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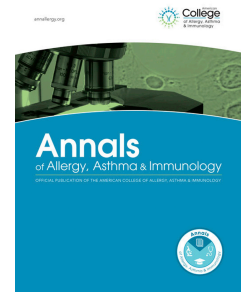
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Invited Review for The Annals of Allergy, Asthma, and Immunology

Title: “Early Immunologic Changes During the Onset of Atopic Dermatitis”

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Abbreviations:

AD: Atopic dermatitis

AMP: Antimicrobial peptide

CAMP: Cathelicidin antimicrobial peptide

CLA: Cutaneous lymphocyte antigen

DEFB4B: Defensin Beta 4B, the gene for human beta defensin 2

FLG: Filaggrin

GWAS: Genome-wide association study

IFN: Interferon

IL1RL1: Interleukin 1 receptor-like 1

LOR: Loricrin

IL-4R: Interleukin 4 receptor

SCORAD: Scoring Atopic Dermatitis

ST2: Suppression of tumorigenicity 2

STAT1: Signal transducer and activator of transcription 1

TEWL: Transepidermal water loss

TLR: Toll-like receptor

TSLP: Thymic stromal lymphopoietin

1 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 AD is a highly heritable disease, showing strong associations with filaggrin (FLG) gene null mutations and Th2 signaling pathways,² that have implications for epithelial barrier and skin inflammatory properties in affected individuals, respectively. AD has a worldwide rise in prevalence,³ posing a significant socioeconomic burden on society.⁴ In about 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,⁵ affecting up to 20% of all children. While a significant proportion of individuals outgrow their disease until the age of 18 years,^{6,7} the prevalence in adults is still about 7-10%.^{3,8} Despite an increasing understanding on immune mechanisms responsible for skin inflammation in AD,⁹ 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, rhinoconjunctivitis, 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.¹⁰ 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 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 to longstanding, adult AD.

Early changes in blood

Given the fact that 85% of all AD cases start before the age of 5 years,¹¹ it can be assumed that this period is critical for AD development, and a potential window for future disease-modifying or even curative treatment approaches. Due to the difficulty of obtaining skin biopsies in infants and young children, the majority of studies in this age group focus on peripheral or cord blood samples¹²⁻³² to characterize AD risk biomarkers or the early disease phenotype.

At birth, most peripheral T cells are naïve, which then gradually develop into memory subsets,⁹ a process that is accelerated in individuals with AD.²⁷ Overall, neonates have lower percentages of CD4⁺ and CD8⁺ IFN- γ -producing PBMCs than children and adults.^{33,34} Already in cord blood, elevated levels of Th2 and further downregulation of Th1 numbers have been demonstrated to increase the risk of AD development (Figure 1),¹²⁻¹⁴ and allergen-specific IFN- γ secretion is reduced in blood of high-risk children who develop atopy.¹²

IL-10, the lead cytokine of regulatory T-cells, has important anti-inflammatory and tolerogenic properties.³⁵ 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.³⁶

Most T-cells within AD lesions are CD45RO⁺ memory cells that express the skin-homing receptor cutaneous lymphocyte antigen (CLA).³⁷ Toddlers and children with AD show a suppressed and delayed development of skin-homing Th1 cells compared to control subjects,^{18,25,27} with expanded numbers of skin-homing CLA⁺ Th2 cells, as opposed to other blood T-cell subsets such as Th22 cells, that are expanded only later in life in AD individuals.²⁵ In line with this increased Th2/Th1 ratio in early AD patients,³⁸⁻⁴⁰ blood levels of Th2-associated mediators (e.g. IL-31, CCL17/TARC, CCL22, CCL27, eosinophils, 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.^{24,28-32,41} Thus, Th2 responses likely fuel atopic inflammation, whereas Th1 responses might be protective.

Early skin immune changes in pediatric AD show several differences from longstanding adult AD

Because of the challenge of obtaining skin biopsies from pediatric individuals, our general understanding of AD skin inflammation has long been based on studies from adults with longstanding disease. Adult AD skin lesions harbor robust Th2 and Th22 activation, with smaller IL-17 skewing.⁴² Chronic lesions (usually defined as >72 hours in duration^{42,43}) show intensification of these immune axes, with an additional activation of Th1 responses.⁴² However, AD has turned out to be quite a heterogeneous disease, much more heterogeneous than other chronic inflammatory skin diseases such as psoriasis.⁴³ Besides differences in IgE levels (intrinsic vs. extrinsic AD)⁴⁴ and patterns of allergic sensitization, between acute vs. chronic lesions,⁴² and differences depending on the ethnic background,⁴⁵⁻⁴⁸ there are also differences in

AD immune gene expression depending on the age of the patient,⁴⁹ especially during the first months of disease when AD is chronically established.^{41,50,51} Already 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.⁵ 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 longstanding adult AD.^{50,51} These investigations showed various differences on a transcriptomic level (Figure 2). While both adult and pediatric AD lesions were characterized by strong Th2 activation,^{50,51} other immune axes showed considerable differences (Figure 3). Th22-associated inflammation was stronger in pediatric AD, as evidenced by higher increases in IL-22 and S100A levels than in adult AD.⁵⁰ Markers of Th-17-associated inflammation were strongly upregulated only in pediatric AD samples, including IL17A, IL17F, IL-36A, IL-36B, IL-36G, IL-20, CCL20, and PI3/Elafin.^{50,51} Overall, this merged Th2/Th17 profile in early pediatric AD is also reflected by a strong upregulation 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.^{52,53} 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.⁵⁰

Despite the fact that the pediatric samples were fully developed, strongly acanthotic lesions, Th1/IFN- γ -dependent inflammation (CXCL10, IL12RB2, STAT1, IFNGR1, CXCL9), that is typically seen in chronic lesions in adult AD, was virtually absent in early pediatric AD,⁵⁰ consistent with lower Th1 responses in high-risk infants and young children with AD.¹²⁻¹⁴

AD has previously been shown to lack adequate upregulation of antimicrobial peptides (AMP) in skin lesions from adult patients,^{54,55} most likely due to Th2 cytokines that suppress their production.⁵⁶ This deficit in antimicrobial activity is consistent with frequent superinfections of AD skin. Conversely, psoriasis usually shows profound upregulation of AMPs, and is rarely superinfected,⁵⁴ although both diseases harbor a barrier defect.⁵⁷ However, already in pediatric healthy control skin, certain AMPs, including CAMP (cathelicidin antimicrobial peptide/LL37) and DEFB4B (human beta defensin 2), were significantly upregulated compared to adult control skin.^{50,51} AMP upregulation showed further, strong increases in pediatric AD lesions, much higher than in adult AD, to levels that were comparable with adult psoriasis.^{50,51} As 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,⁵⁸ 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,^{59,60} but this speculation needs further investigation. Other molecules upregulated in early pediatric AD, that have previously been labeled “psoriasis markers”, include TNF- α and epirelugin,⁵⁰ further highlighting the differences between early pediatric and adult AD.

AD is usually the first manifestation of atopic diseases, followed by food allergies.⁶¹ Food allergies have recently shown a strong increase in hospital admission rates for food-induced anaphylaxis in young children,⁶² while challenge-proven food allergy is quite rare in adults.⁶³ Interestingly, skin from healthy children harbors increased levels of IL-33 compared to adults,⁵¹ which might predispose children early in life to food allergy. IL-33 is an epithelial-derived

cytokine,⁶⁴ similar to TSLP and IL-25.⁷⁹ There is evidence from murine models 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).⁶⁵ ST2 is critically involved in type 2 immunity⁶⁶ and allergic reactions,⁶⁷ and both ST2 and IL-33 showed strong associations with allergic diseases in genome-wide association studies (GWAS).⁶⁴ Transcriptomic analyses of whole blood from toddlers and young children with AD revealed that ST2 was significantly increased compared to healthy control children,⁴¹ and was positively correlated with TEWL of respective lesional skin.⁴¹ Thus, epidermal IL-33 might orchestrate allergic inflammation via ST2 on mast cells, basophils, innate lymphoid cells, and eosinophils.⁶⁴ Together with IL-9, which is similarly increased in early pediatric AD and has also been implicated in food allergy development,⁶⁸ 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.⁶² Filaggrin (encoded by the FLG gene) constitutes a key component of the barrier function within the stratum corneum,⁶⁹ and FLG mutations are associated with increases in transepidermal water loss, a measure of skin barrier dysfunction.⁷⁰ FLG null mutations increase the risk of developing AD,⁷¹ 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,⁷¹ which are then recognized

by dendritic cells, prompting them to mount an inflammatory immune response.⁷² AD 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.⁷³ Nevertheless, animal data have consistently shown that percutaneous exposure of allergens are highly immunogenic, as opposed to the oral route that is usually tolerogenic.¹⁰ These data suggest AD as a potential “entry point” for subsequent allergic diseases due to a breach in epidermal barrier.¹⁰

While filaggrin (FLG) gene null mutations are still the most significant known risk factor for the development of AD,² such mutations are present only in a subset of patients,^{48,74} and not all individuals harboring FLG mutations develop AD. Importantly, Th2 cytokines such as IL4 and IL-13 downregulate filaggrin expression in keratinocytes *in vitro*,⁷⁵ and skin biopsies from adult AD indeed harbor defective filaggrin deposition within the stratum corneum, suggesting a relevant role for low filaggrin expression in adult AD, independent of the FLG mutation status.⁷²

Surprisingly, this kind of filaggrin downregulation is not observed in early pediatric AD, which shows normal levels of FLG mRNA expression and protein filaggrin deposition in the stratum corneum, at least in FLG wild type individuals.^{50,51} The reason for this difference is not yet clear, and needs further investigation. Similarly, other components of the epidermal differentiation complex that mediate terminal differentiation and cornification of keratinocytes, such as loricrin (LOR), were downregulated only to a very small degree in pediatric AD, while adults usually show strong downregulation.⁵¹ Thus, other factors must contribute to the decreased skin barrier in adult and early pediatric AD, that is present in virtually all AD patients, reflected by unanimous increases in transepidermal water loss (TEWL).⁷⁶ Potential candidates are tight

junction components such as certain claudins⁷⁷ that are strongly reduced in early pediatric AD.⁵⁰ Also, stratum corneum lipid deposition is markedly decreased in non-lesional and lesional pediatric AD (Figure 4).⁵⁰ In line, decreases in enzymes involved in skin lipid deposition were associated with increased TEWL levels in correlation studies,⁵⁰ 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.^{50,51}

The pathogenic importance of the skin barrier for early AD development is supported by a study from Kelleher et al., 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.⁷⁸ In line, low TEWL levels at 2 days and 2 months of life were protective against the development of AD,⁷⁸ with similar results reported by Horimukai et al.⁷⁹ Interestingly, TEWL at birth also predicts food allergy at 2 years of age, even in those individuals who do not develop AD,⁸⁰ 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 (TSLP)⁸¹ and STAT6⁸² (a transcription factor for Th2 cytokines), and with abnormalities in IFN-gamma responses.^{83,84} FLG mutations also increase the risk of eczema herpeticum,⁸⁵ but also of sustained infection with Molluscum contagiosum,⁸⁶ or increased colonization with *S. aureus*.⁸⁷ Importantly, skin colonization by *S.*

aureus often precedes the clinical diagnosis of AD in infancy,⁸⁸ and *S. aureus* presence at the time of AD diagnosis is associated with earlier age of AD onset (6 vs. 3 months).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.⁸⁹ Mechanistically, there is evidence from mouse studies that colonization by *S. epidermidis* during the neonatal period favors development of regulatory T cells towards this commensal, while delaying *S. epidermidis* exposure until adulthood prevented this protective effect and promoted inflammatory skin responses to this bacterium.⁹⁰ In line with this observation, regulatory T-cells are enriched in pediatric compared to adult human skin.⁹¹ Thus, early commensal microbe colonization and local chemokine production potentially work together to recruit regulatory T-cells into neonatal skin,⁹² that 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,⁹⁰ 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,^{79,80} attempts to restore this defective epidermal barrier were performed to prevent the advent of AD. Randomized trials starting within the first weeks of life until 6-8 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 As infants with early-onset (<6months of age) and more severe AD are more likely to develop food allergy,⁶² early interference 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 one study reported that no infant whose parents used emollients for at least 5 days per week developed food sensitization.⁹⁵ Several studies have looked at maternal diet, but have failed to alter the incidence of AD or food allergy of the child.⁹⁶ Early use of probiotics has given mixed results at 2 years, but was without effects at 4 years of age.¹⁰ There is still insufficient evidence 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 to 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 >6 months of age (NCT03346434, NCT02612454), but results are not yet available.

Conclusion

Atopic diseases, including AD, are currently a major health concern, as they are the most common chronic illnesses in children in Western societies.⁶² Overall, children and adult AD skin

230 samples share Th2/Th22 activation and lipid metabolism and tight junction alterations, but Th1
231 activation and epidermal differentiation complex defects are only consistently present in adults
232 with AD, while early in life, lack of Th1 responses, and upregulation of the Th17 axis in skin
233 might have a role in disease initiation. These differences might necessitate diverse targeted
234 treatment approaches for the respective age groups.

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FIGURE LEGENDS

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.

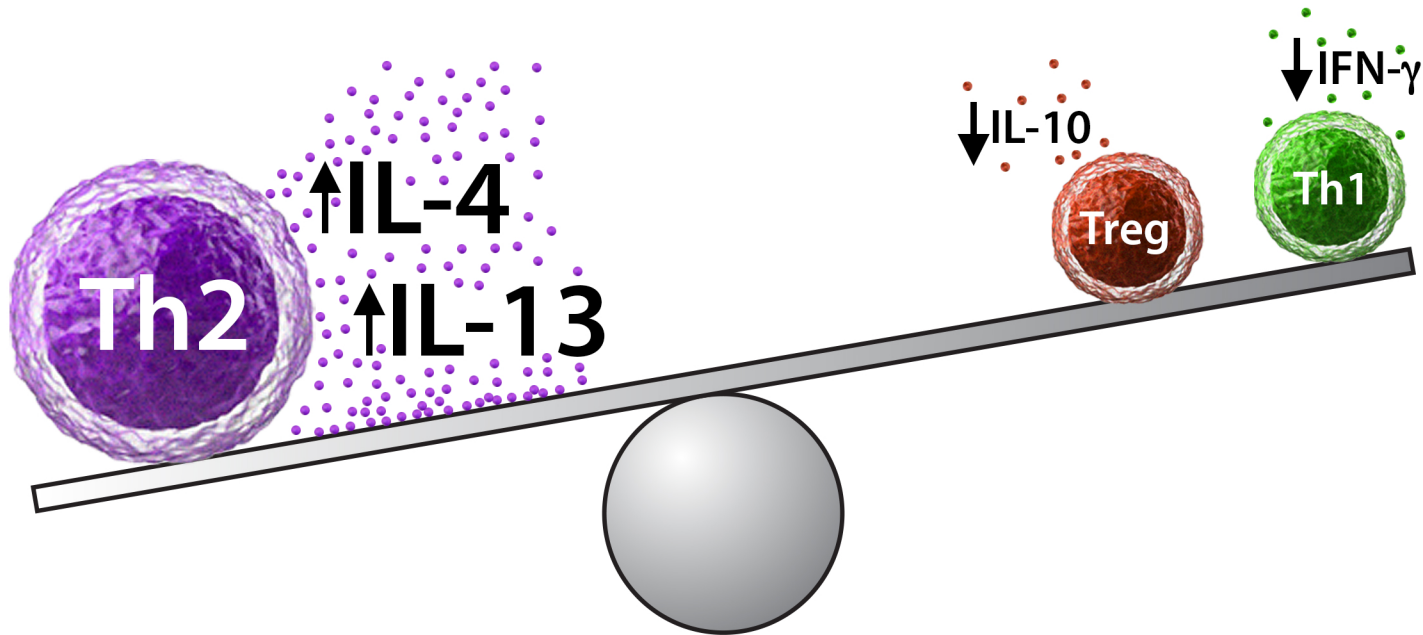
Figure 2. Principal component analysis based on microarray data reveals separate groupings of pediatric and adult AD biopsies, both from lesional and non-lesional skin, as well as healthy control samples. Reproduced with permission from Brunner et al.¹

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;

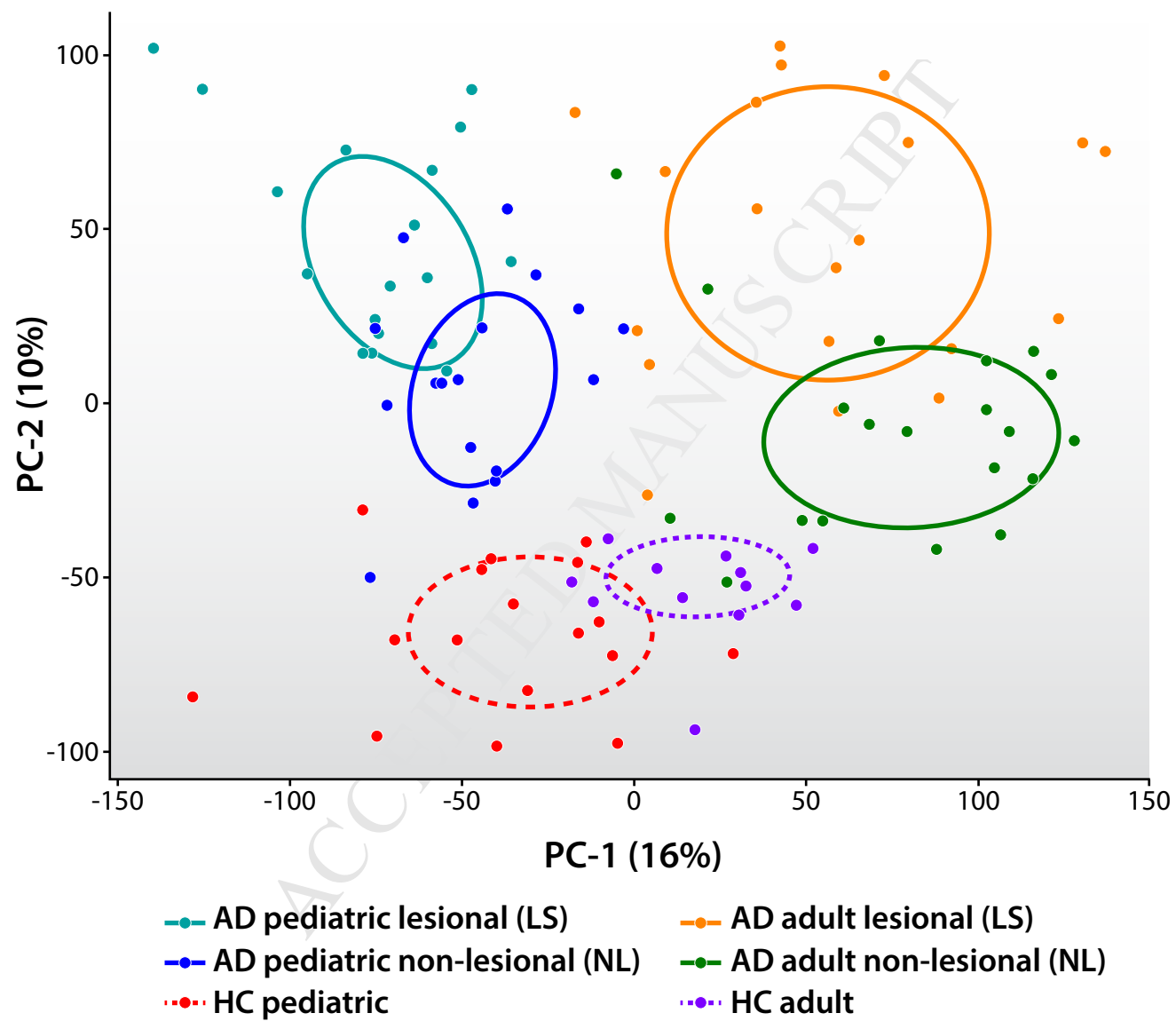
Figure 4. Skin lipid stain using Nile red in pediatric AD samples, compared to age-matched healthy controls. Arrows: Organized lipid layer in stratum corneum; arrowheads: Absence/near absence of organized lipid layer, indicating a barrier defect. Inserts show a representative detail of the stratum corneum for each group. Reproduced with permission from Brunner et al.¹

REFERENCE

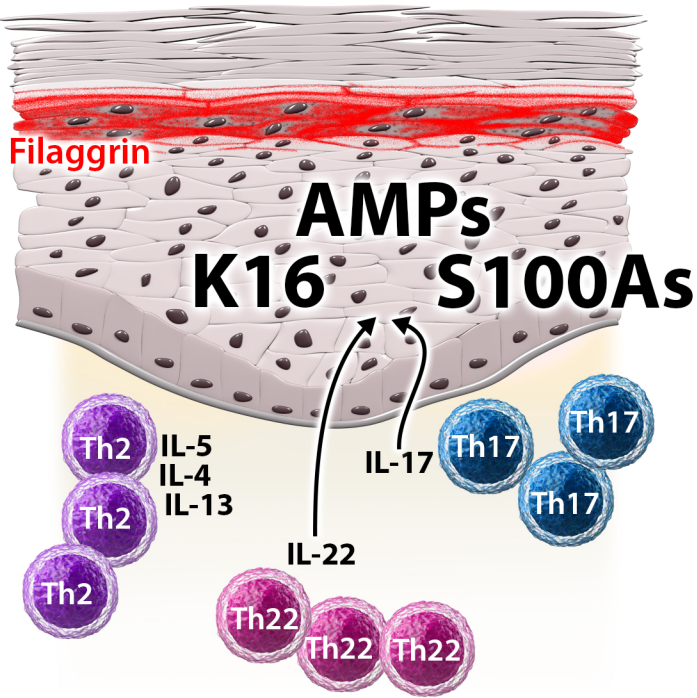
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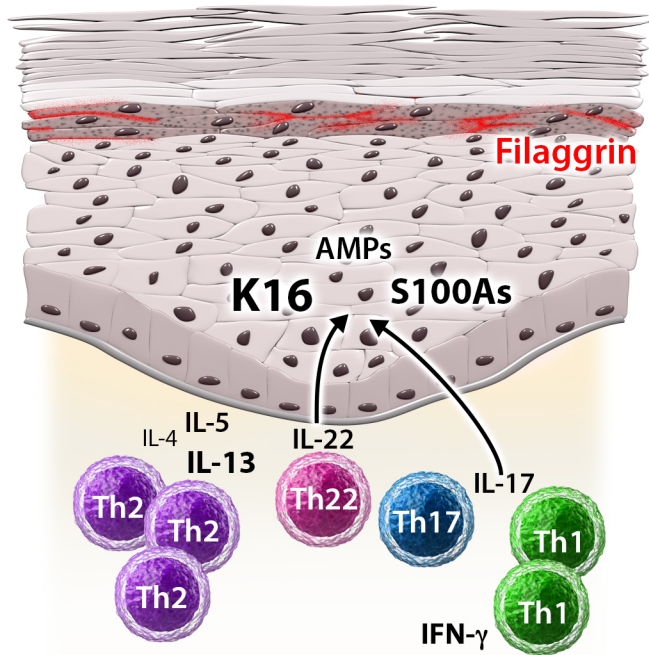
Neonatal Atopic Dermatitis Risk Profile



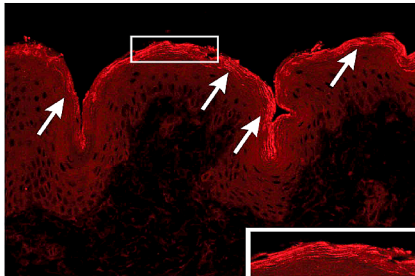
Pediatric Atopic Dermatitis



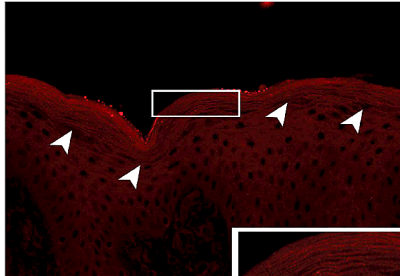
Adult Atopic Dermatitis



Healthy Control



Non-lesional AD



Lesional AD

