Review

Early immunologic changes during the onset of atopic dermatitis

Patrick M. Brunner, MD, MSc
Department of Dermatology, Medical University of Vienna, Austria

Key Messages
- Increases in Th2 and decreases in Th1 responses in blood of newborns predispose to atopic dermatitis (AD) development, suggesting that the Th1 immune axis might have some protective properties.
- Both early pediatric and longstanding adult AD lesions show strong Th2 activation, whereas Th1 responses are only present in longstanding adult AD.
- Pediatric AD shows stronger Th22/Th17 activation than adult AD, with concomitant increases in antimicrobial peptide expression.
- Both pediatric and adult AD lesions show defective skin lipid deposition in the stratum corneum, whereas down-regulation of filaggrin is only seen in adult lesions.
- Restoration of the epidermal barrier by emollients is currently the mainstay of primary prevention strategies for AD.

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ABSTRACT

Objective: Atopic dermatitis (AD), which is commonly called eczema, is the most common chronic inflammatory skin disease. The pipeline of new targeted treatments is currently expanding, a development that is largely based on our increasing understanding of disease mechanisms. Mechanistic insights have long been based on long-standing adult AD. Recently, studies also investigated early pediatric AD at disease onset, and revealed several differences in barrier and immune properties when compared with long-standing adult AD. This review focuses on immunological changes very early in life that predispose to the development of AD, and summarizes characteristics of the molecular AD phenotype in this age group.

Data Sources: Review of published literature.

Study Selections: Studies investigating human AD at disease onset in newborns, toddlers, and young children, in comparison with adults with long-standing disease.

Results: Already in cord blood, increased Th2 and decreased Th1 levels were found to increase the risk of AD development. Both pediatric and adult AD share Th2/Th22 activation and defects in lipid barrier deposition and tight junction formation, but Th1 activation and epidermal differentiation complex defects are largely absent in pediatric AD.

Conclusion: Immune changes predisposing to AD development are present very early in life. During the first months of disease, AD shows various differences in immune and barrier properties from long-standing adult AD, which might necessitate tailored treatment approaches depending on the age of the patient.

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Introduction

Atopic dermatitis (AD) is the most common chronic inflammatory skin disease, characterized by a fluctuating course, intense itch, and strongly inflamed skin lesions,1 putatively caused by a complex interaction of genetic, immune, and environmental factors.2 It is a highly heritable disease, showing strong associations with filaggrin (FLG) gene null mutations and Th2 signaling pathways,3 which has implications for epithelial barrier and skin inflammatory properties.
in affected individuals, respectively. Atopic dermatitis has seen a worldwide rise in prevalence, posing a significant socioeconomic burden on society. In approximately 60% of patients, this chronic disease starts during the first 12 months after birth, and most cases begin within the first 5 years of life, affecting up to 20% of all children. Although a significant proportion of individuals outgrow their disease until the age of 18 years, the prevalence in adults is still approximately 7% to 10%. Despite an increasing understanding of immune mechanisms responsible for skin inflammation in AD, factors determining whether someone develops lifelong disease vs outgrows the disease are still unknown. Besides skin inflammation, AD children also have an increased risk of developing other atopic comorbidities such as allergic asthma, rhinconjunctivitis, or food allergy. The exact mechanisms of how this “atopic march” evolves remain to be elucidated, but overall risk factors include disease severity and persistence, earlier age of onset, parental atopic history, FLG mutation status, polysensitization, and non-rural environment. However, biomarkers that can reliably predict the advent of the atopic march, or the activity of AD until adulthood, have not yet been identified. Such developments have been hampered by the fact that until only recently, AD mechanisms have largely been studied in adult but not early pediatric AD skin samples. Characterizing the very first phase of the disease, however, is eminently important to develop treatment strategies for this age group, and to potentially find targets for disease modification, or even a cure. This review focuses on results from human investigations of children at high risk of developing AD, and on blood and skin studies of the early phase of AD occurring in infants and young children, within the first months of disease onset, in comparison with long-standing adult AD.

Early Changes in Blood

Given the fact that 85% of all AD cases start before the age of 5 years, this period can be assumed to be critical for AD development, and a potential window for future disease-modifying or even curative treatment approaches. Because of the difficulty of performing skin biopsies in infants and young children, most studies in this age group focus on peripheral or cord blood samples to characterize AD risk biomarkers or the early disease phenotype.

At birth, most peripheral T cells are naïve, and then gradually develop into memory subsets, a process that is accelerated in individuals with AD. Overall, neonates have lower percentages of CD4+ and CD8+ interferon gamma (IFN-γ)-producing peripheral blood mononuclear cells than children and adults. Already in cord blood, elevated levels of Th2 and further down-regulation of Th1 numbers have been demonstrated to increase the risk of AD development (Fig 1). And allergen-specific IFN-γ secretion is reduced in blood of high-risk children who develop atopy.

Interleukin (IL)-10, the lead cytokine of regulatory T-cells, has important anti-inflammatory and tolerogenic properties. In line, low IL-10 production from cord blood cells stimulated with antigens from commensal bacteria is a risk factor for AD development in neonates. Most T cells within AD lesions are CD45RO+ memory cells that express the skin-homing receptor cutaneous lymphocyte antigen (CLA). Toddlers and children with AD show a suppressed and delayed development of skin-homing Th1 cells compared with control subjects, with expanded numbers of skin-homing CLA+ Th2 cells, as opposed to other blood T-cell subsets such as Th22 cells, which are expanded only later in life in AD individuals.

In line with this increased Th2/Th1 ratio in early AD patients, blood levels of Th2-associated mediators [eg, IL-31, CCL17/TARC, CCL22, CCL27, eosinophils, immunoglobulin E (Ige)] have been linked to clinical disease severity in pediatric AD, whereas Th1/IFN-γ responses in blood are negatively correlated with clinical severity measures. Thus, Th2 responses likely fuel atopic inflammation, whereas Th1 responses might be protective.

Early Skin Immune Changes in Pediatric AD Show Several Differences from Long-standing Adult AD

Because of the challenge of performing skin biopsies in pediatric individuals, our general understanding of AD skin inflammation has long been based on studies from adults with long-standing disease. Adult AD skin lesions harbor robust Th2 and Th22 activation, with smaller IL-17 skewing. Chronic lesions (usually defined as >72 hours in duration) show intensified expression of these immune axes, with an additional activation of Th1 responses. However, AD has turned out to be quite a heterogeneous disease, much more heterogeneous than other chronic inflammatory skin diseases such as psoriasis. Besides differences in Ige levels (intrinsirc vs extrinsic AD) and patterns of allergic sensitization, between acute vs chronic diseases, and differences depending on the ethnic background, differences in AD immune gene expression are also seen, depending on the age of the patient, especially during the first months of disease when AD is chronically established.

On a clinical level, early pediatric AD shows various differences from adult AD, including involvement of the face, trunk, and extensor limb surfaces in infants, as opposed to adults with more flexural and neck/shoulder involvement. Only recently, studies have investigated the molecular phenotype of early pediatric AD (in children younger than 5 years of age, within 6 months of disease onset), in comparison with long-standing adult AD. These investigations showed various differences on a transcriptomic level. Although both adult and pediatric AD lesions were characterized by strong Th2 activation, other immune axes showed considerable differences (Fig 3). Th22-associated inflammation was stronger in pediatric AD, as evidenced by higher increases in IL-22 and S100A12 levels than in adult AD. Markers of Th17–associated inflammation were strongly up-regulated in pediatric AD samples, including IL-17A, IL-17F, IL-36A, IL-36B, IL-36G, IL-20, CCL20, and P13/Elafin. Overall, this merged Th2/Th17 profile in early pediatric AD is also reflected by a strong up-regulation of IL-19, which is a pro-inflammatory cytokine typically induced by IL-17, and that can further stimulate production of Th2-associated cytokines, thus bridging Th17 to Th2 immune axes. A potential functional role of Th17 activation in early-onset pediatric AD is suggested by the fact that IL-26, a Th17-associated cytokine, showed a strong positive correlation with transepidermal water loss (TEWL) and thus skin barrier impairment.

Despite the fact that the pediatric skin samples were fully developed, strongly acanthotic lesions, Th1/IFN-γ–dependent inflammation (CXCL10, IL12RB2, STAT1, IFNGR1, CXCL9), which is typically seen in chronic lesions in adult AD, was virtually absent in

Figure 1. At birth, low amounts of gamma interferon and increased amounts of type 2 cytokine producing cord blood T cells enhance the risk of the subsequent development of AD.
early pediatric AD, consistent with lower Th1 responses in high-risk infants and young children with AD. Atopic dermatitis has previously been shown to lack adequate up-regulation of antimicrobial peptides (AMP) in skin lesions from adult patients, most likely because of Th2 cytokines that suppress their production. This deficit in antimicrobial activity is consistent with frequent superinfections of AD skin. Conversely, psoriasis usually shows profound up-regulation of AMPs, and is rarely superinfected, although both diseases harbor a barrier defect. However, already in pediatric healthy control skin, certain AMPs, including cathelicidin antimicrobial peptide/LL37 and human beta defensin 2, were significantly up-regulated compared with adult control skin. Up-regulation of AMPs showed further, strong increases in pediatric AD lesions, much higher than in adult AD, to levels that were comparable with adult psoriasis. Because increases in AMP expression levels are counterintuitive to the increased susceptibility of younger children with AD to infections, exact mechanisms leading to AD superinfection in early AD remain to be elucidated. The observed abundance of AMPs in early pediatric AD might be induced by Th17 cytokines and contribute to the pro-inflammatory milieu within skin lesions, possibly via similar toll-like receptor (TLR)-mediated stimulation on myeloid cells, as previously shown for psoriasis, but this speculation needs further investigation. Other molecules up-regulated in early pediatric AD, which have been labeled “psoriasis markers,” include tumor necrosis factor alpha and epiregulin, further highlighting the differences between early pediatric and adult AD.

Atopic dermatitis is usually the first manifestation of atopic diseases, followed by food allergies. Food allergies have recently led to a strong increase in hospital admission rates for food-induced anaphylaxis in young children, whereas challenge-proven food allergy is quite rare in adults. Interestingly, skin from healthy children harbors increased levels of IL-33 compared with adults, which might predispose children early in life to food allergy. Interleukin-33 is an epithelial-derived cytokine similar to thymic stromal lymphopoietin and IL-25. Evidence from murine models suggests that IL-33 promotes food anaphylaxis in epicutaneously sensitized mice via engagement of the IL-33 receptor ST2 (which is encoded by the IL1TL1 gene). ST2 is critically involved in type 2 immunity and allergic reactions, and both ST2 and IL-33 showed strong associations with allergic diseases in genomewide association studies. Transcriptomic analyses of whole blood from toddlers and young children with AD revealed that ST2 was significantly increased compared with healthy control children and was positively correlated with TEWL of respective lesional skin. Thus, epidermal IL-33 might orchestrate allergic inflammation via ST2 on mast cells, basophils, innate lymphoid cells, and eosinophils. Together with IL-9, which is similarly increased in early pediatric AD and has also been implicated in food allergy development, these cytokines might form a basis for the propensity of children with AD to develop food allergies.

Early Changes in the AD Skin Barrier: Defects in Tight Junction Formation and Skin Lipid Deposition

In addition to aberrant immune activation, a defect in skin barrier is a characteristic feature of both early pediatric as well as adult AD. Filaggrin (encoded by the FLG gene) constitutes a key component of

Figure 2. Principal component analysis based on microarray data reveals separate groupings of pediatric and adult AD biopsies, from both lesional and nonlesional skin, as well as healthy control samples. Reproduced with permission from Brunner PM, Israel A, Zhang N, et al. Early-onset pediatric atopic dermatitis is characterized by TH2/TH17/TH22-centered inflammation and lipid alterations. J Allergy Clin Immunol. 2018;141:2094-2106.

Figure 3. Schematic representation of immune activation and epithelial barrier properties in pediatric (left) and adult (right) AD lesions; AMP Antimicrobial peptide; K16 Keratin 16.
the barrier function within the stratum corneum,\textsuperscript{70} and FLG mutations are associated with increases in transepidermal water loss, a measure of skin barrier dysfunction.\textsuperscript{71} The FLG null mutations increase the risk of developing AD,\textsuperscript{48} especially of early onset and a more severe clinical course. Mechanistically, it is assumed that a decreased epidermal barrier leads to enhanced penetration of allergens and danger signals into the skin,\textsuperscript{49} which are then recognized by dendritic cells, prompting them to mount an inflammatory immune response.\textsuperscript{73} Atopic dermatitis is also associated with several other atopic disorders, especially food allergy, allergic rhinitis, and asthma. The concept of an “atopic march”—with AD as the initial step of a process leading to other atopic/allergic comorbidities—has recently been challenged as a unidirectional mechanism.\textsuperscript{74} Nevertheless, animal data have consistently shown that percutaneous exposure of allergens are highly immunogenic, as opposed to the oral route, which is usually tolerogenic.\textsuperscript{10} These data suggest AD as a potential “entry point” for subsequent allergic diseases because of a breach in epidermal barrier.\textsuperscript{10}

Although FLG gene null mutations are still the most significant known risk factor for the development of AD,\textsuperscript{2} such mutations are present only in a subset of patients,\textsuperscript{48,75} and not all individuals harboring FLG mutations develop AD. Importantly, Th2 cytokines such as IL-4 and IL-13 down-regulate FLG expression in keratinocytes in vitro,\textsuperscript{10} and skin biopsy specimens from adult AD indeed harbor defective FLG deposition within the stratum corneum, suggesting a relevant role for low FLG expression in adult AD, independent of the FLG mutation status.\textsuperscript{75} Surprisingly, this kind of FLG down-regulation is not observed in early pediatric AD, which shows normal levels of FLG mRNA expression and protein FLG deposition in the stratum corneum, at least in FLG wild-type individuals.\textsuperscript{50,51} The reason for this difference is not yet clear, and it needs further investigation. Similarly, other components of the epidermal differentiation complex that mediate terminal differentiation and cornification of keratinocytes, such as loricrin, were down-regulated only to a very small degree in pediatric AD, whereas adults usually show strong down-regulation.\textsuperscript{73} Thus, other factors must contribute to the decreased skin barrier in adult and early pediatric AD, which is present in virtually all AD patients, reflected by unanimous increases in TEWL.\textsuperscript{77} Potential candidates are tight junction components such as certain claudins\textsuperscript{91} that are strongly reduced in early pediatric AD.\textsuperscript{50} Also, stratum corneum lipid deposition is markedly decreased in nonlesional and lesional pediatric AD (Fig 4).\textsuperscript{50} In line, decreases in enzymes involved in skin lipid deposition were associated with increased TEWL levels in correlation studies,\textsuperscript{77} pointing to a functional relevance in early AD. Overall, lipid and tight junction abnormalities, but not terminal differentiation abnormalities, seem to characterize the barrier defects in atopic skin at disease onset.\textsuperscript{50,51}

The pathogenic importance of the skin barrier for early AD development is supported by a study from Kelleher et al,\textsuperscript{79} who demonstrated that increased TEWL measured at 2 days and 2 months of life predates and predicts AD at 1 year, an effect that was independent of FLG mutation status or of parental atopy.\textsuperscript{79} In line, low TEWL levels at 2 days and 2 months of life were protective against the development of AD,\textsuperscript{73} with similar results reported by Horimukai et al.\textsuperscript{82} Interestingly, TEWL at birth also predicts food allergy at 2 years of age, even in those individuals who do not develop AD,\textsuperscript{86} supporting the concept of transcutaneous allergen sensitization even in the absence of clinically manifest inflammation.

**Microbial Changes**

Bacterial and viral superinfections are frequent in AD. Eczema herpeticum is associated with genetic variants of thymic stromal lymphopoietin,\textsuperscript{81} and STAT6\textsuperscript{15} (a transcription factor for Th2 cytokines), and with abnormalities in IFN-γ responses.\textsuperscript{83,84} Filaggrin mutations also increase the risk of eczema herpeticum,\textsuperscript{85} but also of sustained infection with *Molluscum contagiosum*,\textsuperscript{86} or increased colonization with *Staphylococcus aureus*.\textsuperscript{87} Importantly, skin colonization by *S aureus* often precedes the clinical diagnosis of AD in infancy,\textsuperscript{88} and *S aureus* presence at the time of AD diagnosis is associated with earlier age of AD onset (6 vs 3 months).\textsuperscript{88} Overall, greater *S aureus* predominance in pediatric patients with AD can be seen in more severe cases, and *S epidermidis* predominance in less severe disease.\textsuperscript{89} Mechanistically, evidence from mouse studies suggests that colonization by *S epidermidis* during the neonatal period favors development of regulatory T cells toward this commensal, while delaying *S epidermidis* exposure until adulthood prevented this protective effect and promoted inflammatory skin responses to this bacterium.\textsuperscript{90} In line with this observation, regulatory T cells are enriched in pediatric compared with adult human skin.\textsuperscript{91} Thus, early commensal microbe colonization and local chemokine production potentially work together to recruit regulatory T cells into neonatal skin,\textsuperscript{92} which can protect from inflammatory skin diseases, but the exact mechanisms in human disease are yet to be elucidated.

Taken together, the epidermal barrier and the cutaneous microbiome, together with skin immune defense mechanisms, are likely the key players determining the balance between healthy and diseased skin early in life,\textsuperscript{93} but further research needs to clarify the exact immunological circuits involved.

**Early Intervention Strategies to Prevent AD Development**

Given that elevated levels of TEWL at birth are a risk factor for the subsequent development of AD,\textsuperscript{85,86} attempts to restore this defective epidermal barrier were performed to prevent the advent

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**Figure 4.** Skin lipid stain using Nile red in pediatric AD samples, compared to age-matched healthy controls. Arrows: Organized lipid layer in stratum corneum; arrowheads: Absence/near absence of organized lipid layer, indicating a barrier defect. Insets show a representative detail of the stratum corneum for each group. Reproduced with permission from Brunner PM, Israel A, Zhang N, et al. Early-onset pediatric atopic dermatitis is characterized by TH2/TH17/TH22-centered inflammation and lipid alterations. *J Allergy Clin Immunol.* 2018;141:2094-2106.
of AD. Randomized trials starting within the first weeks of life until 6 to 8 months of age have shown that regular application of emollients is associated with an approximate halving of the risk of developing AD.62,93–95 With additional, larger studies ongoing to investigate long-term effects of early emollient intervention.62

Because infants with early-onset (<6 months of age) and more severe AD are more likely to develop food allergy,62 early diversity also aims at stalling the atopic march. Whether this early barrier-directed intervention will also decrease sensitization to food and environmental allergens remains to be determined, but 1 study reported that no infant whose parents used emollients for at least 5 days per week developed food sensitization.94 Several studies have looked at maternal diet, but they have failed to alter the incidence of AD or food allergy of the child.95 Early use of probiotics has given mixed results at 2 years, but it was without effects at 4 years of age.96 Insufficient evidence is available for other nutritive interventions, including hydrolyzed formulas, vitamin D supplementation, or prebiotics, for the primary prevention of allergic diseases.4 However, early introduction of allergenic foods such as peanut to high-risk infants suffering from pre-existing food sensitization significantly reduces the rate of challenge-proven peanut allergies compared with placebo,97 which is currently one of the most promising strategies for future treatment approaches.

Interventions directly targeting early changes in the immune system have not been performed. Currently, studies involving the IL-4R blocker dupilumab are ongoing in children with active AD who are older than 6 months of age (NCT03346434, NCT02612454), but results are not yet available.

Conclusion

Atopic diseases, including AD, are currently a major health concern, because they are the most common chronic illnesses in children in Western societies.62 Overall, children and adult AD skin samples share Th2/Th22 activation and lipid metabolism and tight junction alterations, but Th1 activation and epidermal differentiation complex defects are only consistently present in adults with AD, whereas early in life, lack of Th1 responses and up-regulation of the Th17 axis in skin might have a role in disease initiation. These differences might necessitate diverse targeted treatment approaches for the respective age groups.

References


