Here we revisit the inside-out hypothesis in light of these new findings. Reviews have focused on the role of skin barrier defects in AD [7,8]. The clinical manifestations of the disease. A growing number of recent studies of human skin and mechanistic studies on keratinocytes and mouse models of AD. We discuss the importance of the skin barrier and review recent findings on the pro-Th2 cytokines TSLP, IL-25, and IL-33, notably their ability to polarize dendritic cells and promote Th2 responses. After a brief update on the contribution of different T-cell subsets to AD, we focus on Th2 cells and the respective contributions of each of the Th2 cytokines (IL-4, IL-13, IL-5, IL-31, and IL-10) to AD. We conclude with a brief discussion of the current gaps in our knowledge and technical limitations.

Introduction

Atopic dermatitis (AD), also named atopic eczema, is a chronic relapsing skin disease, often occurring within the first year of life and affecting up to 20% of children, the majority of whom outgrow the disease within a few years. As a result, and despite the occurrence of late-onset AD in some adults, the prevalence of AD in the adult population has been estimated to be much lower (2-9%) [1]. Clinical features of AD have been detailed elsewhere [6]. In early infancy, the absence of atopic dermatitis as result of an environmental alteration, would lead to sensitization and eventually be sensitized to an array of aeroallergen and food allergens. Thus, the vast majority of AD patients will develop an atopic disease [5] and should be better referred to as non-atopic eczema. Clinical features of AD have been detailed elsewhere and have been contrasted to psoriasis [2,3].

Atopic diseases are characterized by IgE sensitization to environmental allergens. The gene-environment interactions leading to the development of AD are only partially understood [4]. Distinguishing between primary events leading to AD and secondary events resulting from AD is particularly challenging and complicated by the fact that a majority of young patients rapidly outgrow the disease and therefore offer a short window of investigation. Traditionally, two competing hypothesis are presented to explain the pathogenesis of atopic dermatitis. The inside-out hypothesis suggests that an immunological defect predisposes to atopy and that this IgE-mediated sensitization will result in AD, whereas the outside-in hypothesis proposes that disruption of the skin barrier, either resulting from an intrinsic genetic defect in epidermal skin barrier formation or as a result of an environmental alteration, would lead to sensitization and atopic disease.

The proposed existence of an intrinsic form of AD not associated with IgE-mediated sensitization contradicts the classic definition of an atopic disease [5] and should be better referred to as non-atopic eczema. Clinical features distinguishing intrinsic AD from extrinsic AD has recently been reviewed elsewhere [6]. In early infancy, the absence of allergen-specific IgE is most likely the result of an immature adaptive immune system that will gradually develop IgE-mediated sensitization to environmental allergens. Thus, the vast majority of AD patients will eventually be sensitized to an array of aeroallergen and food allergens [5]. Gene-environmental interactions will determine the timing and strength of the sensitization as well as the likelihood of outgrowing the clinical manifestations of the disease. A growing number of recent reviews have focused on the role of skin barrier defects in AD [7,8]. Here we revisit the inside-out hypothesis in light of these new findings with a particular emphasis on what genetic manipulation in murine models has taught us about Th2 involvement in development of experimental AD.

Skin barrier defects in atopic dermatitis

The outermost layer of skin, the epidermis, is composed of four layers – a basal layer of proliferating keratinocytes, a spinous layer, a granular layer, and the stratum corneum, a brick and mortar structure, composed of dense layers of corneocytes resulting from the differentiation of keratinocytes in the epidermal layers below. These cells are held together by tight junctions, which restrict circulation of large molecules or pathogens through the skin. Tight junctions are formed by transmembrane proteins (notably Claudin-1 to 24) held together by scaffolding proteins (including zonulae occludens ZO-1 to 3). A growing body of evidence suggests that skin barrier dysfunctions promote the development and severity of AD [8]. Recently, impaired epidermal expression of claudin-1 has been reported in non-lesional skin of AD patients compared to psoriasis patients and non-atopic controls with a concomitant association with disease severity [9]. Claudin-1 deficient mice suffer from severe dehydration and skin barrier dysfunction, assessed by measuring increased trans-epidermal water loss (TEWL), and die shortly after birth [10].

Over 30 studies have reported positive associations between polymorphisms in skin barrier genes and AD [11]. A number of the barrier genes are localized on chromosome 1 in a cluster called the epidermal differentiation complex (EDC) and include filaggrin (FLG), loricrin, and involucrin. Most of the genetic studies relate to FLG, a keratinocyte gene that encodes profilaggrin, a large molecule composed of a dozen filaggrin repeats and a major component of keratohyaline granules, that characterize the granular layer of the epidermis [12].

Keywords: Barrier defects; IL-4; IL-13; IL-5; IL-10; IL-31; TSLP; Keratinocytes; T cells

Abstract

Atopic dermatitis (AD), a chronic relapsing inflammatory skin disease, is increasing in prevalence around the world. Intensive research is ongoing to understand the mechanisms involved in the development of AD and offer new treatment options for patients suffering from AD. In this review, we highlight the importance of allergic Th2 responses in the development of the disease and summarize relevant literature, including genetic studies, studies of human skin and mechanistic studies on keratinocytes and mouse models of AD. We discuss the importance of the skin barrier and review recent findings on the pro-Th2 cytokines TSLP, IL-25, and IL-33, notably their ability to polarize dendritic cells and promote Th2 responses. After a brief update on the contribution of different T-cell subsets to AD, we focus on Th2 cells and the respective contributions of each of the Th2 cytokines (IL-4, IL-13, IL-5, IL-31, and IL-10) to AD. We conclude with a brief discussion of the current gaps in our knowledge and technical limitations.
growing number of rare mutations have been described in the numerous repeats of the FLG gene, most of which differ between European and Asian populations [13]. Most studies are not powered to detect rare variants within a specific population, hampering an accurate estimate of the real prevalence of these filaggrin mutations in AD patients and non-atopic skin diseases. While filaggrin mutations are important contributors to AD, anecdotal evidence indicates that filaggrin loss-of-function mutations do not necessarily lead to AD. Despite being generally associated with more severe AD, carriers of these filaggrin mutations can outgrow the disease [14] suggesting that breakdown in the skin barrier is not sufficient for the development of AD.

Filaggrin is dephosphorylated and cleaved by serine proteases, that in turn are tightly regulated, most notably by the serine peptidase inhibitor Kazal type 5 (SPINK5). Mutations in SPINK5 have been associated with AD in several studies [11,15].

Mice with a natural mutation in their filaggrin gene develop dry a scaly skin a few days after birth but this phenotype subsides as they start growing fur. These so called “flaky tail (ft)” mice have a frameshift point mutation in their FLG gene resulting in an abnormal profilaggrin polypeptide that is not cleaved to generate filaggrin [16,17]. Filaggrin is involved in the collapse of the keratin cytoskeleton resulting in the formation of the cornified layers that help maintain hydration and possibly also skin pH. One of the earliest features of AD is dry skin, resulting in increased trans-epidermal water loss (TEWL) and in impaired protection against environmental pathogens and molecules (irritants, endotoxins, allergens) [16,18,19]. As the ft mice age, they start developing clinical feature of AD like erythema, pruritic lesions and edema as well as increased blood IgE levels [18,19]. Interestingly, the nature of the immune responses evolves with time, starting with elevated skin levels of Th17 associated cytokines (IL-6, IL-17A and IL-23). Several months later, skin levels of Th2 cytokines (IL-4 and IL-13) started increasing whereas IFNy levels remained unchanged. The filaggrin mutation in the ft mice occurred spontaneously resulting from a mutation in the matted (ma) gene located close by. After these ft mice were backcrossed until the ma mutation was lost, increased skin inflammation dominated by eosinophils could still be observed [16]. Increased sensitization to ovalbumin, an allergen which usually fails to induce an immune response when applied to intact wild type skin, was observed in OVA exposed filaggrin deficient mice [16,19]. The nature of the environmental factors leading to the early Th17 response and the mechanisms behind the emergence of a Th2 response still need to be addressed.

Skin barrier removal by tape stripping has been recently shown to polarize skin dendritic cells to promote a Th2 response upon allergen exposure [20]. Interestingly, damaging the skin by tape stripping increased skin levels of TSLP and the resulting Th2 response was dependent on TSLP signaling [20]. Together these data suggest that skin barrier dysfunction is an important contributor to the development of AD.

Th2 promoting cytokines

IL-25, IL-33 and TSLP are epithelial cell derived cytokines that promote Th2 cytokine responses either directly by Th2 cytokine expressing cells or indirectly via dendritic cell polarization [21,22]. Interestingly IL-25 and IL-33 have been recently shown to induce the generation of Th2 cytokine producing innate immune cell populations [23]. Their nature and importance in Th2 responses is under active investigation and their role in skin diseases unknown. Th2 cytokines can also be secreted by basophils, eosinophils and mast cells. Basophils have recently been detected in AD skin lesions but their role in AD remains unknown [24]. Eosinophils recruitment into the skin is characteristic of AD. However, in mice lacking the chemokine receptor necessary for eosinophil recruitment (CCR3), the absence of skin eosinophilia had no significant impact on experimental AD [25]. The role of mast cells in AD has been recently reviewed elsewhere [26]. Modulation of Th2 cytokine release by basophils, mast cells and eosinophils are beyond the scope of this review. We will review here, what is known about IL-25, IL-33 and TSLP in AD, then focus on dendritic cells before reviewing the role of T-cells in AD and specifically focus on the Th2 cytokines.

IL-33: IL-33 is a member of the IL-1 family of cytokines [21] and is known to induce expression of the Th2 cytokines IL-5 and IL-13 in vitro, as well as induction of splenomegaly, increased blood eosinophils and serum immunoglobulins when delivered in vivo [27]. In addition to T cells, IL-33 can also induce the activation and maturation of human mast cells [28]. In humans, IL-33 mRNA levels are induced almost 10-fold in the skin of AD patients compared to healthy skin [29]. In mice, subcutaneous injections of IL-33 resulted in cutaneous fibrosis than was dependent on eosinophils and IL-13 but not IL-4 [30]. However the role of IL-33 in atopic dermatitis remains to be evaluated.

TSLP Thymic stromal lymphopoietin (TSLP) was first identified in a mouse thymic stromal cell line. However, epithelial cells, including keratinocytes, have been shown to be the major producers of TSLP. The human and mouse homologs exert similar biologic effects despite sharing only 43% amino acids. TSLP signals through a heterodimeric receptor composed of the TSLP receptor (TSLPR) and the IL-7 receptor alpha (IL7Rα) [31].

The importance of TSLP in AD was first shown when elevated levels of TSLP were observed in lesional skin of AD patients but not in nickel-induced contact allergic dermatitis or in cutaneous lupus erythematosus lesions [32]. In a small study using atopic individuals, dermal injection of a relevant allergen into normal skin triggered a rapid TSLP expression in the dermis, primarily by elastase+ neutrophils, CD31+ endothelial cells, tryptase+ mast cells and CD68+ macrophages. Interestingly, epidermal expression was not observed, possibly because the needle bypassed the epidermis [33]. This was followed by recruitment of TSLP+CD11c+ cells into the skin within 24h of allergen exposure. In contrast, another study using explants from normal human skin demonstrated TSLP was produced by keratinocytes exclusively when exposed to a combination of pro-inflammatory cytokines (TNF-α or IL-1α) and Th2 cytokines (IL-4 and IL-13) [34], but not with either class of cytokine alone. Similar findings were seen in keratinocyte cultures [35,36].

The contribution of innate immune ligands to TSLP expression was not investigated until recently. Heat-killled S. aureus (Staphylococcus aureus) and its subcellular fractions induced TSLP’s release, with the membrane fraction having the greatest activity [37]. TSLP release was enhanced by a combination of Th2 cytokines and TNF-α and partially suppressed by IFN-γ and TGF-β [37]. Small interfering RNA-mediated knockdown of either TLR-2 or TLR-6 inhibited TSLP gene expression. Two recent studies suggest that polyinosinic-polycytidylic acid (poly I:C) a synthetic TLR3 ligand as well as flagellin, a natural TLR5 ligand derived from the flagella of Gram-negative bacteria are both able to induce TSLP production from primary human keratinocytes [35,36]. These data suggest that the bacterial colonization of skin, so common in patients with AD, could further exacerbate the Th2 response.
Overexpression of TSLP in keratinocytes either constitutively (under the K14 promoter) or inducible (K15 promoter) resulted in the development of an AD like phenotype dominated by Th2 cytokines and associated with elevated systemic IgE levels [38]. Importantly, the inflammation resulting from the induction of TSLP in keratinocytes was demonstrated to be T-cell independent using TCRB deficient mice whereas in mice intradermally injected with recombinant TSLP, the resulting Th2 response was abrogated in RAG2 deficient mice (lacking T-cells). TSLP induced inflammation was T-cell dependent but only partially dependent on IL-4 or IL-13 [39]. Interestingly, IL-5 skin mRNA levels were significantly increased in IL-4 deficient mice, IL-13 deficient mice and STAT6 deficient mice pointing to a potential role for eosinophils in this process. Indeed, eosinophil deficient mice were partially protected from TSLP-induced inflammation [39]. Accordingly, intradermal injection of anti-TSLP blocked the recruitment of eosinophils and the ability of T-cells to produce Th2 cytokines following epicutaneous allergen challenge of OVA sensitized mice [40].

In this classic murine model of AD resulting from repeat applications of ovalbumin patches to tape-stripped skin, the increase in skin levels of Th2 cytokines was ablated in mice lacking the TSLP receptor [40]. Interestingly, skin eosinophil numbers but not CD4 T-cells numbers were decreased in the skin of OVA-exposed TSLP deficient mice. TSLPR deficient dendritic cells showed no defect in migration or expression of activation markers. After being transferred into naive mice, TSLPR deficient T-cells showed no defect in recruitment to the skin but failed to upregulate IL-4 and IL-13 following epicutaneous OVA challenge. These results are consistent with previous studies showing that B and T-cell appear to develop normally in TSLPR deficient mice but that TSLP preferentially expanded CD4+ T cells both in vitro and in vivo [41]. Indeed, TSLP has been demonstrated to induce differentiation of naïve murine CD4+ T-cells into IL-4 expressing Th2 cells [42]. In humans, positive staining for receptor expression or TSLPR mRNA levels was absent in PBMC derived-T-cells [43]. Similarly, TSLPR mRNA expression was lacking in freshly isolated human memory cells and CRTH2 CD4+ T-cells [33]. However, upon TCR activation CD4+ T-cells expressed TSLPR and demonstrated increased proliferation and expression of CD25 in the presence of TSLP [43]. Since human CD4+ T-cells don’t constitutively express TSLPR but CD11c+ dendritic cells do, it appears likely that TSLP acts on T-cell differentiation via dendritic cell activation. Indeed several studies have demonstrated that DCs primed with TSLP strongly promote Th2 differentiation [32,44].

IL-25: IL-25, also known as IL-17E, is important in modulating Th2 responses [22]. Two studies have demonstrated that mRNA and protein levels of IL-25 and it cognate receptor, IL-25R are elevated in the skin of individuals with AD, and their expression is higher in lesional skin compared to non-lesional skin [44,45]. The authors also demonstrate that IL-25 is produced by multiple cell types including DCs, basophils and eosinophils. TSLP activated DCs induce Th2 polarization, and IL-25 augments this effect on Th2 cells [44]. In primary keratinocytes allergen exposure alone is sufficient to induce IL-25 expression [44]. And filaggrin expression is attenuated in primary keratinocytes treated with IL-25 [45]. Together this data suggests that IL-25 has dual effects: 1) induction of a Th2 response and 2) promoting barrier breakdown by directly acting on keratinocytes. However, the effects on keratinocytes are based on in vitro studies, and remain to be validated in vivo.

Dendritic cells: By taking up, processing and presenting antigen to T-cells, dendritic cells (DCs) are central players in the initiation of an adaptive immune response. The nature of this adaptive immune response is shaped by the multiple environmental signals the dendritic cells are exposed to, including bacterial compounds and cytokines like IL-12 (pro-Th1) and TSLP (pro-Th2). In the presence of TSLP receptor signaling and the anti-inflammatory cytokine IL-10, DCs activated by mechanical injury trigger Th2 skewing and increased expression of IL-4 and IL-13 [20].

The epidermal layer of the skin is infiltrated with a unique DC subset called Langerhans cells (LCs). In addition, there are several distinct DC subsets in the dermal layer. The recent discovery of a dermal DC expressing langerin (CD207), a surface marker thought to be specific for Langerhans cells, let to a reassessment of the role of Langerhans cells in skin diseases. There are several extensive reviews published in the last year that have examined the role of DCs in AD [46-49] and we refer the readers to these reviews for details. Because of the variety of DC subsets and their recent identification, the specific role of each subset remains to be evaluated.

DCs express toll like receptors (TLRs) that recognize pathogen-derived antigens, as well as FcεRI, the high affinity receptor for IgE. Crosslinking of this receptor by antigen-specific IgE, along with TLR activation leads to the maturation of the DCs and their ability to shape adaptive immune responses in the skin. However, human and murine DCs differ in their expression of some TLRs and FcεRI is present on human LC but not on murine LC. As discussed earlier, not all patients with AD show elevated IgE levels. In a murine AD model induced by epicutaneous exposure to ovalbumin, Abboud et al demonstrated that IgE signaling through the FcεRI receptor partially contributed to the AD phenotype, but IgG signaling through the FcγRIII/CD16 low affinity receptor also contributed significantly to the phenotype [50]. In the absence of FcεRI, there was a partial decrease in skin thickening and cellular inflammation, along with attenuated expression of Th1, Th2 and Th17 cytokines. In the absence of the IgG receptor, skin thickening was completely abolished, as was inflammation. However expression of IFNγ was unaffected, while IL-4 and IL-13 expression was decreased. Both mice showed an increase in expression of IL-10 and the transcription factor Foxp3, a marker of the anti-inflammatory regulatory T cells. This data suggests that both IgG and IgE binding to their cognate receptors can contribute to AD. It remains to be seen if IgG plays a significant role in AD, notably in patients that do not show elevated IgE levels.

In summary, while skin DCs are most likely critically involved in the development of the Th2 responses that characterize AD, the respective contribution of LC and dermal DC subsets to AD development is an area of intense investigation.

T cells and atopic dermatitis

T cell polarization in AD is biphasic – in the acute phase there is a predominantly Th2 response while the chronic phase is characterized by a predominantly Th1 response [4,51]. A marker on skin homing Th2 cells is the chemoattractant receptor-homologous molecule (CRTH2). CRTH2 is one of the receptors for prostaglandin D2 and in addition to T cells is also expressed on eosinophils, basophils, and keratinocytes [52,53]. Studies on T cells in patients show that most of the circulating Th2 cells were CD4+ CRTH2+ and cells expressing bothCLA and CRTH2 were increased in AD patients compared to healthy controls [54]. Comparison of expression profiles of unstimulated CD4+ T cells from normal controls and AD patients revealed increased baseline
expression of CRTH2 [55]. Mouse models of cutaneous allergen exposure reveal that CRTH2 signaling is critical for inflammation in the skin [56-58]. While the exact role of CRTH2 is unclear, all this data taken together suggests that Th2 cells are important players in cutaneous allergic inflammation, an integral part of AD. In this review we present genetic and functional data that supports a role specifically for altered Th2 responses, in the development of AD. Here we discuss evidence linking the Th2 cytokines IL-4, IL-5, IL-13, IL-31, and the anti-inflammatory cytokine IL-10 that downregulates Th2 responses to AD. But first we briefly summarize recent work implicating other T cell subtypes in AD.

γδ+ T cells: In addition to αβ+ T cells, the mouse epidermis contains resident γδ+ T cells, also known as dendritic epidermal T cells (DTEC), that serve as a link between innate and adaptive immunity [59]. Using knockout mice lacking the δ chain, Woodward et al have demonstrated that αβ+ T cells, but not the γδ+ T cells, are critical for mediating the inflammation and IgE production following epicutaneous ovalbumin exposure [60]. The presence of γδ+ T cells in humans remains controversial, although some studies have detected a few of these cells in the epidermis [61]. The functional relevance of these cells in humans is yet to be determined.

CD8+ T cells: T cells expressing cutaneous lymphocyte-associated antigen (CLA, a marker for homing to the skin), are elevated in AD patients. These cells can be either CD4+ or CD8+ and both are equally functional [62]. These CD8+ T cells can also contribute to inflammation and sensitization in AD [63]. In a recent study of atopy patch testing of AD patients to dust mite, CD8+ T cells were recruited very early into the skin [64]. Studies in mouse models of AD have confirmed a role for CD8+ T cells in AD. CD8+ T cells were recruited within 24 hours of allergen exposure, and depletion of these cells attenuated dust mite induced inflammation [65]. In an independent study on FITC induced AD in the NC/Nga mice (that spontaneously develop AD), the absence of CD4+ T cells did not affect the disease phenotype because of an increased recruitment of CD8+ T cells that also expressed IL-17 [66]. Together these data suggest that CD8+ T cells contribute to AD, but details of their mechanistic role early in AD remain to be elucidated.

CD4+ T cell subsets: CD4+ T cells have been divided according to their effector cytokines. Beside the classic Th1 cells (IFNγ), Th2 cells (IL-4, IL-5, IL-13, IL-31), Treg (TGFβ, IL-10, IL-35) and Th17 cells (IL-17A, IL-17F, IL-21, IL-22), new subsets are being described most notably Th9 cells (IL-9, IL-10) and Th22 cells (IL-22). Recent studies have also identified a unique subpopulation of CD4+ T cells in humans that express IL-22 and IL-13 along with skin homing chemokine receptors [67,68].

The presence of IL-22 producing cells was increased in AD compared to psoriasis [69], and a recent study showed that IL-22 down regulates filaggrin expression in keratinocytes [70]. Th22 cells were first identified in patients with psoriasis, but are also detected in patients with AD [71]. IL-22 expression can be induced by the staphylococcal enterotoxin B (SEB) and α-toxin, both produced by S. aureus [72]. This study also demonstrated that IL-22 expression is induced by α-toxin in T cells and PBMCs isolated from patients with AD as compared to patients with psoriasis. However the function of IL-22 and the Th22 cells in AD is yet to be determined.

A recent review has exhaustively covered the role of regulatory T cells in AD [73] and is beyond the scope of this review.

IL-9 is predominantly expressed by Th9 and Th2 cells. To date there is only one genetic study linking polymorphisms in the IL-9 and IL-9 receptor genes with an increased risk of developing AD in a Korean population [74]. The role of IL-9 in AD remains unknown.

Th17 cells are important in the regulation of innate immunity particularly in neutrophil recruitment, and have more recently been implicated in allergic disorders. While the expression of IL-17 is higher in acute AD skin lesions, compared to chronic lesion or normal skin [75], little is known about Th17 cytokines in nascent AD and its importance in AD lesions is minor especially when compared to psoriasis [76]. In vitro treatment of keratinocytes with IL-17 induced expression of several anti-microbial peptides including β-defensin [76] suggesting the presence of IL-17 may protect against bacterial infections. In the mouse skin, He and colleagues have demonstrated that IL-17 expression is induced following OVA sensitization in the absence of the Th2 cytokines IL-4 and IL-13 [77]. While T cell numbers are unaffected by the absence of IL-4 and IL-13, there is a complete absence of eosinophils in the skin [77]. The consequent effects on TEWL, epidermal thickness, pruritus, spongiosis etc. remain to be determined. Since the filaggrin mutant mice showed a baseline Th17 profile, and a mixed Th1, Th2, Th17 profile following cutaneous ovalbumin challenge [19], the precise role of Th17 cells, and the relationship with barrier defects and Th2 cells merits additional exploration.

In this review, we specifically focus on what is known about Th2 cytokines and their role in AD. Human studies frequently demonstrate association between altered expression of a specific gene, either at the mRNA or protein level, and disease presence or severity. Functional relevance is derived primarily from mouse models since such studies are impossible in human subjects. A critical limitation is that there is no single mouse model for AD that recapitulates all the characteristics seen in human AD patients [46]. Once a cellular or molecular target has been identified, experiments in human primary cells or derived cell lines then validate the relevance of these mechanisms. Throughout this review, where available, we first present data from human subjects supporting further examination of the role of the cytokine in AD, followed by data from one or more mouse models, and finally, the findings from in vitro studies in human cell lines. Figure 1 summarizes these findings. We then discuss known genetic polymorphisms in the Th2 cytokines that have been associated with AD.

Th2 cytokines

Numerous studies have examined the production of Th2 cytokines in T cells, PBMC’s, and skin samples from patients with AD. The findings are frequently conflicting likely due to one or more of the following reasons – variability in the source of the sample (blood or skin), whether T cells were isolated (or a mixed pool of cells was used), the time the sample was collected, the heterogeneous genetic profiles of the patients, and the variability in the assays used, or cultivation of cells in vitro before analysis, rather than immediately after isolation.

While Machura et al find no differences in the ability of PBMC’s from subjects with AD and healthy controls to produce either Th2 or Th1 cytokines [78], other studies have demonstrated increased IL-10 and IL-13 producing CD4+ and CD8+ T cells in the peripheral blood of patients with AD [79]. In the case of T cells lines established from the skin, the cells showed a Th1 profile upon isolation, differentiated rapidly into Th2 cells upon stimulation with anti-CD3 and anti-CD28, and upon prolonged exposure, differentiated back into Th1 cells [80]. In another study, levels of IL-4, IL-5, IL-13 and IL-10 were elevated in the skin of patients with AD, and IL-5 and IL-13 levels were further
increased in patients with elevated IgE levels [81]. A recent study compared the expression of multiple cytokines in the serum of adults with acute AD, children with acute AD, and adults with chronic AD. They found that adults with acute AD had worse SCORAD (an index of AD severity) and higher IgE levels than the children with AD. Within the adults with acute AD, there was a significant association between SCORAD and IgE levels. No correlation with IL-4 or IL-10 was observed [82]. In an independent study, there was no correlation between cord blood IgE levels and AD [83]. Together these data, albeit conflicting, suggest that the role of Th2 cytokines in AD deserves to be examined in greater detail. Given the complex nature of AD – with genetic, and environmental risk factors – resulting in either defective skin barrier function, or altered immune function, a great deal of work remains to be done to delineate the components that contribute to AD, both in maintaining skin barrier integrity, and modulation of immune responses.

**IL-4:** IL-4 is a key Th2 cytokine critical for Th2 cell differentiation, IgE production, and eosinophil recruitment, among other functions. Analysis of CD4+ cord blood T cells showed that elevated IL-4 levels (in conjunction with decreased IFN-γ) in these cells were associated with an increased risk for developing AD [84]. Multiple mouse models have established a definitive role for IL-4 in the pathogenesis of AD. A transgenic mouse overexpressing IL-4 in the epidermis developed all the hallmarks of AD including pruritis, increased inflammatory cells in the skin, bacterial infection in the skin, and elevated IgG1 and IgE [85]. These mice also manifested the typical pattern of early expression of Th2 cytokines, with a transition to a more Th1 phenotype with disease progression [86]. IgG1 and IgE (Th2 phenotype) levels were also increased in early disease with elevated levels of IgG2a (Th1 phenotype) in later stages [87]. Generation of a variant of this IL-4 transgenic mouse (on the SKH1 hairless mouse background) yielded an IL-4 transgenic where IgE levels did not increase. However all the other markers of AD were comparable to the transgenic mice on the CByB6 background [88]. When the IL-4 transgenic was backcrossed to pro-Th2 Balb/c or pro-Th1 C57Bl/6 backgrounds, the Balb/c mice developed more severe AD than the C57Bl/6 strain [89]. Together the data suggest that IL-4 mediated increases in IgE are not necessary for the development of AD. These findings are also consistent with the data from the FcεR1 mice demonstrating that IgE signaling is not necessary for AD.

The second line of evidence comes from the NC/Nga mice that develop AD characterized by elevated IgE, increased pro-inflammatory cytokines (including IL-4), and increased migration of inflammatory cells into the skin [90]. These mice develop spontaneous AD-like phenotypes, even in the absence of STAT6, a transcription factor critical for mediating IL-4 effects in asthma. This suggests that in the skin, IL-4 has effects independent of STAT6 [91].

In a third model, transgenic mice overexpressing IL-4, IL-13, and IL-5 spontaneously developed AD with elevated IgE levels, increased...
inflammation and skin lesions [92]. This is consistent with the studies discussed above with the IL-4, and below with the IL-13 skin transgenic mice.

In a converse approach, Spargel and colleagues examined the effect of allergen challenge in the absence of IL-4 or IL-5 or the Th1 cytokine IFN-γ. In the IL-4 deleted mice, there was no effect on epidermal thickness, but there were fewer infiltrating eosinophils and T cells [93]. These findings in the skin are consistent with the role of IL-4 in the lung in asthma.

In another study from the same group, mice lacking both IL-4 and IL-13 showed attenuated IgE levels and skin eosinophilia, but normal numbers of CD4+ T cells [77]. In the absence of IL-4, there was an increase in baseline expression of the EDC genes loricrin, involucrin, and filaggrin (amongst other genes) [94]. These mice showed significantly less uptake of allergen, suggesting that IL-4 expression weakens the epidermal barrier. Overexpression of a constitutively active Stat6 (Stat6VT) in T cells can induce skin inflammation. While in the STAT6VT mice, there was increased skin thickening and inflammation, IL-4 expression increased with time in these mice. When IL-4 expression was low, Flg expression was unchanged. However as IL-4 expression increased, Flg expression decreased, with a concomitant increase in skin inflammation. STAT6VT mice that do not express IL-4 show decreased inflammation and restored EDC gene expression. These mice also recover more rapidly from inflammation induced by retinoic acid. The effect of allergen exposure in these mice remains to be evaluated, but the findings, in conjunction with the studies from the STAT6 null mice, suggest that STAT6 activation is sufficient to induce AD phenotypes, but STAT6 is not necessary for the development of AD.

Numerous in vitro studies have examined the effects of IL-4 on different cell types found in the skin. Normal human epidermal keratinocytes treated with IL-4 show increased expression of CCL26 (eotaxis-3), a key chemokine for eosinophil recruitment [95]. Dermal fibroblasts isolated from patients with acute AD skin lesions were far more responsive to IL-4 in their ability to express CCL11 (eotaxis-1), another critical cytokine for eosinophil recruitment [96]. Human dermal fibroblasts treated with IL-4 and IL-13 increase collagen synthesis by activation of the ERK pathway [97]. As discussed earlier, IL-4 and IL-13 can attenuate filaggrin expression. One mechanism for this is through the downregulation of the anti-microbial peptide S100/A11, a calcium binding protein [98], that in turn attenuates expression of human β-defensin 3 (HBD-3) and filaggrin. IL-4 and IL-13 also attenuate expression of involucrin and loricin in a STAT6 dependent manner [99]. Taken together, all this data makes a strong case for the inside-out hypothesis of AD, where changes to IL-4 expression can result in a defective barrier with increased permeability to allergens.

**IL-13:** IL-13 is a critical mediator of allergic inflammation [100]. While IL-4 and IL-13 share 20-25% sequence homology and effector functions, several studies in human subjects and human keratinocyte cell lines support a unique role for IL-13 independent of IL-4 in AD. IL-13 is expressed in both acute and chronic lesions of AD [101]. However, there is a selective significant up-regulation of IL-13 message in subacute and chronic AD lesions (with elevated IgE levels) in 27 of 28 patients, while increased IL-4 expression is seen in only 3 patients [102]. Differences in the time-course of IL-4 and IL-13 expression, and limitations in the ability to detect IL-4 might explain this discrepancy. Levels of CD4+IL-4+ and CD4+IL-13+ T cells were elevated in the peripheral blood of children with AD, and CD4+IL-13+ cells and eosinophil counts additionally correlated with disease severity [103]. Production of IL-13 from cord blood mononuclear cells at birth has been associated with subsequent development of AD [104,105]. Elevated IL-13 expression was also observed in the serum of AD patients, the expression was highest in the most severe cases, and it strongly correlated with IgE levels in an Egyptian population [106]. PBMCs isolated from AD patients produced elevated levels of IL-13 independent of the IgE status [107].

Unlike IL-4, there are few studies evaluating the role of IL-13 in mouse models of AD. A definitive role for IL-13 comes from a skin specific IL-13 transgenic mouse that developed all the key features of AD, including pruritic dermatitis, elevated IgG1 and IgE levels, inflammatory cell infiltration into the skin, upregulation of TSLP and other cytokines and chemokines, and skin fibrosis [108].

IL-13 signals through a heterodimeric receptor composed of IL-4Ra and IL-13Ra1 [109]. In addition, IL-13 also binds to IL-13Ra2, postulated to be a decoy receptor that lacks an intracellular signaling motif [110-112]. One study found that IL-13Ra2 contributed to pulmonary fibrosis instead of functioning as a decoy receptor [113]. These data suggest that there may be multiple roles of IL-13Ra2 and merit further study. Indirect evidence for a role for IL-13 in AD comes from our work on mice lacking IL-13Ra2. The expression of the decoy receptor IL-13Rα2 is regulated by Th2 cytokines in keratinocytes [114], and microarray analysis of keratinocytes from skin lesions of patients with AD has revealed elevated IL-13Ra2 levels [115]. We evaluated the role of IL-13Ra2 in the skin during inflammation and observed that mice lacking IL-13Ra2 had significantly increased transepidermal water loss, cutaneous inflammation, peripheral eosinophilia, IgG1, and IgE levels compared to wild type mice [116]. Depletion of IL-13Ra2 in a keratinocyte cell line resulted in increased STAT6 signaling in response to IL-13. Taken together, the data suggest that IL-13Ra2 serves as a decoy receptor in the keratinocytes, and that increased IL-13 signaling via STAT6 contributes to the pathogenesis of allergic inflammation and loss of skin barrier function.

Because of the functional overlap of IL-4 and IL-13, most in vitro studies evaluate the effects of both IL-4 and IL-13 on downstream effects in keratinocytes. The effects of IL-4 on attenuating expression of the EDC genes filaggrin, loricin, and involucrin, and the effects on collagen synthesis in dermal fibroblasts also apply to IL-13. Like IL-4, IL-13 can act directly on keratinocytes to enhance production of CCL26 (eotaxis-3), thereby increasing recruitment of eosinophils to eczematous skin [117]. IL-13 treated keratinocytes also increase migration of CD4+CCR4+ skin homing T cells [118], thereby promoting an inflammatory response.

HaCaT keratinocytes and primary human keratinocytes express functional IL-13 receptors and we observed that IL-4 and IL-13 increase IL-13Ra2 without altering IL-13Ra1 expression [116]. While these findings replicate other studies in HaCaT cells [114, 119, 120], it is inconsistent with one study in primary human keratinocytes [121], where IL-4 or IL-13 treatment did not up-regulate IL-13Ra2 expression. Potential reasons for the disparate results could be the methodologies used and the time point of the assay. In summary, it seems that inappropriate regulation of expression of IL-13 and its decoy receptor IL-13Ra2 could contribute to AD.

**IL-5:** IL-5 is a critical cytokine for eosinophil development, survival and proliferation [122]. PBMCs from patients with both extrinsic and intrinsic AD produced elevated levels of IL-5 as compared to normal...
with its role in other tissues – recruitment of eosinophils to the site of inflammation. Together the data suggest the role of IL-5 in the skin is consistent with its role in other tissues – recruitment of eosinophils to the site of allergen exposure. Whether the recruitment of eosinophils is necessary for the AD phenotype is yet to be addressed.

**IL-31:** IL-31 is a more recently identified cytokine, produced primarily by Th2 cells [123], and also by mast cells in response to antimicrobial peptides [124]. The receptor for IL-31 (a heterodimer of IL-31RA and OSMR) is expressed in the skin, and normal human epidermal keratinocytes, along with eosinophils, and activated monocytes and macrophages [123,125,126]. IL-31 expression is higher in lesional AD skin compared to non-lesional skin [127] and in the skin homing CLA+ T cells [128]. Serum IL-31 levels are also increased and there is a significant correlation with disease severity in both adults and children [129,130]. Levels of IL-31 also correlate with levels of IL-4 and IL-13 in the skin of subjects with AD [131]. And expression of the receptor subunit IL-31RA is induced following IFN-γ treatment in the PBMCs from AD patients but not normal controls [126].

Consistent with the human data, IL-31 transgenic mice developed spontaneous pruritus and skin lesions, hallmarks of AD. These effects were independent of elevated IgE levels and T cells. Injection of IL-31 directly into the skin resulted in increased recruitment of inflammatory cells into the skin, suggesting a role for IL-31 in chemotaxis [123]. Using *in vitro* co-culture studies, Cheung et al demonstrated that expression of pro-inflammatory molecules by eosinophils was induced by IL-31 and the effect was synergistic in the presence of keratinocytes [125]. In the NC/Nga mice that spontaneously develop AD, IL-31 levels correlate with scratching behavior. Treating these mice with anti-IL-31 antibodies ablates the scratching response. Since the scratch response is due to the activation of skin nerve endings, and since IL-31 receptor expression is higher in the dorsal root ganglia of the sensory neurons [127], all the data collectively support a role for IL-31 in regulation of scratching behavior in AD.

An interesting observation is that staphylococcal exotoxins induce IL-31RA expression on monocytes and macrophages [126] that then further induce expression of several pro-inflammatory cytokines. This could be one possible explanation for the increased susceptibility of AD patients to bacterial infections: the increased expression of the receptor renders the cells more sensitive to IL-31 (produced by T cells and mast cells), and the consequent increased scratching disrupts the skin barrier, allowing bacteria increased access to the lower dermal layers.

In this context, the role of bacterial toxins in AD merits further discussion. The skin of over 90% of patients with AD is colonized with *Staphylococcus aureus* (*S. aureus*), as compared to about 5% of healthy subjects [132]. Staphylococcal proteins are recognized by Toll-like receptor 2 (TLR-2) and polymorphisms in TLR-2 are associated with AD, and with impaired inflammatory responses in AD patients [133-135]. Another possible explanation for increased *S. aureus* infections is the decreased expression of the antimicrobial peptides (AMPs) - β-defensin 2 and cathelicidin - in AD lesions. In *in vitro* studies in keratinocytes have demonstrated that the Th2 cytokines can attenuate expression of these AMPs [136]. This would suggest that the persistent *S. aureus* colonization is a consequence of attenuated innate immune responses.

Conversely, epicutaneous delivery of the *S. aureus* exotoxin, SEB, to Balb/c mice is sufficient to induce a Th2 response, increased dermal eosinophilia, and immunoglobulins [137]. In addition, the chemokine CCL18 is the most highly expressed chemokine in AD lesions whose expression is induced by SEB [138]. Several chemokines induced in patients with AD (including CCL17 and CCL18) recruit T cells to sites of inflammation [139]. Together the data suggest that *S. aureus* infection predisposes individuals to develop Th2 type responses. It remains to be established whether defective immune responses predispose AD patients to *S. aureus* infections, or if *S. aureus* infections trigger Th2 responses and AD.

SEB has been demonstrated to induce IL-31 production [127]. *In vitro* studies in primary keratinocytes demonstrated that the TLR-2 ligand Pam3Cys can induce IL-31R expression [140]. In this study, pre-activation of keratinocytes with Pam3Cys renders cells more sensitive to IL-31 mediated downstream effects. This effect was not observed in keratinocytes from patients with AD where TLR-2 activation was impaired.

When all this evidence is examined together, it is interesting to speculate that a defective innate immune response leads to increased *S. aureus* colonization in the skin, which then triggers increased Th2 responses (including increased IL-31 production) consequently inducing pruritis, wounding and worse bacterial infection.

**IL-10:** IL-10 is a critical anti-inflammatory cytokine, whose expression is induced after the pro-inflammatory mediators. It helps tone down immune responses thereby minimizing tissue damage. IL-10 is produced by numerous cell types including Th2 cells [141]. Data from studies examining IL-10 in subjects with AD are conflicting. Consistent with its role as an anti-inflammatory cytokine, fewer IL-10 producing CD4+ T cells were found in individuals with severe AD as compared to mild AD and normal controls. IL-10 was also lower in all but 6% of AD skin homing CD4+ CLA+ T cells [142]. Plasma levels of IL-10 inversely correlate with severity of AD [143]. While there were no significant differences in the ability of LPS to induce IL-10 expression in PBMCs isolated from normal or asthmatic subjects [144], PBMCs isolated from patients with AD showed constitutively lower levels of IL-10 after culturing for 48 hours [145]. These cells also showed attenuated IL-10 production in response to various environmental triggers. IL-10 production from monocyte-derived dendritic cells is lower in AD subjects (while not statistically significant) [146]. Studies have also found attenuated expression of the IL-10 receptor subunit, IL-10RI, in acute atopic skin lesions [147]. Together these data suggest that IL-10 modulates disease severity.

In contrast to these findings, other studies have shown either elevated IL-10 levels in PBMC’s isolated from AD patients [148], in lesional skin [149-151], or increased induction of IL-10 in PHA stimulated blood cultures from subjects with AD [152] or PBMC’s...
expression of IL-31 in PBMCs [163]. Once again, the functional “intrinsic” dermatitis, and this mutation induces a 4 fold induction the IL-5 receptor α chain and peripheral blood eosinophil levels. in intrinsic AD [162]. This study also found an association with SNPs in were associated with extrinsic AD (with elevated IgE levels) but not occurrence of AD. In a Korean population study, two SNPs in IL-5 although there was no association between this IL-5 SNP and the finding, another study in a Japanese population found a significant association between IgE levels and an IL-5 promoter SNP [161], the risk of AD development, the risk was higher in conjunction with [158]. In a Polish study, while the presence of a filaggrin SNP increased keratinocytes, STAT6 phosphorylation, and expression of genes including filaggrin and other EDC genes or tight junction genes been published [46]. These include the age at which mice develop AD of AD, no study has comprehensively followed these patients and evaluated changes in barrier permeability or the expression of barrier genes including filaggrin and other EDC genes or tight junction genes over time.

Mechanistic studies have their own limitations. In vitro studies with human cell linesor primary cells do not take into account crosstalk between different cell types, in this case between immune cells and keratinocytes. A recent study has established a novel human skin equivalent model where IL-4 and IL-13 treatment are sufficient to induce all the hallmarks of AD such as edema, apoptosis of the keratinocytes, STAT6 phosphorylation, and expression of genes known to be induced in AD [170]. This model will prove to be useful in studying the skin barrier. Once again, it might prove beneficial to develop a human skin model where the communication between the skin barrier and the immune cells can be addressed.

Mouse models only partially recapitulate all of the clinical features of AD. A review of the limitations of existing mouse models has recently been published [46]. These include the age at which mice develop AD compared to humans, the type of inflammatory cells observed in the skin, and the nature of the physical changes to the barrier, suggesting that some of the events occurring in murine skin lesions might not translate to human disease. However, when the role of a specific cytokine is demonstrated in more than one type of mouse model and supported in relevant human cells in vitro, the proposed role of this cytokine in disease pathophysiology is strengthened.

The functional relevance of most of the SNPs identified in the Th2 cytokine family members that are associated with AD remain [148] and monocytes [151] stimulated with LPS. Elevated numbers of CD4+ and CD8+ cells expressing IL-10 were also observed in patients with AD [79]. As with the conflicting data on Th2 cytokines, the differences between the two groups of findings could be explained by the type of assay, the timing of the analysis, whether cells were cultured or immediately processed before assay. In either case, the data suggest that further evaluating the role of IL-10 in AD is merited.

One study has examined the role of IL-10 in a mouse model of AD. In this case the absence of IL-10 skewsthe T cells towards a Th1 rather than a Th2 phenotype, with attenuated expression of IL-4, IL-5 and eotaxin and decreased eosinophilia, suggesting a pro-inflammatory role for IL-10. In this model, the relevant IL-10 producing cell type was the dendritic cells. The study did not examine the effect of IL-10 on the other markers of AD such as epidermal thickness and pruritis [153]. This study also showed an early transient increase in IL-10 expression in the skin following damage by tape stripping. This is consistent with the findings by Oyoshi et al. [20] that dendritic cells isolated from IL-10 knockout mice showed increased expression of the Th1 cytokine IFN-γ and attenuated expression of IL-4 and IL-13.

In vitro studies in skin explants from patients with AD have shown that IL-10 down-regulated expression of the anti-microbial peptides necessary for host defense [150], consistent with the mouse data. In an apparent feedback mechanism, treatment of keratinocytes with low doses of IL-4 induces expression of the IL-10 receptor 1 subunit on these cells [147]. At higher doses, the expression of IL-10R1 decreases in a dose dependent manner. The role of IL-10 signaling in keratinocytes remains to be elucidated.

Genetic polymorphisms in Th2 cytokines and atopic dermatitis

Several recent reviews provide a comprehensive survey of the genetics of AD [11,154,155]. Here we summarize some of the key studies linking single nucleotide polymorphisms (SNPs) in the Th2 cytokines and AD. SNPs in both the IL-4 and IL-13 promoters that alter expression of the cytokines are significantly associated with the occurrence of AD [156,157]. Genetic polymorphisms in IL-13, IL-13Rα1, and STAT6 are associated with elevated cord blood IgE levels [158]. In a Polish study, while the presence of a filaggrin SNP increased the risk of AD development, the risk was higher in conjunction with SNPs in IL-13 or IL-10 [159]. A SNP in IL-13 and a haplotype consisting of SNPs in IL-13 and IL-4 are strongly associated with the development of AD at 2 years of age [160]. Whether these mutations are functional remains to be established.

A polymorphism in IL-5 was specifically associated with elevated cord blood IgE levels in African Americans [158]. Consistent with this finding, another study in a Japanese population found a significant association between IgE levels and an IL-5 promoter SNP [161], although there was no association between this IL-5 SNP and the occurrence of AD. In a Korean population study, two SNPs in IL-5 were associated with extrinsic AD (with elevated IgE levels) but not intrinsich AD [162]. This study also found an association with SNPs in the IL-5 receptor a chain and peripheral blood eosinophil levels.

One study has identified a SNP in IL-31 that is associated with “intrinsic” dermatitis, and this mutation induces a 4 fold induction expression of IL-31 in PBMCs [163]. Once again, the functional consequence of this SNP is not known.

As with the biological data, the genetic data on IL-10 are also conflicting. SNPs in the IL-10 promoter are associated with AD severity [164], blood eosinophil counts [165] and with serum IgE levels [166]. In contrast, a German study found no association between IL-10 SNP and AD [167]. However this study picked only one SNP in IL-10 that was not significant in any of the studies above. The other three studies examined multiple SNPs either individually or as a haplotype that showed significant association with AD. Once again, the functional effects of the SNPs are unknown. In summary, the genetic data, especially if taken in conjunction with the molecular and mechanistic data support a role for perturbed immune responses as a contributor to the development of AD.

Current barriers and future studies

As discussed above, barrier defects, particularly mutations in the EDC genes, are associated with AD. However 55% of patients with filaggrin mutations do not have the disease and that less than a third of the AD patients carry filaggrin mutations. Interestingly, an association between common mutations in the filaggrin gene and peanut allergy was recently described [168]. Importantly, not all the patients carrying the mutation had concomitant AD. In addition, filaggrin expression is decreased in AD patients, even in the absence of the known mutations, and in vitro studies in keratinocytes show that the Th2 cytokines IL-4 and IL-13 can attenuate filaggrin expression [169]. While functional mutations in EDC genes can disrupt the integrity of the skin barrier, elevated Th2 cytokines levels, characteristic of atopic diseases, have the potential to alter the skin barrier too. Both mechanisms most likely influence disease development albeit not to the same degree in all patients.

Very few human studies have addressed what happens in the early stages of AD development. While studies have shown an association between cord blood levels of some Th2 cytokines and the development of AD, no study has comprehensively followed these patients and evaluated changes in barrier permeability or the expression of barrier genes including filaggrin and other EDC genes or tight junction genes over time.

Mechanistic studies have their own limitations. In vitro studies with human cell lines or primary cells do not take into account crosstalk between different cell types, in this case between immune cells and keratinocytes. A recent study has established a novel human skin equivalent model where IL-4 and IL-13 treatment are sufficient to induce all the hallmarks of AD such as edema, apoptosis of the keratinocytes, STAT6 phosphorylation, and expression of genes known to be induced in AD [170]. This model will prove to be useful in studying the skin barrier. Once again, it might prove beneficial to develop a human skin model where the communication between the skin barrier and the immune cells can be addressed.
to be established. As the study by Lesiak et al demonstrates, genetic studies might be far more likely to find associations with AD when both immune and barrier genes are considered together. Retrospective studies using existing genetic databases could easily serve as a first step before embarking on more extensive prospective studies.

Topical therapies such as emollients, calcineurin inhibitors and steroids are more commonly used to treat AD. In cases where there are no improvements, systemic steroids are even employed [171]. Therapies directed specifically at Th2 responses are being developed mainly targeted at IL-5 and IL-5. As with the inconsistencies in the data regarding the involvement of these mediators, the therapies also yield inconclusive results, with anti-IL-5 therapy being the most promising [171]. Therapies targeting the skin barrier either directly (emollients) or indirectly (by targeting mast cell that induce scratching or the cytokines that downregulate barrier genes) will most likely be beneficial. The hope remains that a better understanding of the molecular and cellular processes that contribute to AD will result in the development of improved therapies and as a consequence, an improved quality of life for AD patients.

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**References**


