanut allergy risk in children with at least one *FLG* mutation, with the risk of peanut allergy almost doubling in children with early AD[52]. High levels of peanut protein in household dust have also been associated with an increased risk of peanut sensitisation and likely allergy in children with AD; this risk was augmented in children with more severe AD[53]. Finally, Flohr et al showed that AD increased the risk of food sensitisation more than 6-fold in 3-month old infants, with a stepwise increase in risk with more severe AD - even after adjusting for *FLG* mutations. Importantly, skin barrier dysfunction (raised TEWL) remained an independent risk factor for food sensitisation[54]. Conversely, the paradigm-shifting LEAP Study has confirmed that sustained peanut consumption in high-risk infants (severe AD, egg allergy or both) starting in the first year of life, is highly effective in preventing peanut allergy at 5 years of age[55].

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Environmental factors

Taken together, the above evidence implies that AD is initiated by a defective skin barrier in at least a considerable proportion of affected individuals. Once the skin barrier is disrupted, transcutaneous antigen exposure and allergic priming is facilitated by antigen-presenting cells in the epidermis. It follows that any environmental factor adversely impacting on skin barrier integrity should enhance both AD risk and severity. The most important local environmental factor is mechanical damage, including from repetitive scratching, use of detergents and release of exogenous proteases (eg. from house dust mite[56]).

Washing of the skin may also contribute to mechanical damage, and controversy surrounds the optimal bathing frequency for children with AD. One review reported that studies favouring frequent bathing outnumber those favouring infrequent bathing[57]. Frequent washing is also a marker of hygiene levels, and notably a UK birth cohort study reported that high hygiene scores at 15 months of age were independently associated with subsequent AD. This association was even stronger for severe AD[58].

Inherent properties of the water used for washing may also play an important role. A recent UK population-based study reported that living in a hard water area was associated with up to 87% increased risk of AD at 3 months of age; the risk tended to be higher in children with *FLG* mutations[59]. These findings were recently confirmed in a cohort study from Denmark, where early exposure to hard domestic water was associated with an increase in the relative prevalence of AD in the first 18 months of life[60]. Although this may have implications for AD prevention in early life, a previous RCT showed no benefit of water softeners on established AD in children up to 16 years of age[61].

Finally, climatic effects on the skin barrier are also thought to be relevant. An autumn/winter birth has also been associated with an increase in AD prevalence[60], and a USA study found that AD prevalence was higher in areas with more indoor heating, lower relative humidity, lower mean temperatures, higher precipitation and lower UV index[62]. Most recently, a worldwide ecological analysis[63] using ISAAC (The International Study of Asthma and Allergies in Childhood) Phase III data reported a positive linear association between country-level AD prevalence and certain metrics of UVR exposure, and nonlinear relationships with other metrics of UVR exposure. These associations were significant amongst 13- to 14-year olds, but not in 6- to 7-year olds.

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Immunological dysfunction

Impairment in epidermal barrier function may directly trigger the cutaneous inflammation of AD, a concept supported by murine models such as filaggrin-deficient mice exhibiting a spontaneous eczematous dermatitis and increased IgE[64,65]. As outlined above, filaggrin deficiency is associated with enhanced percutaneous microbial and allergen penetration, and reduced inflammatory thresholds to irritants and haptens[66-68]. Epithelial damage leads to innate immune activation, including release of pro-inflammatory cytokines and chemokines by keratinocytes[69,70], and antigen presentation by skin-resident Langerhans cells and dermal dendritic cells[68,71,72] (Figure 2).

This pro-inflammatory cascade is characterised by sequential and progressive infiltration of different inflammatory cell subsets. Based on transcriptome analyses - albeit using small and diverse patient populations - it appears that areas of active dermatitis are dominated by $CD4^+$ T helper 2 (T_H2) cells, defined by production of the cytokines interleukin (IL)-4, IL-5 and IL-13[73]. Even non-lesional skin in AD patients has been found to harbour an increased proportion of T_H2 cells[74,75]. As acute dermatitis develops, the skin becomes infiltrated with more T_H2 cells and additional $CD4^+$ subsets, including T_H22 and T_H17 cells[74]. In addition to $CD4^+$ T cells, other lymphocyte subsets are found in increased numbers, including type 2 cytokine-producing $CD8^+$ T cells and type 2 innate lymphoid cells[75,76]. The role of these cells remains to be elucidated; however, they and potentially other innate lymphocyte populations are likely to play a role in early sensing of tissue damage and the initiation of inflammatory cascades prior to the development of antigen-driven adaptive immune responses. T_H2 inflammation leads to the recruitment of additional immune cell subsets including eosinophils and mast cells, which further contribute to pathological inflammation via release of mediators such as histamine.

In chronic AD, skin recruitment of T_H1 lymphocytes (defined by production of interferon-γ) appears to result in a mixed T_H1, T_H2 and T_H22 infiltrate[73]. These activated lymphocytes adopt a tissue-resident memory T cell phenotype with tissue priming and limited capacity to recirculate out of the skin, facilitating rapid recall responses when re-exposed to antigens. Skin-resident lymphocyte subsets may also be cross-reactive to autoantigens or antigens commonly encountered at the epidermis (eg. house dust mite and *Malasezzia furfur*)[77], thus contributing to disease chronicity.

As mentioned above, the pathogenesis of AD is further complicated by the impact of skin inflammation on skin barrier function. Inflammatory responses may lead to widespread secondary changes that further contribute to the AD phenotype, such as suppression of epidermal differentiation complex genes – including *FLG* – by T_H2, T_H22 and T_H1 cytokines[41,78,79];

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inhibition of antimicrobial peptide production by T_H2 cytokines[80]; up-regulation of S100As by IL-17 and IL-22[74]; and induction of epidermal hyperplasia by IL-22[74]. Interestingly, pro-inflammatory factors including cytokines, including IL-31, and additional secreted molecules such as histamine, can affect cutaneous somatosensory nerves both directly and via release of pruritogens[81]. This crosstalk is poorly understood, but may contribute to the severe itch of AD. Consequent repetitive scratching may damage the epidermis and alter peripheral and central neural sensitisation, thereby provoking further pruritus – the 'itch-scratch cycle'[82].

AD is associated with systemic immune effects, including T_H2 skewing of the systemic immune system. In addition to food sensitisation and respiratory hypersensitivity (atopic asthma) (as previously discussed), many individuals with AD have high levels of circulating specific and non-specific IgE, with associated immediate-type allergies, such as to food. IgE can directly activate mast cells and skin-resident antigen presenting cells (eg. Langerhans cells) via high affinity FcεR1 receptors[83]; however, the precise role of IgE in AD remains unclear. Subsequent exposure to allergens in sensitised individuals can drive flares and disease chronicity[84], and the production of allergen-specific IgE is seen in murine models of AD, such as following the topical application of ovalbumin to 'flaky tail' (*FLG* null) mice[66]. However, it may be that IgE formation is primarily an epiphenomenon that develops secondary to severe skin inflammation[85].

Microbiome

The skin microbiome encompasses the global array of microorganisms living in the skin microenvironment. Established AD is associated with marked changes (dysbiosis) in the composition of the skin bacterial microbiome, forming part of a complex interplay with skin barrier integrity. Numerous culture-based studies support the clinical link between *S. aureus* burden and AD severity[86,87]. Skin barrier disruption (as measured by TEWL) is correlated with higher *S. aureus* colonisation[88]. In addition, staphylococcal peptides and their superantigens have also been implicated in driving eczematous inflammation: staphylococcal delta toxin causes mast cell degranulation whilst alpha toxin induces keratinocyte apoptosis; T cells are stimulated by staphylococcal enterotoxins; and certain staphylococcal surface proteins modulate inflammation[89-92]. More recently, sequencing of skin microbiota has also demonstrated that *S. epidermidis* may be associated with established AD[93]. Disease flares are known to be associated with a significant fall in skin microbiota diversity and an increase in abundance of both *S. aureus* and *S. epidermidis.* Following topical anti-inflammatory and anti-microbial treatment these changes appear to be reversed, with restoration of skin bacterial diversity[93].

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It remains uncertain whether skin bacterial dysbiosis plays a causal role in the development of AD and/or disease flares. The ADAM17(fl/fl)Sox9-(Cre) mouse has been used to model a rare genetic dermatosis manifesting as an eczematous dermatitis with skin dysbiosis[94]. *Corynebacterium mastitidis*, *S. aureus*, and *Corynebacterium bovis* emerged during the onset of dermatitis, and antibiotics specifically targeting these bacteria both reversed dysbiosis and prevented skin inflammation[94]. However, it is also possible that the observed expansion of *S. aureus* and reduction in bacterial diversity seen in AD may also represent an epiphenomenon resulting from the impaired and inflamed skin barrier. A more recent small study of neonates with and without AD found no significant differences in bacterial diversity between groups; however, early colonisation with commensal staphylococci at 2 months was associated with a lower risk of AD at 1 year[95].

Therapeutic approaches

Conventional systemic treatments for moderate-to-severe AD broadly target cutaneous inflammation through use of immunosuppressive drugs, such as corticosteroids, ciclosporin, methotrexate and azathioprine, and are often limited by variable efficacy and toxicity. However, our increasing understanding of AD pathogenesis is informing the development of targeted treatment strategies. To date, most of these have focused on addressing the immunological pathways outlined above.

As already discussed, lymphocyte activation is central to the inflammation of AD, and key to this are T_H2 cells and other lymphocyte subsets that produce the cytokines IL-4, IL-5 and IL-13. Selective antagonists targeting these pro-inflammatory mediators, such as monoclonal antibody (mAb) biologics, represent promising new therapeutic agents. Dupilumab binds to and blocks the common alpha chain of the IL-4 receptor (IL-4Rα), thereby blocking the actions of IL-4 and IL-13. Phase IIb randomised controlled trials (RCTs) in moderate-to-severe AD showed a major improvement in disease activity (Eczema Area and Severity Index; EASI) of 68–74% (high-dose dupilumab groups) at 16 weeks, compared with 18% in the placebo group[96]. In addition, dupilumab treatment was associated with reduced epidermal hyperplasia and an improvement in molecular markers of disease activity. Notably, there was a transition in the mRNA transcriptome of involved skin towards that of non-lesional skin, clearly demonstrating the

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importance of these cytokines to the pathogenesis of AD[97]. This early outcome data has been supported by two recent phase III RCTs in which 44-51% of patients treated with dupilumab 300mg every other week achieved a 75% reduction in the Eczema Area and Severity Index (EASI 75) by week 16, compared with 12-15% of patients in the placebo group. Apart from conjunctivitis and slightly higher rates of injection site reactions, there were no clinically relevant safety issues over the 16-week observation period[98]. Trial results from additional biologics targeting the IL-4Rα (pitrakinra [phase II]) and IL-13 (tralokinumab [phase II], lebrikizumab [phase II]) are awaited.

Additional cytokines of interest include IL-5 and thymic stromal lmphopoietin (TSLP) (Figure 2). Mepolizumab is a mAb against the type 2 cytokine IL-5, which is important in eosinophil recruitment and proliferation. Despite efficacy in asthma, this biologic only led to a moderate improvement in patients with AD[99], although a reduction in peripheral eosinophilia was noted. These data may indicate that IL-5 is less critical to the pathogenesis of AD. TSLP is a keratinocyte-derived cytokine involved in establishing the T_H2 response in AD, which in mouse models appears key to mechanisms underlying systemic sensitisation[100]. An anti-TSLP mAb tezepelumab has already completed phase I (AMG 157; NCT00757042) and phase IIa (MEDI9929; NCT02525094) trials in AD[101].

Omalizumab is a mAb against IgE that is effective in patients with asthma and chronic spontaneous urticaria; however, it is not clearly effective in chronic AD, despite abrogating acute allergic responses[102,103]. This, together with the observation that dupilumab (anti-IL-4Rα) appears to be effective irrespective of IgE status, suggests that the development of allergen-specific IgE is not a key driver of AD, and as mentioned earlier may actually be an epiphenomenon. Nevertheless, other novel methods to deplete circulating IgE in patients with AD are currently under investigation, including IgE immunoadsorption[104].

Finally, recognition of the importance of T_H1 and T_H17 in the pathogenic immune dysregulation of AD has also informed trials of biologics currently used in psoriasis. In a recently reported small phase II study of the mAb IL-12/23 antagonist ustekinumab, 31% of the intervention group achieved a ≥50% improvement in AD severity at 16 weeks; however, this was not statistically significant compared to the placebo group[105]. A phase II trial using IL-17A (secukinumab) is underway.

Aside from the biologics, another strategy to modulate the immunological pathways active in AD is the use of small molecule antagonists that specifically target components of intracellular signaling. Preliminary data has suggested that the phosphodiesterase 4 (PDE4) inhibitor

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apremilast has similar efficacy to conventional systemic immunosuppressants, such as ciclosporin[106], with additional phase II results yet to be reported. The efficacy of the topical PDE4 inhibitor crisaborole has been recently reported in a phase III study, with 31–33% of treated patients with mild-to-moderate AD reporting clear/almost clear responses compared with 18–25% for the vehicle-treated group[107].

Another potential target is the aryl hydrocarbon receptor (AHR)[108]. AHR-deficient mice have impaired skin barrier function as measured by transepidermal water loss[109]. A study in humans with AD showed that the effects of coal tar are mediated through AHR activation, which restored *FLG* expression in *FLG*-haploinsufficient keratinocytes and blocked T_H2 cytokine signaling[110]. The topical AhR agonist tapinarof thus aims to target both inflammation and barrier function, with promising preliminary results in AD[101].

Also under investigation is the prostaglandin D2 receptor, which is expressed on inflammatory T_H2 cells, type II innate lymphoid cells, eosinophils, and basophils[101]. Mast cells may represent a source of prostaglandin D2 in AD skin, which would act on its receptor and initiate migration of T_H2 cells into the skin. Investigation of prostaglandin D2 receptor antagonists in AD are underway (OC000459 and fevipripant/QAW039), albeit with disappointing preliminary results (NCT01785602) thus far[101].

Other small molecules of interest include janus kinase inhibitors, which block cytokine-signaling pathways and are increasingly used in other chronic inflammatory conditions. A phase II study comparing twice-daily topical tofacitinib (a JAK1/3 inhibitor) with a vehicle control reported a significant EASI improvement in patients with mild-to-moderate disease after 4 weeks of treatment (-82% vs. -30%)[111]. This finding is supported by small case series that have reported a beneficial effect of oral tofacitinib in AD[112]. However, further studies are required.

Specifically targeting pruritus represents another emerging therapeutic approach. IL-31 appears to play a role; in both humans and mice, it is predominantly produced by T_H2 cells and its receptor IL-31RA is expressed on C fibers[113]. A 12-week phase II trial showed that monthly injections of the anti-IL-31RA antibody nemolizumab, significantly reduced pruritus in patients with moderate to severe AD (up to 63% improvement), with some associated improvement in AD severity[114]. In addition, an IL-31 neutralizing antibody (BMS-981164) has completed a phase I trial in AD[101]. Another target is the type 4 histamine receptor (H4R), expressed on keratinocytes and T_H2 cells; H4 stimulation leads to IL-31 production. Blocking H4R reduced scratching behavior and improved dermatitis in mice, and in humans a H4R antagonist (ZPL-389) has undergone phase II clinical studies in AD with encouraging results[101]. Neuropeptide signaling is another target for anti-pruritus therapies – plasma levels of substance P are

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associated with AD disease activity, and neurokinin 1 receptor (NK1R) antagonists that block substance P have also been found to reduce scratching behavior in AD mouse models[115]. Two small molecule NK1R antagonists (tradipitant, NCT02651714; and serlopitant, NCT02975206) are currently in phase II trials in AD[101].

Finally, as already mentioned, the skin microbiome also provides a focus for potential therapeutic strategies. *S. aureus* directly influences skin barrier integrity by stimulating endogenous proteolytic activity[116], and produces toxins that activate various inflammatory cell types. Dilute bleach baths as maintenance therapy for AD may therefore exert benefit through reducing the burden of *S. aureus*, although mechanistic studies also suggest direct effects on inflammation and upregulation of barrier protein expression[101]. The reduction in microbial diversity associated with AD is an area of equally active investigation. In a mouse model of AD, transplantation of Gram-negative bacteria from healthy volunteers, but not from patients with AD, was associated with enhanced barrier function, innate immunity activation, control of *S. aureus* and improved AD outcomes[117]. In humans, a small RCT showed that a cream containing nonpathogenic *Vitroscilla filiform*is significantly improved AD, as well as reducing *S. aureus* colonisation and transepidermal water loss[118]. Another study found that coagulase-negative *Staph* strains with antimicrobial activity are common in the normal population, but rare in AD patients. Reintroduction of coagulase-negative *Staphylococci* to AD patients decreased *S. aureus* colonisation[119]. Ongoing studies in AD are investigating topical application of the Gram-negative coccobacillus *Roseomonas mucosa* (NCT03018275) and a targeted microbiome transplant lotion containing a combination of commensal bacteria (NCT03151148)[101].

The novel treatment approaches outlined above principally focus on downstream immunological targets. Attempting to repair the defective epidermal barrier may prove a powerful and synergistic therapeutic strategy. Indeed, experiments on 'flaky tail' mice and human skin equivalents *in vitro* have succeeded in restoring key barrier components such as filaggrin, which in mice also improved histological features of diseased skin[120,121]. However, increasing recognition of skin barrier dysfunction as central to the initiation and progression of AD suggests that the more fundamental value of interventions directed at the skin barrier may lie in the potential to prevent AD altogether.

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Preventative approaches

The development of novel prevention strategies focused on the skin barrier is an area of active research. Emerging evidence on the impact of water hardness[59] has raised the question of whether installing domestic water softeners around the time of birth for high-risk children may prevent the condition developing in infants. A UK intervention trial to test this is in progress (NCT03270566).

Other groups have hypothesized that using emollients to enhance a defective skin barrier early in life may prevent or delay the onset of AD. One pilot study and two RCTs[11,12] found that daily full-body emollient therapy virtually from birth in high-risk children, reduced the cumulative incidence of AD by 30-50% during the first year of life. A large-scale RCT of >1300 infants comparing daily emollients versus standard skin care advice is currently underway (Barrier Enhancement for Eczema Prevention [BEEP], NCT01142999).

With AD historically classified as an allergic disease, other prevention strategies have previously focused on allergen avoidance[122]. However, a Cochrane review including 334 pregnant women found no protective effect of maternal dietary antigen avoidance during pregnancy or lactation on AD incidence up to 18 months of life[123]. Another Cochrane review concluded that exclusive breastfeeding for 6-7 months conferred no protection against developing AD at 5-7 years compared with exclusive breastfeeding for 3-4 months[124].

Nutritional supplements have also been investigated as a preventative approach. A review of the impact of maternal vitamin D intake on AD risk in children was inconclusive[125]. Probiotics and prebiotics have been used based on the observation that children who demonstrate reduced gut bacterial diversity in early life seem to have a higher risk of developing AD. This may be due to a lack of microbial exposure during infancy, resulting in skewing of the developing immune system towards T_H2 responses[126]. Several meta-analyses have reported that probiotics reduce the risk of AD when used in the last trimester of pregnancy, during breastfeeding and when given to infants[127,128]. Accordingly, for offspring thought to be at high risk of AD, the World Allergy Organisation guidelines currently support the use of probiotics in women during pregnancy and breastfeeding and in infants. However, these guidelines remain vague on the optimal delivery protocol, including timing (prenatal, postnatal or both), recipients (mother, baby or both), and whether single or mixed bacterial strains should be used[129].

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Conclusion

We have shown how diverse inherited and acquired abnormalities in epidermal structural and enzymatic proteins converge to impair skin barrier function, resulting in increased susceptibility to AD. We have also explored the impact of the defective skin barrier on immune responses and the skin microbiome. Although primary pathogenic events in AD are uncertain, it is the complex interplay between the epidermal barrier and host immune activation that determines the response to environmental factors, including allergens and microbes. Ongoing research to characterise this relationship will lead to a new era of combined management for AD, aiming to simultaneously address epidermal susceptibility and cutaneous inflammation. For now, identification of effective biologics and targeted small molecules in AD is heralding real progress in developing an armamentarium to rival that of psoriasis. Finally, the emerging 'outside-in' paradigm of AD is driving research into preventative strategies for this and indeed other atopic conditions, via early 'barrier therapy' that may also minimise transcutaneous allergic sensitisation and thus reduce the risk of food and respiratory allergies.

References

1. Flohr C, Mann J. New insights into the epidemiology of childhood atopic dermatitis. Allergy. 2014 Jan;69(1):3-16.
2. Beattie P, Lewis-Jones M. A comparative study of impairment of quality of life in children with skin disease and children with other chronic childhood diseases. Br J Dermatol 2006 Jul;155:145-51.
3. Herd RM, Tidman MJ, Prescott RJ, Hunter JA. The cost of atopic eczema. Br J Dermatol. 1996 Jul;135(1):20-3.
4. Mancini AJ, Kaulback K, Chamlin SL. The socioeconomic impact of atopic dermatitis in the United States: a systematic review. Pediatr Dermatol. 2008 Jan-Feb;25(1):1-6.
5. Williams HC, Burney PG, Hay RJ et al. The U.K. Working Party's Diagnostic Criteria for Atopic Dermatitis. I. Derivation of a minimum set of discriminators for atopic dermatitis. Br J Dermatol. 1994 Sep;131(3):383-96.
6. Hanifin J, Rajka G. Diagnostic features of atopic dermatitis. Acta Derm Venereol 1980; 92(Suppl): 44–47.
7. Weidinger S, Novak N. Atopic dermatitis. Lancet. 2016 Mar 12;387(10023):1109-22.
8. Silverberg JI, Silverberg NB. Childhood atopic dermatitis and warts are associated with increased risk of infection: a US population-based study. J Allergy Clin Immunol. 2014 Apr;133(4):1041-7.
9. Patel D, Jahnke MN. Serious Complications from Staphylococcal aureus in Atopic Dermatitis. Pediatr Dermatol. 2015 Nov-Dec;32(6):792-6.
10. Paternoster L, Standl M, Waage J et al. Multi-ancestry genome-wide association study of 21,000 cases and 95,000 controls identifies new risk loci for atopic dermatitis. Nat Genet. 2015 Dec;47(12):1449-56.
11. Simpson EL, Chalmers JR, Hanifin JM et al. Emollient enhancement of the skin barrier from birth offers effective atopic dermatitis prevention. J Allergy Clin Immunol. 2014

This article is protected by copyright. All rights reserved.

Accepted Article

Oct;134(4):818-23.
12. Horimukai K, Morita K, Narita M et al. Application of moisturizer to neonates prevents development of atopic dermatitis. J Allergy Clin Immunol. 2014 Oct;134(4):824-830.
13. Candi E, Schmidt R, Melino G. The cornified envelope: a model of cell death in the skin. Nat Rev Mol Cell Biol 2005;6:328-40.
14. Flohr C, England K, Radulovic S et al. Filaggrin loss-of-function mutations are associated with early-onset eczema, eczema severity and transepidermal water loss at 3 months of age. Br J Dermatol 2010;163:1333-6.
15. Kezic S, Kemperman PM, Koster ES et al. Loss-of-function mutations in the filaggrin gene lead to reduced level of natural moisturizing factor in the stratum corneum. J Invest Dermatol 2008; 128:2117-9.
16. Cork MJ, Robinson DA, Vasilopoulos Y et al. New perspectives on epidermal barrier dysfunction in atopic dermatitis: gene-environment interactions. J Allergy Clin Immunol. 2006 Jul;118(1):3-21.
17. Smith FJD, Irvine AD, Terron-Kwiatkowski A et al. Loss-of-function mutations in the gene encoding filaggrin cause ichthyosis vulgaris. Nat Genet 2006; 38:337–42.
18. Palmer CN, Irvine AD, Terron-Kwiatkowski A et al. Common loss-of-function variants of the epidermal barrier protein filaggrin are a major predisposing factor for atopic dermatitis. Nat Genet. 2006 Apr;38(4):441-6.
19. Gruber R, Elias PM, Crumrine D et al. Filaggrin genotype in ichthyosis vulgaris predicts abnormalities in epidermal structure and function. Am J Pathol. 2011 May;178(5):2252-63.
20. Hachem JP, Wagberg F, Schmuth M et al. Serine protease activity and residual LEKTI expression determine phenotype in Netherton syndrome. J Invest Dermatol 2006;126:1609-21.
21. Nylander-Lundqvist E, Egelrud T. Formation of active IL-1 beta from pro-IL-1 beta catalyzed by stratum corneum chymotryptic enzyme in vitro. Acta Derm Venereol 1997;77:203-6.
22. Vávrová K, Henkes D, Strüver K et al. Filaggrin deficiency leads to impaired lipid profile and altered acidification pathways in a 3D skin construct. J Invest Dermatol. 2014 Mar;134(3):746-753.
23. Irvine AD, McLean WH, Leung DY. Filaggrin mutations associated with skin and allergic diseases. N Engl J Med. 2011 Oct 6;365(14):1315-27.
24. Tsakok T, Flohr C. Filaggrin and atopic dermatitis (Chapter 16). Thyssen & Maibach's Filaggrin Molecules in Health and Disease. Springer USA 2014.
25. Wang IJ, Lin TJ. FLG P478S polymorphisms and environmental risk factors for the atopic march in Taiwanese children: a prospective cohort study. Ann Allergy Asthma Immunol. 2015;114(1):52–7.
26. Meng L, Wang L, Tang H et al. Filaggrin gene mutation c.3321delA is associated with various clinical features of atopic dermatitis in the Chinese Han population. PLoS ONE. 2014;9(5):e98235.
27. Kim SY, Yang SW, Kim HL et al. Association between P478S polymorphism of the filaggrin gene & atopic dermatitis. Indian J Med Res. 2013;138(6):922–7.
28. Yu HS, Kang MJ, Jung YH et al. Mutations in the filaggrin are predisposing factor in Korean children with atopic dermatitis. Allergy Asthma Immunol Res. 2013;5(4):211–5.
29. Li M, Liu JB, Liu Q et al. Interactions between FLG mutations and allergens in atopic dermatitis. Arch Dermatol Res. 2012;304(10):787–93.
30. Cai SC, Chen H, Koh WP et al. Filaggrin mutations are associated with recurrent skin infection in Singaporean Chinese patients with atopic dermatitis. Br J Dermatol. 2012;166(1):200–3.
31. Wang IJ, Lin TJ, Kuo CF et al. Filaggrin polymorphism P478S, IgE level, and atopic phenotypes. Br J Dermatol. 2011;164(4):791–6.

This article is protected by copyright. All rights reserved.

Accepted Article

32. Margolis DJ, Gupta J, Apter AJ,et al. Filaggrin-2 variation is associated with more persistent atopic dermatitis in African American subjects. J Allergy Clin Immunol. 2014;133(3):784–9.
33. Noda S, Suárez-Fariñas M, Ungar B et al. The Asian atopic dermatitis phenotype combines features of atopic dermatitis and psoriasis with increased TH17 polarization. J Allergy Clin Immunol. 2015 Nov;136(5):1254-64.
34. Weidinger S1, O'Sullivan M, Illig T et al. Filaggrin mutations, atopic eczema, hay fever, and asthma in children. J Allergy Clin Immunol. 2008 May;121(5):1203-1209.e1.
35. Howell MD, Kim BE, Gao P et al. Cytokine modulation of atopic dermatitis filaggrin skin expression. J Allergy Clin Immunol. 2007 Jul;120(1):150-5.
36. Brown SJ, Kroboth K, Sandilands A et al. Intragenic copy number variation within filaggrin contributes to the risk of atopic dermatitis with a dose-dependent effect. J Invest Dermatol. 2012 Jan;132(1):98–104.
37. Pellerin L, Henry J, Hsu CY et al. Defects of filaggrin-like proteins in both lesional and nonlesional atopic skin. J Allergy Clin Immunol. 2013 Apr;131(4):1094-102.
38. De Benedetto A, Slifka MK, Rafaels NM et al. Reductions in claudin-1 may enhance susceptibility to herpes simplex virus 1 infections in atopic dermatitis. J Allergy Clin Immunol. 2011 Jul;128(1):242-246.e5.
39. De Benedetto A, Rafaels NM, McGirt LY et al. Tight junction defects in patients with atopic dermatitis. J Allergy Clin Immunol. 2011 Mar;127(3):773-86.e1-7.
40. Cubero JL, Isidoro-García M, Segura N et al. Filaggrin gene mutations and new SNPs in asthmatic patients: a cross-sectional study in a Spanish population. Allergy Asthma Clin Immunol. 2016 Jul 26;12:31.
41. Kubo A, Nagao K, Amagai M. Epidermal barrier dysfunction and cutaneous sensitization in atopic diseases. J Clin Invest. 2012 Feb;122(2):440-7.
42. Kodama M, Asano K, Oguma T et al. Strain-specific phenotypes of airway inflammation and bronchial hyperresponsiveness induced by epicutaneous allergen sensitization in BALB/c and C57BL/6 mice. Int Arch Allergy Immunol. 2010;152 suppl 1:67–74.
43. Lack G. Epidemiologic risks for food allergy. J Allergy Clin Immunol. 2008 Jun;121(6):1331-6.
44. Tsakok T, Marrs T, Mohsin M et al. Does atopic dermatitis cause food allergy? A systematic review. J Allergy Clin Immunol. 2016 Apr;137(4):1071-8.
45. Lack G, Fox D, Northstone K, Golding J. Avon Longitudinal Study of Parents and Children Study Team. Factors associated with the development of peanut allergy in childhood. N Engl J Med 2003 Mar;348:977-85.
46. Brown SJ, Asai Y, Cordell HJ et al. Loss-of-function variants in the filaggrin gene are a significant risk factor for peanut allergy. J Allergy Clin Immunol. 2011 Mar;127(3):661–7.
47. Brough HA, Liu AH, Sicherer S et al. Atopic dermatitis increases the effect of exposure to peanut antigen in dust on peanut sensitization and likely peanut allergy. J Allergy Clin Immunol. 2015 Jan;135(1):164-70.
48. Flohr C, Perkin M, Logan K et al. Atopic dermatitis and disease severity are the main risk factors for food sensitization in exclusively breastfed infants. J Invest Dermatol. 2014 Feb;134(2):345-50.
49. Du Toit G, Roberts G, Sayre PH et al. Randomized trial of peanut consumption in infants at risk for peanut allergy. N Engl J Med. 2015 Feb 26;372(9):803-13.
50. Cardona ID, Stillman L, Jain N. Does bathing frequency matter in pediatric atopic dermatitis? Ann Allergy Asthma Immunol. 2016 Jul;117(1):9-13.
51. Sherriff A, Golding J; Alspac Study Team. Hygiene levels in a contemporary population cohort are associated with wheezing and atopic eczema in preschool infants. Arch Dis Child. 2002 Jul;87(1):26-9.
52. Perkin MR, Craven J, Logan K et al. Association between domestic water hardness, chlorine, and atopic dermatitis risk in early life: A population-based cross-sectional

This article is protected by copyright. All rights reserved.

Accepted Article

study. J Allergy Clin Immunol. 2016 Apr. pii: S0091-6749(16)30187-7.
53. Engebretsen KA, Bager P, Wohlfahrt J et al. Prevalence of atopic dermatitis in infants by domestic water hardness and season of birth: Cohort study. J Allergy Clin Immunol. 2017 May;139(5):1568-1574.e1.
54. Thomas KS, Dean T, O'Leary C et al. A randomised controlled trial of ion-exchange water softeners for the treatment of eczema in children. PLoS Med. 2011 Feb 15;8(2):e1000395.
55. Silverberg JI, Hanifin J, Simpson EL. Climatic factors are associated with childhood eczema prevalence in the United States. J Invest Dermatol. 2013 Jul;133(7):1752-9.
56. Oyoshi MK, Murphy GF, Geha RS. Filaggrin-deficient mice exhibit TH17-dominated skin inflammation and permissiveness to epicutaneous sensitization with protein antigen. J Allergy Clin Immunol. 2009 Sept. 124(3):485-93.
57. Kawasaki H, Nagao K, Kubo A et al. Altered stratum corneum barrier and enhanced percutaneous immune responses in filaggrin-null mice. J Allergy Clin Immunol. 2012 Jun;129(6):1538-46.e6.
58. Fallon PG, Sasaki T, Sandilands A et al. A homozygous frameshift mutation in the mouse Flg gene facilitates enhanced percutaneous allergen priming. Nat Genet. 2009 May;41(5):602-8.
59. Kezic S, O'Regan GM, Lutter R et al. Filaggrin loss-of-function mutations are associated with enhanced expression of IL-1 cytokines in the stratum corneum of patients with atopic dermatitis and in a murine model of filaggrin deficiency. J Allergy Clin Immunol. 2012 Apr;129(4):1031-9.
60. Scharschmidt TC, Man MQ, Hatano Y et al. Filaggrin deficiency confers a paracellular barrier abnormality that reduces inflammatory thresholds to irritants and haptens. J Allergy Clin Immunol. 2009 Sep;124(3):496-506.
61. Dickel H, Gambichler T, Kamphowe J, Altmeyer P, Skrygan M. Standardized tape stripping prior to patch testing induces upregulation of Hsp90, Hsp70, IL-33, TNF-α and IL-8/CXCL8 mRNA: new insights into the involvement of 'alarmins'. Contact Dermatitis. 2010 Oct;63(4):215-22.
62. Leyva-Castillo JM, Hener P, Michea P et al. Skin thymic stromal lymphopoietin initiates Th2 responses through an orchestrated immune cascade. Nat Commun. 2013;4:2847.
63. Black AP, Ardern-Jones MR, Kasprowicz V et al. Human keratinocyte induction of rapid effector function in antigen-specific memory CD4+ and CD8+ T cells. Eur J Immunol. 2007 Jun;37(6):1485-93.
64. Jarrett R, Salio M, Lloyd-Lavery A et al. Filaggrin inhibits generation of CD1a neolipid antigens by house dust mite-derived phospholipase. Sci Transl Med. 2016 Feb;8(325):325ra18.
65. Suarez-Farinas M, Tintle SJ, Shemer A et al. Nonlesional atopic dermatitis skin is characterized by broad terminal differentiation defects and variable immune abnormalities. J Allergy Clin Immunol. 2011 Apr;127(4):954-64.
66. Gittler JK, Shemer A, Suárez-Fariñas M et al. Progressive activation of T(H)2/T(H)22 cytokines and selective epidermal proteins characterizes acute and chronic atopic dermatitis. J Allergy Clin Immunol. 2012 Dec;130(6):1344-54.
67. Hijnen D, Knol EF, Gent YY et al. CD8(+) T cells in the lesional skin of atopic dermatitis and psoriasis patients are an important source of IFN-γ, IL-13, IL-17, and IL-22. J Invest Dermatol. 2013 Apr;133(4):973-9.
68. Salimi M, Barlow JL, Saunders SP et al. A role for IL-25 and IL-33-driven type-2 innate lymphoid cells in atopic dermatitis. J Exp Med. 2013 Dec;210(13):2939-50.

This article is protected by copyright. All rights reserved.

Accepted Article

69. Glatz M, Bosshard PP, Hoetzenecker W, Schmid-Grendelmeier P. The Role of Malassezia spp. in Atopic Dermatitis. J Clin Med. 2015 May;4(6):1217-28.
70. Gutowska-Owsiak D, Schaupp AL, Salimi M, Taylor S, Ogg GS. Interleukin-22 downregulates filaggrin expression and affects expression of profilaggrin processing enzymes. Br J Dermatol. 2011 Sep;165(3):492-8.
71. Kim BE, Leung DY, Boguniewicz M, Howell MD. Loricrin and involucrin expression is down-regulated by Th2 cytokines through STAT-6. Clin Immunol. 2008 Mar;126(3):332-7.
72. Kopfnagel V, Harder J, Werfel T. Expression of antimicrobial peptides in atopic dermatitis and possible immunoregulatory functions. Curr Opin Allergy Clin Immunol. 2013 Oct;13(5):531-6.
73. Sonkoly E, Muller A, Lauerma AI et al. IL-31: a new link between T cells and pruritus in atopic skin inflammation. J Allergy Clin Immunol. 2006 Feb;117(2):411-7.
74. Mollanazar NK, Smith PK, Yosipovitch G. Mediators of Chronic Pruritus in Atopic Dermatitis: Getting the Itch Out? Clin Rev Allergy Immunol. 2016 Dec;51(3):263-292.
75. Novak N. An update on the role of human dendritic cells in patients with atopic dermatitis. J Allergy Clin Immunol. 2012 Apr;129(4):879-86.
76. Werfel T, Heratizadeh A, Niebuhr M et al. Exacerbation of atopic dermatitis on grass pollen exposure in an environmental challenge chamber. J Allergy Clin Immunol. 2015 Jul;136(1):96-103.
77. Strid J, Sobolev O, Zafirova B et al. The intraepithelial T cell response to NKG2D-ligands links lymphoid stress surveillance to atopy. Science. 2011 Dec;334(6060):1293-7.
78. Gong JQ, Lin L, Lin T et al. Skin colonization by Staphylococcus aureus in patients with eczema and atopic dermatitis and relevant combined topical therapy: a double-blind multicentre randomized controlled trial. Br J Dermatol. 2006 Oct;155(4):680-7.
79. Leyden JJ, Marples RR, Kligman AM. Staphylococcus aureus in the lesions of atopic dermatitis. Br J Dermatol. 1974 May;90(5):525-30.
80. Jinnestal CL, Belfrage E, Bäck O et al. Skin barrier impairment correlates with cutaneous Staphylococcus aureus colonization and sensitization to skin-associated microbial antigens in adult patients with atopic dermatitis. Int J Dermatol. 2014 Jan;53(1):27-33.
81. Brauweiler AM, Goleva E, Leung DY. Th2 cytokines increase Staphylococcus aureus alpha toxin-induced keratinocyte death through the signal transducer and activator of transcription 6 (STAT6). J Invest Dermatol. 2014 Aug;134(8):2114-21.
82. Ezepchuk YV, Leung DY, Middleton MH et al. Staphylococcal toxins and protein A differentially induce cytotoxicity and release of tumor necrosis factor-alpha from human keratinocytes. J Invest Dermatol. 1996 Oct;107(4):603-9.
83. Nakamura Y, Oscherwitz J, Cease KB et al. Staphylococcus δ-toxin induces allergic skin disease by activating mast cells. Nature. 2013 Nov;503(7476):397-401.
84. Zollner TM, Wichelhaus TA, Hartung A et al. Colonization with superantigen-producing Staphylococcus aureus is associated with increased severity of atopic dermatitis. Clin Exp Allergy. 2000 Jul;30(7):994-1000.
85. Kong HH, Oh J, Deming C et al. Temporal shifts in the skin microbiome associated with disease flares and treatment in children with atopic dermatitis. Genome Res. 2012 May;22(5):850-9.

This article is protected by copyright. All rights reserved.

Accepted Article

86. Kobayashi T, Glatz M, Horiuchi K et al. Dysbiosis and Staphylococcus aureus Colonization Drives Inflammation in Atopic Dermatitis. Immunity. 2015 Apr 21;42(4):756-66.
87. Kennedy EA, Connolly J, Hourihane JO et al. Skin microbiome before development of atopic dermatitis: Early colonization with commensal staphylococci at 2 months is associated with a lower risk of atopic dermatitis at 1 year. J Allergy Clin Immunol. 2017 Jan;139(1):166-172.
88. Thaçi D, Simpson EL, Beck LA et al. Efficacy and safety of dupilumab in adults with moderate-to-severe atopic dermatitis inadequately controlled by topical treatments: a randomised, placebo-controlled, dose-ranging phase 2b trial. Lancet. 2016 Jan;387(10013):40-52.
89. Beck LA, Thaçi D, Hamilton JD et al. Dupilumab treatment in adults with moderate-to-severe atopic dermatitis. N Engl J Med. 2014 Jul;371(2):130-9.
90. Simpson EL, Bieber T, Guttman-Yassky E et al. Two Phase 3 Trials of Dupilumab versus Placebo in Atopic Dermatitis. N Engl J Med. 2016 Dec;375(24):2335-2348.
91. Oldhoff JM, Darsow U, Werfel T et al. Anti-IL-5 recombinant humanized monoclonal antibody (mepolizumab) for the treatment of atopic dermatitis. Allergy. 2005 May;60(5):693-6.
92. Demehri S, Morimoto M, Holtzman MJ, Kopan R. Skin-derived TSLP triggers progression from epidermal-barrier defects to asthma. PLoS Biol 2009;7:e1000067.
93. Paller AS, Kabashima K, Bieber T. Therapeutic pipeline for atopic dermatitis: End of the drought? J Allergy Clin Immunol. 2017 Sep;140(3):633-643.
94. Heil PM, Maurer D, Klein B et al. Omalizumab therapy in atopic dermatitis: depletion of IgE does not improve the clinical course - a randomized, placebo-controlled and double blind pilot study. J Dtsch Dermatol Ges. 2010 Dec;8(12):990-8.
95. Hotze M, Baurecht H, Rodríguez E et al. Increased efficacy of omalizumab in atopic dermatitis patients with wild-type filaggrin status and higher serum levels of phosphatidylcholines. Allergy. 2014 Jan;69(1):132-5.
96. Kasperkiewicz M, Schmidt E, Frambach Y et al. Improvement of treatment-refractory atopic dermatitis by immunoadsorption: a pilot study. J Allergy Clin Immunol. 2011 Jan;127(1):267-70, 270.e1-6.
97. Khattri S, Brunner PM, Garcet S et al. Efficacy and safety of ustekinumab treatment in adults with moderate-to-severe atopic dermatitis. Exp Dermatol. 2017 Jan;26(1):28-35.
98. Samrao A, Berry TM, Goreshi R, Simpson EL. A pilot study of an oral phosphodiesterase inhibitor (apremilast) for atopic dermatitis in adults. Arch Dermatol. 2012 Aug;148(8):890-7.
99. Paller AS, Tom WL, Lebwohl MG et al. Efficacy and safety of crisaborole ointment, a novel, nonsteroidal phosphodiesterase 4 (PDE4) inhibitor for the topical treatment of atopic dermatitis (AD) in children and adults. J Am Acad Dermatol. 2016 Sep;75(3):494-503.e4.
100. Di Meglio P, Duarte JH, Ahlfors H et al. Activation of the aryl hydrocarbon receptor dampens the severity of inflammatory skin conditions. Immunity. 2014 Jun 19;40(6):989-1001.
101. Haas K, Weighardt H, Deenen R et al. Aryl Hydrocarbon Receptor in Keratinocytes Is Essential for Murine Skin Barrier Integrity. J Invest Dermatol. 2016 Nov;136(11):2260-2269.
102. van den Bogaard EH, Bergboer JG, Vonk-Bergers M et al. Coal tar induces AHR-dependent skin barrier repair in atopic dermatitis. J Clin Invest 2013;123:917-27.

This article is protected by copyright. All rights reserved.

Accepted Article

103. Bissonnette R, Papp KA, Poulin Y et al. Topical tofacitinib for atopic dermatitis: a phase IIa randomized trial. Br J Dermatol. 2016 Nov;175(5):902-911.
104. Levy LL, Urban J, King BA. Treatment of recalcitrant atopic dermatitis with the oral Janus kinase inhibitor tofacitinib citrate. J Am Acad Dermatol. 2015 Sep;73(3):395-9.
105. Dillon SR, Sprecher C, Hammond A et al. Interleukin 31, a cytokine produced by activated T cells, induces dermatitis in mice. Nat Immunol 2004;5:752-60.
106. Ruzicka T, Hanifin JM, Furue M et al. Anti-interleukin-31 receptor a antibody for atopic dermatitis. N Engl J Med 2017;376:826-35.
107. Ohmura T, Hayashi T, Satoh Y, Konomi A, Jung B, Satoh H. Involvement of substance P in scratching behaviour in an atopic dermatitis model. Eur J Pharmacol 2004;491:191-4.
108. Williams MR, Nakatsuji T, Sanford JA et al. Staphylococcus aureus Induces Increased Serine Protease Activity in Keratinocytes. J Invest Dermatol. 2017 Feb;137(2):377-384.
109. Myles IA, Williams KW, Reckhow JD et al. Transplantation of human skin microbiota in models of atopic dermatitis. JCI Insight. 2016 Jul 7;1(10). pii: e86955.
110. Gueniche A, Knaudt B, Schuck E et al. Effects of nonpathogenic gram-negative bacterium Vitreoscilla filiformis lysate on atopic dermatitis: a prospective, randomized, double-blind, placebo-controlled clinical study. Br J Dermatol. 2008 Dec;159(6):1357-63.
111. Nakatsuji T, Chen TH, Narala S et al. Antimicrobials from human skin commensal bacteria protect against Staphylococcus aureus and are deficient in atopic dermatitis. Sci Transl Med. 2017 Feb 22;9(378).
112. Stout TE, McFarland T, Mitchell JC et al. Recombinant filaggrin is internalized and processed to correct filaggrin deficiency. J Invest Dermatol. 2014 Feb;134(2):423-9.
113. Otsuka A, Doi H, Egawa G et al. Possible new therapeutic strategy to regulate atopic dermatitis through upregulating filaggrin expression. J Allergy Clin Immunol. 2014 Jan;133(1):139-46.e1-10.
114. Flohr C, Mann J. New approaches to the prevention of childhood atopic dermatitis. Allergy. 2014 Jan;69(1):56-61.
115. Kramer MS, Kakuma R. Maternal dietary antigen avoidance during pregnancy or lactation, or both, for preventing or treating atopic disease in the child. Cochrane Database Syst Rev. 2012 Sep;(9):CD000133.
116. Kramer MS, Kakuma R. Optimal duration of exclusive breastfeeding. Cochrane Database Syst Rev. 2012 Aug;(8):CD003517.
117. Christesen HT, Elvander C, Lamont RF, Jørgensen JS. The impact of vitamin D in pregnancy on extraskeletal health in children: a systematic review. Acta Obstet Gynecol Scand. 2012 Dec;91(12):1368-80.
118. Flohr C, Yeo L. Atopic dermatitis and the hygiene hypothesis revisited. Curr Probl Dermatol. 2011;41:1-34.
119. Cuello-Garcia CA, Brożek JL, Fiocchi A et al. Probiotics for the prevention of allergy: A systematic review and meta-analysis of randomized controlled trials. J Allergy Clin Immunol. 2015 Oct;136(4):952-61.
120. Fiocchi A, Pawankar R, Cuello-Garcia C et al. World Allergy Organization-McMaster University Guidelines for Allergic Disease Prevention (GLAD-P): Probiotics. World Allergy Organ J. 2015 Jan;8(1):4.

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Figure legends

Figure 1. Genetic susceptibility to atopic dermatitis. Genetic susceptibility identified by GWAS has indicated components of the epidermal barrier, environmental sensing, immune regulation and tissue response as important aspects of the immunopathogenesis of atopic dermatitis, with the strongest association with null mutations in the *FLG* gene that encodes the epidermal protein filaggrin.

Figure 2. Immunopathogenesis of atopic dermatitis. Epidermal barrier dysfunction and stress from environmental factors is associated with increased epidermal permeability and enhanced microbial and allergen contact with the cutaneous immune system. Damaged epithelial cells activate innate immune mechanisms, such as the release of the pro-inflammatory cytokines IL-1, TSLP, IL-25 and IL-33, and additional chemokines. This leads to activation of innate lymphocyte subsets and antigen presenting cells, with subsequent T cell activation and recruitment. Atopic dermatitis is largely driven by $CD4^+$ T helper 2 (T_H2) cells, but other T cell subsets are found, including $CD4^+$ T_H22 cells. The type 2 cytokines IL-4, IL-5 and IL-13 drive further pathological changes, including eosinophil and mast cell recruitment and activation, B cell IgE production, and IL-31 secretion. There is also crosstalk between inflammatory cytokines, secreted molecules and cutaneous nerves to mediate pruritus, with resultant scratching causing further mechanical disruption of the skin barrier. The inflammatory environment also leads to further impairment in epidermal barrier function and favours *S. aureus* colonisation, leading to bacterial dysbiosis and positive reinforcement of the atopic dermatitis disease cycle.

Epidermal Differentiation Complex (EDC); Interleukin (IL); Thymic stromal lymphopoietin (TSLP); Immunoglobulin (Ig); T helper cell (T_H), Dendritic cell (DC); Langerhans cell (LC); ILC (innate lymphoid cell)

1 Flohr C, Mann J. New insights into the epidemiology of childhood atopic dermatitis. *Allergy* 2014; **69**: 3-16.
2 Beattie PE, Lewis-Jones MS. A comparative study of impairment of quality of life in children with skin disease and children with other chronic childhood diseases. *Br J Dermatol* 2006; **155**: 145-51.
3 Herd RM, Tidman MJ, Prescott RJ *et al.* The cost of atopic eczema. *Br J Dermatol* 1996; **135**: 20-3.
4 Mancini AJ, Kaulback K, Chamlin SL. The socioeconomic impact of atopic dermatitis in the United States: a systematic review. *Pediatr Dermatol* 2008; **25**: 1-6.
5 Williams HC, Burney PG, Pembroke AC *et al.* The U.K. Working Party's Diagnostic Criteria for Atopic Dermatitis. III. Independent hospital validation. *Br J Dermatol* 1994; **131**: 406-16.
6 Hanifin J, Rajka G. Diagnostic features of atopic dermatitis. *Acta Derm Venereol* 1980; **92**: 44-7.
7 Weidinger S, Novak N. Atopic dermatitis. *Lancet* 2016; **387**: 1109-22.

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Accepted Article

8 Silverberg JI, Silverberg NB. Childhood atopic dermatitis and warts are associated with increased risk of infection: a US population-based study. *J Allergy Clin Immunol* 2014; **133**: 1041-7.

9 Patel D, Jahnke MN. Serious Complications from Staphylococcal aureus in Atopic Dermatitis. *Pediatr Dermatol* 2015; **32**: 792-6.

10 Paternoster L, Standl M, Waage J *et al.* Multi-ancestry genome-wide association study of 21,000 cases and 95,000 controls identifies new risk loci for atopic dermatitis. *Nat Genet* 2015; **47**: 1449-56.

11 Simpson EL, Chalmers JR, Hanifin JM *et al.* Emollient enhancement of the skin barrier from birth offers effective atopic dermatitis prevention. *J Allergy Clin Immunol* 2014; **134**: 818-23.

12 Horimukai K, Morita K, Narita M *et al.* Application of moisturizer to neonates prevents development of atopic dermatitis. *J Allergy Clin Immunol* 2014; **134**: 824-30 e6.

13 Candi E, Schmidt R, Melino G. The cornified envelope: a model of cell death in the skin. *Nat Rev Mol Cell Biol* 2005; **6**: 328-40.

14 Flohr C, England K, Radulovic S *et al.* Filaggrin loss-of-function mutations are associated with early-onset eczema, eczema severity and transepidermal water loss at 3 months of age. *Br J Dermatol* 2010; **163**: 1333-6.

15 Kezic S, Kemperman PM, Koster ES *et al.* Loss-of-function mutations in the filaggrin gene lead to reduced level of natural moisturizing factor in the stratum corneum. *J Invest Dermatol* 2008; **128**: 2117-9.

16 Cork MJ, Robinson DA, Vasilopoulos Y *et al.* New perspectives on epidermal barrier dysfunction in atopic dermatitis: gene-environment interactions. *J Allergy Clin Immunol* 2006; **118**: 3-21; quiz 2-3.

17 Hachem JP, Roelandt T, Schurer N *et al.* Acute acidification of stratum corneum membrane domains using polyhydroxyl acids improves lipid processing and inhibits degradation of corneodesmosomes. *J Invest Dermatol* 2010; **130**: 500-10.

18 Elias PM. Stratum corneum acidification: how and why? *Exp Dermatol* 2015; **24**: 179-80.

19 Smith FJ, Irvine AD, Terron-Kwiatkowski A *et al.* Loss-of-function mutations in the gene encoding filaggrin cause ichthyosis vulgaris. *Nat Genet* 2006; **38**: 337-42.

20 Palmer CN, Irvine AD, Terron-Kwiatkowski A *et al.* Common loss-of-function variants of the epidermal barrier protein filaggrin are a major predisposing factor for atopic dermatitis. *Nat Genet* 2006; **38**: 441-6.

21 Elias PM, Feingold KR. Does the tail wag the dog? Role of the barrier in the pathogenesis of inflammatory dermatoses and therapeutic implications. *Arch Dermatol* 2001; **137**: 1079-81.

22 Elias PM, Steinhoff M. "Outside-to-inside" (and now back to "outside") pathogenic mechanisms in atopic dermatitis. *J Invest Dermatol* 2008; **128**: 1067-70.

23 Gruber R, Elias PM, Crumrine D *et al.* Filaggrin genotype in ichthyosis vulgaris predicts abnormalities in epidermal structure and function. *Am J Pathol* 2011; **178**: 2252-63.

24 Vavrova K, Henkes D, Struver K *et al.* Filaggrin deficiency leads to impaired lipid profile and altered acidification pathways in a 3D skin construct. *J Invest Dermatol* 2014; **134**: 746-53.

25 Gao PS, Rafaels NM, Hand T *et al.* Filaggrin mutations that confer risk of atopic dermatitis confer greater risk for eczema herpeticum. *J Allergy Clin Immunol* 2009; **124**: 507-13, 13 e1-7.

26 Miajlovic H, Fallon PG, Irvine AD *et al.* Effect of filaggrin breakdown products on growth of and protein expression by Staphylococcus aureus. *J Allergy Clin Immunol* 2010; **126**: 1184-90 e3.

27 Hachem JP, Wagberg F, Schmuth M *et al.* Serine protease activity and residual LEKTI expression determine phenotype in Netherton syndrome. *J Invest Dermatol* 2006; **126**: 1609-21.

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Accepted Article

28 Nylander-Lundqvist E, Egelrud T. Formation of active IL-1 beta from pro-IL-1 beta catalyzed by stratum corneum chymotryptic enzyme in vitro. *Acta Derm Venereol* 1997; **77**: 203-6.

29 Irvine AD, McLean WH, Leung DY. Filaggrin mutations associated with skin and allergic diseases. *N Engl J Med* 2011; **365**: 1315-27.

30 Tsakok T, Flohr C. Filaggrin and atopic dermatitis. In: *Filaggrin Molecules in Health and Disease*: Springer. 2014.

31 Wang IJ, Lin TJ. FLG P478S polymorphisms and environmental risk factors for the atopic march in Taiwanese children: a prospective cohort study. *Ann Allergy Asthma Immunol* 2015; **114**: 52-7.

32 Meng L, Wang L, Tang H *et al.* Filaggrin gene mutation c.3321delA is associated with various clinical features of atopic dermatitis in the Chinese Han population. *PLoS One* 2014; **9**: e98235.

33 Kim SY, Yang SW, Kim HL *et al.* Association between P478S polymorphism of the filaggrin gene & atopic dermatitis. *Indian J Med Res* 2013; **138**: 922-7.

34 Yu HS, Kang MJ, Jung YH *et al.* Mutations in the Filaggrin are Predisposing Factor in Korean Children With Atopic Dermatitis. *Allergy Asthma Immunol Res* 2013; **5**: 211-5.

35 Li M, Liu JB, Liu Q *et al.* Interactions between FLG mutations and allergens in atopic dermatitis. *Arch Dermatol Res* 2012; **304**: 787-93.

36 Cai SC, Chen H, Koh WP *et al.* Filaggrin mutations are associated with recurrent skin infection in Singaporean Chinese patients with atopic dermatitis. *Br J Dermatol* 2012; **166**: 200-3.

37 Wang IJ, Lin TJ, Kuo CF *et al.* Filaggrin polymorphism P478S, IgE level, and atopic phenotypes. *Br J Dermatol* 2011; **164**: 791-6.

38 Margolis DJ, Gupta J, Apter AJ *et al.* Filaggrin-2 variation is associated with more persistent atopic dermatitis in African American subjects. *J Allergy Clin Immunol* 2014; **133**: 784-9.

39 Noda S, Suarez-Farinas M, Ungar B *et al.* The Asian atopic dermatitis phenotype combines features of atopic dermatitis and psoriasis with increased TH17 polarization. *J Allergy Clin Immunol* 2015; **136**: 1254-64.

40 Weidinger S, O'Sullivan M, Illig T *et al.* Filaggrin mutations, atopic eczema, hay fever, and asthma in children. *J Allergy Clin Immunol* 2008; **121**: 1203-9 e1.

41 Howell MD, Kim BE, Gao P *et al.* Cytokine modulation of atopic dermatitis filaggrin skin expression. *J Allergy Clin Immunol* 2007; **120**: 150-5.

42 Brown SJ, Kroboth K, Sandilands A *et al.* Intragenic copy number variation within filaggrin contributes to the risk of atopic dermatitis with a dose-dependent effect. *J Invest Dermatol* 2012; **132**: 98-104.

43 Pellerin L, Henry J, Hsu CY *et al.* Defects of filaggrin-like proteins in both lesional and nonlesional atopic skin. *J Allergy Clin Immunol* 2013; **131**: 1094-102.

44 De Benedetto A, Slifka MK, Rafaels NM *et al.* Reductions in claudin-1 may enhance susceptibility to herpes simplex virus 1 infections in atopic dermatitis. *J Allergy Clin Immunol* 2011; **128**: 242-6 e5.

45 De Benedetto A, Rafaels NM, McGirt LY *et al.* Tight junction defects in patients with atopic dermatitis. *J Allergy Clin Immunol* 2011; **127**: 773-86 e1-7.

46 Cubero JL, Isidoro-Garcia M, Segura N *et al.* Filaggrin gene mutations and new SNPs in asthmatic patients: a cross-sectional study in a Spanish population. *Allergy Asthma Clin Immunol* 2016; **12**: 31.

47 Kubo A, Nagao K, Amagai M. Epidermal barrier dysfunction and cutaneous sensitization in atopic diseases. *J Clin Invest* 2012; **122**: 440-7.

48 Kodama M, Asano K, Oguma T *et al.* Strain-specific phenotypes of airway inflammation and bronchial hyperresponsiveness induced by epicutaneous allergen sensitization in BALB/c and C57BL/6 mice. *Int Arch Allergy Immunol* 2010; **152 Suppl 1**: 67-74.

49 Lack G. Epidemiologic risks for food allergy. *J Allergy Clin Immunol* 2008; **121**: 1331-6.

This article is protected by copyright. All rights reserved.

Accepted Article

50 Tsakok T, Marrs T, Mohsin M *et al.* Does atopic dermatitis cause food allergy? A systematic review. *J Allergy Clin Immunol* 2016; **137**: 1071-8.

51 Lack G, Fox D, Northstone K *et al.* Factors associated with the development of peanut allergy in childhood. *N Engl J Med* 2003; **348**: 977-85.

52 Brown SJ, Asai Y, Cordell HJ *et al.* Loss-of-function variants in the filaggrin gene are a significant risk factor for peanut allergy. *J Allergy Clin Immunol* 2011; **127**: 661-7.

53 Brough HA, Liu AH, Sicherer S *et al.* Atopic dermatitis increases the effect of exposure to peanut antigen in dust on peanut sensitization and likely peanut allergy. *J Allergy Clin Immunol* 2015; **135**: 164-70.

54 Flohr C, Perkin M, Logan K *et al.* Atopic dermatitis and disease severity are the main risk factors for food sensitization in exclusively breastfed infants. *J Invest Dermatol* 2014; **134**: 345-50.

55 Du Toit G, Roberts G, Sayre PH *et al.* Randomized trial of peanut consumption in infants at risk for peanut allergy. *N Engl J Med* 2015; **372**: 803-13.

56 Takai T, Ikeda S. Barrier dysfunction caused by environmental proteases in the pathogenesis of allergic diseases. *Allergol Int* 2011; **60**: 25-35.

57 Cardona ID, Stillman L, Jain N. Does bathing frequency matter in pediatric atopic dermatitis? *Ann Allergy Asthma Immunol* 2016; **117**: 9-13.

58 Sherriff A, Golding J, Alspac Study T. Hygiene levels in a contemporary population cohort are associated with wheezing and atopic eczema in preschool infants. *Arch Dis Child* 2002; **87**: 26-9.

59 Perkin MR, Craven J, Logan K *et al.* Association between domestic water hardness, chlorine, and atopic dermatitis risk in early life: A population-based cross-sectional study. *J Allergy Clin Immunol* 2016; **138**: 509-16.

60 Engebretsen KA, Bager P, Wohlfahrt J *et al.* Prevalence of atopic dermatitis in infants by domestic water hardness and season of birth: Cohort study. *J Allergy Clin Immunol* 2017; **139**: 1568-74 e1.

61 Thomas KS, Dean T, O'Leary C *et al.* A randomised controlled trial of ion-exchange water softeners for the treatment of eczema in children. *PLoS Med* 2011; **8**: e1000395.

62 Silverberg JI, Hanifin J, Simpson EL. Climatic factors are associated with childhood eczema prevalence in the United States. *J Invest Dermatol* 2013; **133**: 1752-9.

63 Fuertes E, Flohr C, Silverberg JI *et al.* Global Associations between UVR Exposure and Current Eczema Prevalence in Children from ISAAC Phase Three. *J Invest Dermatol* 2017; **137**: 1248-56.

64 Oyoshi MK, Murphy GF, Geha RS. Filaggrin-deficient mice exhibit TH17-dominated skin inflammation and permissiveness to epicutaneous sensitization with protein antigen. *J Allergy Clin Immunol* 2009; **124**: 485-93, 93 e1.

65 Kawasaki H, Nagao K, Kubo A *et al.* Altered stratum corneum barrier and enhanced percutaneous immune responses in filaggrin-null mice. *J Allergy Clin Immunol* 2012; **129**: 1538-46 e6.

66 Fallon PG, Sasaki T, Sandilands A *et al.* A homozygous frameshift mutation in the mouse Flg gene facilitates enhanced percutaneous allergen priming. *Nat Genet* 2009; **41**: 602-8.

67 Kezic S, O'Regan GM, Lutter R *et al.* Filaggrin loss-of-function mutations are associated with enhanced expression of IL-1 cytokines in the stratum corneum of patients with atopic dermatitis and in a murine model of filaggrin deficiency. *J Allergy Clin Immunol* 2012; **129**: 1031-9 e1.

68 Scharschmidt TC, Man MQ, Hatano Y *et al.* Filaggrin deficiency confers a paracellular barrier abnormality that reduces inflammatory thresholds to irritants and haptens. *J Allergy Clin Immunol* 2009; **124**: 496-506, e1-6.

69 Dickel H, Gambichler T, Kamphowe J *et al.* Standardized tape stripping prior to patch testing induces upregulation of Hsp90, Hsp70, IL-33, TNF-alpha and IL-8/CXCL8 mRNA: new insights into the involvement of 'alarmins'. *Contact Dermatitis* 2010; **63**: 215-22.

70 Leyva-Castillo JM, Hener P, Michea P *et al.* Skin thymic stromal lymphopoietin initiates Th2 responses through an orchestrated immune cascade. *Nat Commun* 2013; **4**: 2847.

This article is protected by copyright. All rights reserved.

Accepted Article

71 Black AP, Ardern-Jones MR, Kasprowicz V *et al.* Human keratinocyte induction of rapid effector function in antigen-specific memory CD4+ and CD8+ T cells. *Eur J Immunol* 2007; **37**: 1485-93.

72 Jarrett R, Salio M, Lloyd-Lavery A *et al.* Filaggrin inhibits generation of CD1a neolipid antigens by house dust mite-derived phospholipase. *Sci Transl Med* 2016; **8**: 325ra18.

73 Suarez-Farinas M, Tintle SJ, Shemer A *et al.* Nonlesional atopic dermatitis skin is characterized by broad terminal differentiation defects and variable immune abnormalities. *J Allergy Clin Immunol* 2011; **127**: 954-64 e1-4.

74 Gittler JK, Shemer A, Suarez-Farinas M *et al.* Progressive activation of T(H)2/T(H)22 cytokines and selective epidermal proteins characterizes acute and chronic atopic dermatitis. *J Allergy Clin Immunol* 2012; **130**: 1344-54.

75 Hijnen D, Knol EF, Gent YY *et al.* CD8(+) T cells in the lesional skin of atopic dermatitis and psoriasis patients are an important source of IFN-gamma, IL-13, IL-17, and IL-22. *J Invest Dermatol* 2013; **133**: 973-9.

76 Salimi M, Barlow JL, Saunders SP *et al.* A role for IL-25 and IL-33-driven type-2 innate lymphoid cells in atopic dermatitis. *J Exp Med* 2013; **210**: 2939-50.

77 Glatz M, Bosshard PP, Hoetzenecker W *et al.* The Role of Malassezia spp. in Atopic Dermatitis. *J Clin Med* 2015; **4**: 1217-28.

78 Gutowska-Owsiak D, Schaupp AL, Salimi M *et al.* Interleukin-22 downregulates filaggrin expression and affects expression of profilaggrin processing enzymes. *Br J Dermatol* 2011; **165**: 492-8.

79 Kim BE, Leung DY, Boguniewicz M *et al.* Loricrin and involucrin expression is down-regulated by Th2 cytokines through STAT-6. *Clin Immunol* 2008; **126**: 332-7.

80 Kopfnagel V, Harder J, Werfel T. Expression of antimicrobial peptides in atopic dermatitis and possible immunoregulatory functions. *Curr Opin Allergy Clin Immunol* 2013; **13**: 531-6.

81 Sonkoly E, Muller A, Lauerma AI *et al.* IL-31: a new link between T cells and pruritus in atopic skin inflammation. *J Allergy Clin Immunol* 2006; **117**: 411-7.

82 Mollanazar NK, Smith PK, Yosipovitch G. Mediators of Chronic Pruritus in Atopic Dermatitis: Getting the Itch Out? *Clin Rev Allergy Immunol* 2016; **51**: 263-92.

83 Novak N. An update on the role of human dendritic cells in patients with atopic dermatitis. *J Allergy Clin Immunol* 2012; **129**: 879-86.

84 Werfel T, Heratizadeh A, Niebuhr M *et al.* Exacerbation of atopic dermatitis on grass pollen exposure in an environmental challenge chamber. *J Allergy Clin Immunol* 2015; **136**: 96-103 e9.

85 Strid J, Sobolev O, Zafirova B *et al.* The intraepithelial T cell response to NKG2D-ligands links lymphoid stress surveillance to atopy. *Science* 2011; **334**: 1293-7.

86 Gong JQ, Lin L, Lin T *et al.* Skin colonization by Staphylococcus aureus in patients with eczema and atopic dermatitis and relevant combined topical therapy: a double-blind multicentre randomized controlled trial. *Br J Dermatol* 2006; **155**: 680-7.

87 Leyden JJ, Marples RR, Kligman AM. Staphylococcus aureus in the lesions of atopic dermatitis. *Br J Dermatol* 1974; **90**: 525-30.

88 Jinnestal CL, Belfrage E, Back O *et al.* Skin barrier impairment correlates with cutaneous Staphylococcus aureus colonization and sensitization to skin-associated microbial antigens in adult patients with atopic dermatitis. *Int J Dermatol* 2014; **53**: 27-33.

89 Brauweiler AM, Goleva E, Leung DYM. Th2 cytokines increase Staphylococcus aureus alpha toxin-induced keratinocyte death through the signal transducer and activator of transcription 6 (STAT6). *J Invest Dermatol* 2014; **134**: 2114-21.

90 Ezepchuk YV, Leung DY, Middleton MH *et al.* Staphylococcal toxins and protein A differentially induce cytotoxicity and release of tumor necrosis factor-alpha from human keratinocytes. *J Invest Dermatol* 1996; **107**: 603-9.

91 Nakamura Y, Oscherwitz J, Cease KB *et al.* Staphylococcus delta-toxin induces allergic skin disease by activating mast cells. *Nature* 2013; **503**: 397-401.

This article is protected by copyright. All rights reserved.

Accepted Article

92 Zollner TM, Wichelhaus TA, Hartung A *et al.* Colonization with superantigen-producing Staphylococcus aureus is associated with increased severity of atopic dermatitis. *Clin Exp Allergy* 2000; **30**: 994-1000.

93 Kong HH, Oh J, Deming C *et al.* Temporal shifts in the skin microbiome associated with disease flares and treatment in children with atopic dermatitis. *Genome Res* 2012; **22**: 850-9.

94 Kobayashi T, Glatz M, Horiuchi K *et al.* Dysbiosis and Staphylococcus aureus Colonization Drives Inflammation in Atopic Dermatitis. *Immunity* 2015; **42**: 756-66.

95 Kennedy EA, Connolly J, Hourihane JO *et al.* Skin microbiome before development of atopic dermatitis: Early colonization with commensal staphylococci at 2 months is associated with a lower risk of atopic dermatitis at 1 year. *J Allergy Clin Immunol* 2017; **139**: 166-72.

96 Thaci D, Simpson EL, Beck LA *et al.* Efficacy and safety of dupilumab in adults with moderate-to-severe atopic dermatitis inadequately controlled by topical treatments: a randomised, placebo-controlled, dose-ranging phase 2b trial. *Lancet* 2016; **387**: 40-52.

97 Beck LA, Thaci D, Hamilton JD *et al.* Dupilumab treatment in adults with moderate-to-severe atopic dermatitis. *N Engl J Med* 2014; **371**: 130-9.

98 Simpson EL, Bieber T, Guttman-Yassky E *et al.* Two Phase 3 Trials of Dupilumab versus Placebo in Atopic Dermatitis. *N Engl J Med* 2016; **375**: 2335-48.

99 Oldhoff JM, Darsow U, Werfel T *et al.* Anti-IL-5 recombinant humanized monoclonal antibody (mepolizumab) for the treatment of atopic dermatitis. *Allergy* 2005; **60**: 693-6.

100 Demehri S, Morimoto M, Holtzman MJ *et al.* Skin-derived TSLP triggers progression from epidermal-barrier defects to asthma. *PLoS Biol* 2009; **7**: e1000067.

101 Paller AS, Kabashima K, Bieber T. Therapeutic pipeline for atopic dermatitis: End of the drought? *J Allergy Clin Immunol* 2017; **140**: 633-43.

102 Heil PM, Maurer D, Klein B *et al.* Omalizumab therapy in atopic dermatitis: depletion of IgE does not improve the clinical course - a randomized, placebo-controlled and double blind pilot study. *J Dtsch Dermatol Ges* 2010; **8**: 990-8.

103 Hotze M, Baurecht H, Rodriguez E *et al.* Increased efficacy of omalizumab in atopic dermatitis patients with wild-type filaggrin status and higher serum levels of phosphatidylcholines. *Allergy* 2014; **69**: 132-5.

104 Kasperkiewicz M, Schmidt E, Frambach Y *et al.* Improvement of treatment-refractory atopic dermatitis by immunoadsorption: a pilot study. *J Allergy Clin Immunol* 2011; **127**: 267-70, 70 e1-6.

105 Khattri S, Brunner PM, Garcet S *et al.* Efficacy and safety of ustekinumab treatment in adults with moderate-to-severe atopic dermatitis. *Exp Dermatol* 2017; **26**: 28-35.

106 Samrao A, Berry TM, Goreshi R *et al.* A pilot study of an oral phosphodiesterase inhibitor (apremilast) for atopic dermatitis in adults. *Arch Dermatol* 2012; **148**: 890-7.

107 Paller AS, Tom WL, Lebwohl MG *et al.* Efficacy and safety of crisaborole ointment, a novel, nonsteroidal phosphodiesterase 4 (PDE4) inhibitor for the topical treatment of atopic dermatitis (AD) in children and adults. *J Am Acad Dermatol* 2016; **75**: 494-503 e6.

108 Di Meglio P, Duarte JH, Ahlfors H *et al.* Activation of the aryl hydrocarbon receptor dampens the severity of inflammatory skin conditions. *Immunity* 2014; **40**: 989-1001.

109 Haas K, Weighardt H, Deenen R *et al.* Aryl Hydrocarbon Receptor in Keratinocytes Is Essential for Murine Skin Barrier Integrity. *J Invest Dermatol* 2016; **136**: 2260-9.

110 van den Bogaard EH, Bergboer JG, Vonk-Bergers M *et al.* Coal tar induces AHR-dependent skin barrier repair in atopic dermatitis. *J Clin Invest* 2013; **123**: 917-27.

111 Bissonnette R, Papp KA, Poulin Y *et al.* Topical tofacitinib for atopic dermatitis: a phase IIa randomized trial. *Br J Dermatol* 2016; **175**: 902-11.

112 Levy LL, Urban J, King BA. Treatment of recalcitrant atopic dermatitis with the oral Janus kinase inhibitor tofacitinib citrate. *J Am Acad Dermatol* 2015; **73**: 395-9.

113 Dillon SR, Sprecher C, Hammond A *et al.* Interleukin 31, a cytokine produced by activated T cells, induces dermatitis in mice. *Nat Immunol* 2004; **5**: 752-60.

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Accepted Article

114 Ruzicka T, Hanifin JM, Furue M *et al.* Anti-Interleukin-31 Receptor A Antibody for Atopic Dermatitis. *N Engl J Med* 2017; **376**: 826-35.

115 Ohmura T, Hayashi T, Satoh Y *et al.* Involvement of substance P in scratching behaviour in an atopic dermatitis model. *Eur J Pharmacol* 2004; **491**: 191-4.

116 Williams MR, Nakatsuji T, Sanford JA *et al.* Staphylococcus aureus Induces Increased Serine Protease Activity in Keratinocytes. *J Invest Dermatol* 2017; **137**: 377-84.

117 Myles IA, Williams KW, Reckhow JD *et al.* Transplantation of human skin microbiota in models of atopic dermatitis. *JCI Insight* 2016; **1**.

118 Gueniche A, Knaudt B, Schuck E *et al.* Effects of nonpathogenic gram-negative bacterium Vitreoscilla filiformis lysate on atopic dermatitis: a prospective, randomized, double-blind, placebo-controlled clinical study. *Br J Dermatol* 2008; **159**: 1357-63.

119 Nakatsuji T, Chen TH, Narala S *et al.* Antimicrobials from human skin commensal bacteria protect against Staphylococcus aureus and are deficient in atopic dermatitis. *Sci Transl Med* 2017; **9**.

120 Stout TE, McFarland T, Mitchell JC *et al.* Recombinant filaggrin is internalized and processed to correct filaggrin deficiency. *J Invest Dermatol* 2014; **134**: 423-9.

121 Otsuka A, Doi H, Egawa G *et al.* Possible new therapeutic strategy to regulate atopic dermatitis through upregulating filaggrin expression. *J Allergy Clin Immunol* 2014; **133**: 139-46 e1-10.

122 Flohr C, Mann J. New approaches to the prevention of childhood atopic dermatitis. *Allergy* 2014; **69**: 56-61.

123 Kramer MS, Kakuma R. Maternal dietary antigen avoidance during pregnancy or lactation, or both, for preventing or treating atopic disease in the child. *Cochrane Database Syst Rev* 2012: CD000133.

124 Kramer MS, Kakuma R. Optimal duration of exclusive breastfeeding. *Cochrane Database Syst Rev* 2012: CD003517.

125 Christesen HT, Elvander C, Lamont RF *et al.* The impact of vitamin D in pregnancy on extraskeletal health in children: a systematic review. *Acta Obstet Gynecol Scand* 2012; **91**: 1368-80.

126 Flohr C, Yeo L. Atopic dermatitis and the hygiene hypothesis revisited. *Curr Probl Dermatol* 2011; **41**: 1-34.

127 Cuello-Garcia CA, Brozek JL, Fiocchi A *et al.* Probiotics for the prevention of allergy: A systematic review and meta-analysis of randomized controlled trials. *J Allergy Clin Immunol* 2015; **136**: 952-61.

128 Zhao M, Shen C, Ma L. Treatment efficacy of probiotics on atopic dermatitis, zooming in on infants: a systematic review and meta-analysis. *Int J Dermatol* 2018; **57**: 635-41.

129 Fiocchi A, Pawankar R, Cuello-Garcia C *et al.* World Allergy Organization-McMaster University Guidelines for Allergic Disease Prevention (GLAD-P): Probiotics. *World Allergy Organ J* 2015; **8**: 4.

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