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Early pediatric atopic dermatitis shows only a CLA⁺ Th2/Th1 imbalance, while adults acquire CLA⁺ Th22/Tc22 subsets

Tali Czarnowicki, MD^{1,*}, Hitokazu Esaki, MD^{1,2,*}, Juana Gonzalez, PhD³, Dana Malajian, BA^{1,4}, Avner Shemer, MD⁶, Shinji Noda, MD¹, Sreya Talasila⁵, Adam Berry⁵, Jayla Gray⁵, Lauren Becker, MD⁵, Yeriel Estrada², Hui Xu², Xiuzhong Zheng¹, James G Krueger, MD PhD¹, Amy S Paller, MD^{5,**}, and Emma Guttman-Yassky, MD PhD^{1,2,**}

¹Laboratory for Investigative Dermatology, The Rockefeller University, New York, NY, USA

²Dermatology Department at the Icahn School of Medicine at Mount Sinai, New York, NY, USA

³The Translational Technology Core Laboratory, The Rockefeller University, New York, NY, USA

⁴Columbia University College of Physicians and Surgeons, New York, NY, USA

⁵Departments of Dermatology and Pediatrics, Northwestern University Feinberg School of Medicine, IL, USA

⁶Department of Dermatology, Tel-Hashomer Hospital, Tel-Aviv, Israel

Abstract

Background—Identifying differences and similarities between CLA⁺ polarized T-cell subsets in pediatric vs. adult atopic dermatitis (AD) is critical for directing new treatments towards children.

Objective—To compare activation markers and frequencies of skin-homing (CLA⁺) vs. systemic (CLA⁻) “polar” CD4 and CD8 T-cell subsets in early pediatric AD, adult AD and controls.

Methods—Flow cytometry was used to measure CD69/ICOS/HLA-DR frequency in memory subsets as well as IFN- γ , IL-13, IL-9, IL-17, and IL-22 cytokines, defining Th1/Tc1, Th2/Tc2, Th9/Tc9, Th17/Tc17, and Th22/Tc22 populations in CD4 and CD8 cells, respectively. We compared peripheral blood from 19 children <5 years and 42 adults with well-characterized moderate to severe AD, as well as age-matched controls (17 children and 25 adults).

Results—Selective ICOS activation ($P<0.001$) was seen in children. CLA⁺ Th2 T-cells were markedly expanded in both AD children and adults compared to controls, but decreases in CLA⁺ Th1 T-cells were greater in AD children (17% vs. 7.4%, $P=0.007$). Unlike in adults, no imbalances were detected in pediatric AD CLA⁻ T-cells, nor were there altered frequencies of Th22 T-cells within CLA⁺ or CLA⁻ compartments. Adults with AD had increased frequency of IL-22-

Corresponding Author: Emma Guttman-Yassky, MD PhD, Department of Dermatology, Icahn School of Medicine at Mount Sinai Medical Center, 5 E. 98th Street, New York, NY 10029, Emma.Guttman@mountsinai.org, Telephone: 212-241-9728/3288; Fax: 212-876-8961.

//* These authors contributed equally to the manuscript

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producing CD4 and CD8 T-cells within the skin-homing population (9.5% vs. 4.5%; 8.6% vs. 2.4%, respectively; $P<0.001$) as well as increased HLA-DR activation ($P<0.01$).

Conclusions—These data suggest that Th2 activation within skin-homing T-cells may drive AD in children and that reduced counter-regulation by Th1 T-cells may contribute to excess Th2 activation. Th22 “spreading” of AD is not seen in young children and may be influenced by immune development, disease chronicity or recurrent skin infections.

Clinical Implications—Therapeutic approaches to treat AD in children may be best directed to correction of Th2/Th1 imbalance, while adults might also benefit from IL-22 targeted therapies.

Capsule Summary

While AD in children is characterized by Th1/Th2 dysregulation, AD in adults is accompanied by higher chronic activation and a Th22/Tc22 component.

Keywords

Atopic dermatitis; T-cell; CLA; IL-13; IL-22; IFN- γ ; ICOS; CD69; HLA-DR

Introduction

Despite being one of the most common pediatric disorders,^{1, 2} atopic dermatitis (AD) harbors immunological alterations within the first 6 months of onset that are largely unexplored in children.^{3, 4} Although 85% of AD cases present before 5 years old (y.o.) and immune polarization in children may differ from adults with long-standing disease, studies have largely been limited to adults.^{5–7}

The percentage of CD4 memory T-cells (CD45RO⁺) in the circulation of newborns (5%–10%) is significantly lower than in 3–10 y.o. children (~15%) and adults (30–50%), while the percentage of naïve T-cells (CD45RA⁺) is highest in newborns (~90%).^{8, 9–11} Most T-cells in AD lesions are CD45RO⁺ memory cells that express the skin-homing receptor cutaneous lymphocyte antigen (CLA).^{12–14} Circulating CLA⁺ T-cells are expanded in AD, respond to skin-associated allergens, and play a role in disease pathogenesis.^{14–17} CLA⁺ T-cells have been also demonstrated to be peripheral biomarkers in several inflammatory skin diseases.¹⁸ The need for large biopsies to classify T-cell subsets limits the feasibility of intracellular cytokine staining (ICS) of skin from adults and even more so in children. Therefore, studying polarized, activated circulating CLA⁺ memory T-cell subsets may provide a surrogate to skin phenotyping.

Previous studies indicate low IFN- γ and high Th2 signals in cord blood of newborns that develop AD.^{19–21} Subsequent infant and toddler AD studies mostly demonstrate elevated Th2 signals^{22–24} with controversial data regarding Th1 frequency,^{23, 25–28} CD8 polar subsets^{23, 28} and levels of T-cell activation.^{29–31} Several studies include cohorts of adults and children, but most do not provide direct comparison between these groups.^{22, 23, 26, 32} While we have recently reported a detailed blood phenotype of adult AD,³³ few pediatric studies differentiate cytokine expression in CLA subsets³⁰ and virtually none compare adults to children.

Defining the similarities and differences between activated polarized T-cell subsets in adults and early onset pediatric AD is critical for understanding initial pathogenic immune mechanisms. Furthermore, linking IgE levels with T-cell polarization in newly diagnosed AD might help define the sequence of events that leads to AD development.

In this study, we compared differences between T-cell memory subset activation within the skin-homing/CLA⁺ and systemic/CLA⁻ compartments, as well as frequencies of polarized CD4 and CD8 subsets in blood of pediatric and adult AD patients. We found that while AD onset in children is Th2-dominated and characterized by short-term activation, adult AD extends to additional Th subsets, particularly Th22.

Methods

Patients' characteristics and blood samples

Blood was obtained (with patients' or parents' informed consent) from 19 children (11 females, 8 males; aged 5–70 months; mean 25 months) with moderate-to-severe AD as well as from 17 pediatric controls (7 females, 10 males; aged 6–67 months; mean 32 months) and from 42 adults (18 females, 24 males; aged 18–74; mean 42) with moderate-to-severe AD and 25 adult controls (12 females, 13 males; aged 19–66; mean 39) under Institutional Review Board-approved protocol. No age ($P=0.3$), ethnicity ($P=0.8/P=0.055$; children/adults) or gender ($P=0.8/P=0.5$; children/adults) disparities were observed between the groups. AD children were within 6 months from AD diagnosis.

AD adult serum IgE levels (normal <200 kU/L) ranged from 4–50000 kU/L (mean=6238 kU/L), and AD children (normal <100 kU/L) ranged from 23–1772 kU/L (mean=526 kU/L). Control children ranged 0–43 kU/L (mean=26 kU/L).

Scoring of AD/SCORAD was used to evaluate disease severity (adults: SCORAD range 32–97, mean=65; children: range 30–73, mean=54).

Controls had no personal history of inflammatory disease and no family history of atopy. Demographic and laboratory data are summarized in Table 1.

Isolation of Peripheral Blood Mononuclear Cells (PBMCs)

PBMCs were isolated from whole blood by Ficoll-Paque Plus (GE Healthcare, Sweden). Briefly, the blood was laid under Ficoll gradient; after spinning, PBMCs were collected at the interface between the plasma and the Ficoll gradient (see Methods in the Online Repository).

Stimulation of blood cell populations for cytokine responses

Whole blood was incubated with PMA (25ng/ml) plus ionomycin (2μg/ml) in the presence of brefeldin A (10μg/ml) for 4 hours at 37°C to induce T-cell cytokine responses. After stimulation, RBCs were lysed with FACS Lysing solution to obtain leukocytes (see Methods in the Online Repository).

Cell surface staining and intracellular staining on PBMCs and stimulated and non-stimulated CD4/CD8 T-cells

PBMCs were stained with fluorochrome-labeled Abs to cell surface markers (CD3, CD8, CD4, CD45RO, CCR7, ICOS, HLA-DR and CLA). Stimulated and non-stimulated blood cells were also stained for cell surface markers (CD3, CD4, CD69 and CLA) and then permeabilized with FACS/perm to stain for cytokines including IL-13, IL-22, IL-9, IFN- γ and IL-17 (see Methods in the Online Repository).

Statistical analysis

Data was analyzed using Student's *t*-test to compare between variables. IgE values were log10-transformed before analyses. Variables were correlated using Pearson correlations. $P < 0.05$ was considered significant.

Results

To examine immune activation in AD children vs. adults, surface staining was used to measure expression of early (CD69), mid (inducible co-stimulator molecule/ICOS) and late (HLA-DR) activation markers in central (Tcm/CCR7⁺CD45RO⁺) and effector memory (Tem/CCR7⁺CD45RO⁺) T-cells in skin homing/CLA⁺ and CLA⁻ subsets. CD25⁺CD127⁻CCR4⁺ phenotype was used to exclude Tregs from all PBMCs analyses. Subsequently, ICS was used to measure frequencies of IFN- γ , IL-13, IL-22, IL-17A and IL-9 producing T-cells after PMA/ionomycin activation, defining Th1/Tc1, Th2/Tc2, Th22/Tc22, Th17/Tc17 and Th9/Tc9 subsets in CD4 and CD8 T-cells, respectively, in CLA⁺ and CLA⁻ subsets. Gating strategy appears in Figure E1.

Effector T-cells are uniquely elevated in adult AD

As previously described,¹¹ our data shows that the proportion of naïve T-cells was higher in both control (CD4: 79% vs. 60%, $P < 0.001$ and CD8: 68% vs. 56%, $P = 0.01$; Fig 1A–B) and AD children vs. adults (CD4: 78% vs. 59%, $P < 0.001$ and CD8: 77% vs. 55%, $P < 0.001$; Fig 1A–B), whereas Tcm/Tem subsets were lower in control and AD children compared to adults ($P = 0.001$; Fig 1A–B). No significant differences in memory subsets were observed between AD and controls ($P > 0.05$; Fig 1A–B).

While no significant differences in effector cell frequencies were observed among controls (CD4: 4.5% vs. 3.6%, $P = 0.5$ and CD8: 12% vs. 10%, $P = 0.2$; Fig 1A–B), adult AD had significantly higher effector cells compared with pediatric AD (CD4: 3.8% vs. 7.5%, $P = 0.049$ and CD8: 8% vs. 15%, $P < 0.001$; Fig 1A–B).

Memory subsets are positively correlated with IgE, but not with SCORAD, in AD children

Tcm and Tem are crucial components of the adaptive immune system, and expand upon antigenic stimulation.³⁴ To evaluate potential associations between disease severity and antigenic stimulation, Tcm and Tem frequencies were correlated with IgE levels and disease severity/SCORAD.

Significant correlations were only observed between Tcm/Tem frequencies and IgE levels in AD children (CD4: Tcm: $r=0.88$, $P=0.004$ and Tem: $r=0.82$, $P=0.01$; Fig E2B). Memory subset frequencies were neither correlated with SCORAD in AD populations, nor with IgE levels in control children (Fig E2A&C–E).

ICOS is highly expressed on CD4 Tcm and Tem memory subsets of AD patients

ICOS is a mid-activation surface marker that is expressed up to 24hrs after T-cell receptor stimulation and is essential for effective T-helper responses.^{35, 36} Comparing ICOS expression on CD4 T-cells between pediatric AD, adult AD and controls showed that although AD patients had increased ICOS expression in both CLA⁺ and CLA[−] memory subsets compared to their relative controls (Tcm CLA⁺: children 17% vs. 9%, $P=0.01$; adults 22% vs. 9%, $P<0.001$; Fig 2A), CLA⁺ ICOS levels were substantially higher (Tcm CLA[−]: children 8% vs. 4%, $P=0.01$ and adults 6% vs. 3.7%, $P=0.003$; Fig 2A). No significant CD4 ICOS expression differences were observed between AD groups in both CLA⁺/CLA[−] Tem/Tcm ($P>0.16$; Fig 2A–B).

Contrary to adults, in which higher CD8 ICOS expression was measured in CLA⁺ Tcm/Tem cells compared to controls (Tcm: 19% vs. 12%; $P=0.01$ and Tem: 26.7% vs. 13.5%, $P<0.001$; Fig 2A–B), no parallel differences were observed between AD children and controls ($P>0.48$, Fig 2A–B). Among AD groups, adults had higher ICOS expression in CD8 Tcm/Tem compared to their pediatric counterparts (Tcm: CLA⁺ 19% vs. 6%; CLA[−] 4.7% vs. 1% and Tem: CLA⁺ 27% vs. 10%; CLA[−] 4% vs. 1.8%, $P<0.001$ for all; Fig 2A–B).

HLA-DR is preferentially expressed on Tcm/Tem in adult but not pediatric AD

HLA-DR is a human class II major histocompatibility complex (MHC) antigen that indicates chronic activation.³⁷ While comparable levels of Tcm/Tem HLA-DR expression were observed in children regardless of AD status ($P>0.1$; Fig 3A–B), higher HLA-DR activation was found in adult AD compared to controls, particularly among CLA⁺ populations ($P<0.001$; Fig 3A–B). Adult AD also showed higher HLA-DR activation than pediatric AD, mainly among CD8 subsets (Tcm: CLA⁺ 20% vs. 6.5%; CLA[−] 11.5% vs. 3.3% and Tem: CLA⁺ 19% vs. 8%; CLA[−] 8% vs. 1.5%, $P<0.001$ for all; Fig 3A–B).

CD69 expression is higher in AD children compared to adults

CD69 is the earliest activation marker expressed on T-cells following CD4 and CD8 T-cell receptor activation.³⁸ CD69 was the only activation marker that was higher in children, particularly prominent in AD for both CD4 (CLA⁺: 40% vs. 18%, $P=0.0013$ and CLA[−]: 70% vs. 35%, $P<0.001$, Fig 3C) and CD8 subsets (CLA⁺: 66% vs. 40%, $P<0.001$ and CLA[−]: 62% vs. 36%, $P<0.001$, Fig 3C).

Skin selective Th1/Th2 imbalance in pediatric AD

Because prior T-cell activation drives subset differentiation, we quantified polar T-cell subsets, including Th1/Tc1, Th2/Tc2, Th22/Tc22, Th17/Tc17 and Th9/Tc9 frequencies in CLA⁺/CLA[−] subsets in all children and adult populations.

Adult AD patients had normal to slightly lower IFN- γ ⁺CLA⁺ levels compared to healthy individuals (CD4: 17% vs. 20%, $P=0.18$; Fig 4A), and this difference is significant in severe patients, as we recently described.³³ In contrast, AD children have significantly lower IFN- γ ⁺CLA⁺ levels than control children (7.4% vs. 17%, $P=0.007$; Fig 4A) and lower levels of both CLA⁺ and CLA⁻ CD4 T-cell subsets than AD adults (CLA⁺: 7.4% vs. 17%, $P<0.001$ and CLA⁻: 6.3% vs. 25%, $P<0.001$; Fig 4A).

IL-13 was higher in adult AD compared to controls in CLA⁺/CLA⁻ CD4/CD8 subsets ($P<0.04$; Fig 4B). In children this difference was most prominent among CD4 CLA⁺ T-cells (9% vs. 4%, $P=0.04$; Fig 4B). Unlike other cytokines, similar frequencies of CD4 IL-13⁺CLA⁺ were seen in pediatric and adult AD, highlighting Th2 dominance in early AD. Higher CD8 IL-13⁺CLA⁺ frequency was seen in adults compared to AD children (CLA⁺: 9% vs. 3.8%, $P=0.006$ and CLA⁻: 1% vs. 0.09%, $P=0.002$; Fig 4B), possibly suggesting a less prominent role of CD8 in AD children with early disease.

To further evaluate the Th1/Th2 imbalance in AD, we compared this ratio between groups. AD children had markedly lower Th1:Th2 compared to controls, particularly in CD4 CLA⁺ cells (1.2% vs. 12%, $P=0.01$; Fig 4C). This imbalance was less evident in CD8 T-cells. A significantly lower ratio was also observed in pediatric versus adult AD, particularly in CD4 cells (CLA⁺: 1.2% vs. 4%, $P=0.03$ and CLA⁻: 15% vs. 50%, $P=0.01$; Fig 4C).

Since adult AD is characterized by Th22/Tc22 expansion in skin^{39, 40} and blood,³³ these subsets were measured in children. No significant differences in IL-22 were observed between pediatric AD and controls ($P>0.12$; Fig 5A), whereas significantly higher IL-22 levels were observed in adult vs. pediatric AD (CD4: CLA⁺, 9.5% vs. 4.7%, and CLA⁻, 3% vs. 0.2%; CD8: CLA⁺, 8.6% vs. 4.8%, and CLA⁻, 0.6% vs. 0.1%, $P<0.001$ for all; Fig 5A). Th17/Tc17 levels were higher in adult versus pediatric AD, specifically in the CLA⁻ subsets (CD4: 1.3% vs. 0.3%, $P<0.001$ and CD8: 0.4% vs. 0.1%, $P<0.001$; Fig 5B), with no difference compared to controls. Finally, while no IL-9 differences were observed between AD and controls, adults with AD showed higher Th9 levels in CLA⁺ cells compared to children with AD (2.3% vs. 1.3, $P=0.01$; Fig 5C).

Discussion

While initial pathogenic events in AD development remain elusive, IgE switching, T-cell activation and cytokine polarization have been suggested as key players.^{41, 42} Our current understanding of AD pathogenesis derives mostly from studies in adults with long-standing disease. However, only by studying young children with new-onset AD can we gain insight into primary pathogenic events. This is the first comprehensive report characterizing T-cell polarization and activation within skin homing and systemic memory subsets in early onset disease in young children with well-characterized disease severity, in comparison with age-matched pediatric controls and severity-matched AD adults.

At birth, T-cell repertoire consists mostly of naïve cells. With antigenic exposure, memory cells comprise ~35% of circulating T-cells by the second decade of life.⁴³ Our data on memory T-cell subsets corroborates this naïve-memory CD4/CD8 T-cell shift. Furthermore,

it shows that CD4/CD8 effector cells are the only consistently higher subset in adult vs. pediatric AD with no parallel differences in their controls. Normally, effector cell proliferation is part of the 'expansion phase' of the immune reaction, which is replaced shortly after by a 'death phase' of greater than 90% of these effectors, with remaining cells entering a 'memory phase'.⁴⁴ Since antigenic exposure and microbial phenotype play central roles in shaping T-cell effector profiles,⁴⁴ cumulatively more antigenic exposure and increased infection rates may contribute to increased effector cells in adult AD.^{45–48}

T-cell activation through the T-cell receptor leads to a specific sequence of surface activation marker expression. CD69 is a very early activation marker (upregulated within 1hr from cell activation and peaking by 16–24hrs). ICOS is an intermediate activation marker, appearing within 24hrs, while HLA-DR is a late marker indicating chronic activation.^{49–51}

The ICOS co-stimulatory signal is required for T-cell activation and plays a role in shaping adaptive immunity.^{52–54} Mouse studies show that ICOS is vital in T-cell/B-cell interaction, immunoglobulin isotype switching (IgE production), and germinal center formation.^{55, 56} Here, we show that AD children have higher ICOS expression, particularly among CD4 cells. These results may relate to the role of ICOS in Th2 immunity expansion and IgE switching,⁵⁷ supporting an IgE-Th2 mechanism in pediatric AD. The only parameter that showed a trend of higher ICOS activation in AD children compared to adults was the CLA⁺ CD4 population, which further supports the role of ICOS in promoting non-cutaneous atopy in AD children. One possible implication is that ICOS suppression might be used as a target in patients with extrinsic AD accompanied by a central Th2 component.

These ICOS differences were not observed among CD8 cells in pediatric AD. Besides quantitative CD8 decreases relative to CD4 in infants,^{10, 29} an alternative explanation may be that antigenic stimulation of AD in young populations is not presented by MHC-I molecule.

HLA-DR expression on T-cells indicates a state of chronic activation.⁵⁰ While Bunikowski et al.⁵⁸ have previously reported high HLA-DR⁺CD3⁺ T-cells in AD children above 2 years of age compared to controls, another blood study showed no difference.²⁹ Antunez et al.³⁰ subsequently showed no difference between AD children and controls in CD4/CD8 HLA-DR expression, but results were not stratified to CLA⁺/CLA[−] subsets and all children were above 7 years. While we⁵⁹ and others^{60, 61} reported high CD4/CD8 CLA⁺/CLA[−] Tcm/Tem HLA-DR activation in circulating skin homing/CLA⁺ T-cells of adult AD, parallel pediatric data has been unavailable. Our current data indicate that all memory subsets show low HLA-DR activation in children, both in controls and AD. For most subsets, HLA-DR activation is greater in adults versus pediatric AD, but not in normal adults vs. children. This may result from the long-term, chronic activation in adults vs. young children with newly diagnosed AD. Furthermore, differences between the adult groups were more striking in CD8 subsets (with higher values seen in CLA⁺ cells), indicating a possible role for CD8 in the pathogenesis of chronic, long-standing AD.

CD69 also functions as an immunoregulatory receptor,⁶² and has been reported to have opposing functional roles in inflammatory responses.⁶² In AD, its expression on eosinophils

was associated with high IgE levels and active inflammation,⁶³ and data from other allergic diseases indicate that CD69 promotes the inflammatory process.⁶⁴ Our data show that CD69 is the only activation marker higher in children vs. adults with AD. This is in line with prior reports showing that CD69 was virtually absent on CLA⁺ T-cells from adults with AD, probably due to its transient nature as a very early activation marker.^{60, 65} Thus, not surprisingly it is highest in early developmental stages of AD in young children in whom inflammation is peaking.

Since conversion of naïve to cytokine-producing memory subsets is an indication of T-cell activation, we have also assessed cytokine production.

In adults, Th2/Th22 profile predominates the skin phenotype with Th1/Th17 components in chronic lesions.³⁹ We have recently shown that the blood profile to a large extent resembles the skin phenotype, with skin-selective Th2/Tc2/Th22/Tc22 dominance and a Th1/Tc1 defect.³³ ICS data in children is limited and mostly confined to total cytokine measurements (as opposed to CLA⁺/CLA⁻), largely showing high Th2 frequency with conflicting results regarding Th1/Tc1.^{20, 23, 24, 26, 28, 32} The sole pediatric paper including CLA cytokine data highlights only the IL-13⁺CLA⁺ predominance in CD4 cells.³⁰ Our results in AD children display a skin-selective Th1 defect, accompanied by skin-selective increases in Th2 and an imbalanced CLA⁺ Th1:Th2 ratio. While similar trends were observed in adults, levels and differences from controls were more striking in children. Increased migration to skin,^{39, 66} Th1 exhaustion⁶⁷ and high susceptibility to apoptosis⁶⁸ are all plausible explanations for the low IFN- γ in children. However, in light of the very high Th2 signal and high ICOS activation, we suggest that the main cause for decreased Th1 is suppression by Th2 cells.^{69, 70}

Current IgE models suggest that Th2 T-cell differentiation is required to promote IgE class switching, with a suppressive effect of IFN- γ .^{71, 72} Thus, increased ICOS expression that contributes to Th2 differentiation, and in turn to Th1 suppression, may all drive the allergic phenotype observed in young children with AD, including high IgE levels, increased food allergy and other atopic diseases. Indeed, our data show that Tcm/Tem subsets in adults with AD do not correlate with disease severity or IgE levels, but in AD children they strongly correlate with IgE levels. This data supports the notion that Th2-dependent IgE class switching occurs early in the disease and persists into adulthood. Yet, the pathogenic role of high IgE in early-onset AD (seen in approximately 70% of affected children)^{29, 73, 74} remains unclear. Recent reports in adults suggest that IgE might serve to amplify immune activation rather than having a primary pathogenic role.⁷⁵

Our results show that while adult AD demonstrates significantly higher IL-22⁺CLA⁺ than adult controls or pediatric AD, no differences in IL-22 are observed between healthy and AD children. AD in infants and young children tends to be characterized by acute lesions with erythema and dryness, whereas adults with long-standing disease have marked thickening or lichenification of lesions. Our data, together with the previously recognized role of IL-22 in inducing epidermal hyperplasia,⁷⁶ support a role for IL-22 in disease chronicity and not in disease initiation. Immune reactions tend to have 'epitope' spreading over time, and in a similar way may involve T-helper subset expansion to Th22 seen in chronic AD in adults.

Previous cytokine data in children is discordant regarding relative CD4/CD8 immune dysregulation.^{20, 23, 25, 30, 32} Contrary to adults, and similar to our observations with ICOS, Th1/Th2 imbalance is not evident in CD8 T-cells.

We have shown that CD8 T-cells are a major source of IL-22 in adult AD skin lesions, and that CD8⁺IL-22⁺ T-cells are correlated with disease severity.⁴⁰ Although AD was historically considered a CD4-driven disease, a pathogenic role has recently been elucidated for CD8 cells.^{77–79} Thus, in infants and young children CD8 T-cell immaturity,^{80–82} low counts,^{11, 82} exhaustion from super/antigenic stimulation (environmental, food, bacterial etc.) seen in AD^{46, 83–86} or dysfunctional states⁸⁷ are all putative explanations for the low IL-22 signal in pediatric AD.

In addition to the Th2 bias at birth, bias towards Th17 T-cell development has recently been described in normal infants.⁸⁸ According to our results, AD and control adults had higher IL-17 only among CLA⁺ subsets compared to their respective pediatric counterparts. This may be due to a selectively higher IL-17 signal in CLA⁺ cells in children, but the trend of lower Th17 in AD children compared to controls suggests that AD children are failing to properly build this axis within the cutaneous compartment.

During the naïve-to-memory cell conversion in infancy and early childhood, there is a physiological increased susceptibility to infections.^{44, 89} In addition, increased *S. aureus* colonization, as evidenced by increased T-cell receptor (TCR) V β expression on CLA⁺ T-cells,⁹⁰ underdeveloped IL-22, reduced IL-17 and suppressed IFN- γ , all considered protective cytokines against pathogens,^{91–98} might orchestrate the immune milieu, rendering AD children more susceptible to skin infections.^{99, 100}

Interestingly, IL-9 is higher in adult versus pediatric AD, but only in the skin homing compartment. Ma et al.¹⁰¹ have previously shown that Th9 frequencies were higher in AD compared to controls and that frequency correlated with SCORAD and IgE levels. Furthermore, we showed increased IL-9 in chronic AD lesional skin.³⁹ Alternately, higher Th9 frequency in adults versus children with AD may be attributed to higher allergic contact dermatitis seen in this group, though this is speculative.¹⁰²

Studies have shown that approximately 75% of children "outgrow" their AD by 10 years of age.^{42, 103–105} One possibility, based on our data, is that age-related disease resolution in children may reflect increased differentiation of Th1 T-cells to counter-regulate the increased Th2, as well as reciprocal up-regulation of Th1/Th17 cells, which synergistically suppress Th2 production or Th22 development.

Finally, these results may have relevant therapeutic implications. While targeted anti-IL-22 treatment may be beneficial only in adults (ILV-094; [ClinicaTrial.gov](https://clinicaltrials.gov/ct2/show/study/NCT01941537) identifier: NCT01941537), investigating therapeutics that target Th2 cytokines, such as anti IL-13 mAb (Tralokinumab; [ClinicaTrial.gov](https://clinicaltrials.gov/ct2/show/study/NCT02347176) identifier: NCT02347176) and anti-IL-4R alpha mAb (Dupilumab [REGN668/SAR231893]; [ClinicaTrial.gov](https://clinicaltrials.gov/ct2/show/study/NCT01979016) identifier: NCT01979016) may be particularly valuable in the pediatric AD population.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

AD	Atopic dermatitis
CD69	Cluster of Differentiation 69
CLA	Cutaneous Lymphocyte Antigen
HLA-DR	human leukocyte antigen DR
IFN-γ	Interferon gamma
IL	Interleukin
ICOS	Inducible co-stimulator molecule
MHC	Major histocompatibility complex
PBMC	Peripheral Blood Mononuclear Cell
SCORAD	SCORing of Atopic Dermatitis
Tc1	Type 1 Cytotoxic T-cell
Tc17	Type 17 cytotoxic T-cell
Tc2	Type 2 cytotoxic T-cell
Tc22	Type 22 cytotoxic T-cell
Th	T helper
Th1	Type 1 helper T-cell
Th17	Type 17 helper T-cell
Th2	Type 2 helper T-cell
Th22	Type 22 helper T-cell
Y.O.	Years old

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Figure 1. CD4 and CD8 T-cell subsets in children and adults with AD vs. controls

A–B) Naïve T-cell frequency was higher in both control and AD children compared to adults, while Tcm/Tem subsets were lower in control and AD children than adults. Adults with AD had significantly higher effector cells compared with AD children.

Figure 2. ICOS expression in CLA⁺ and CLA⁻ memory T-cell subsets in adult and pediatric AD
A) AD patients had increased ICOS expression in both CLA⁺ and CLA⁻ memory subsets compared to their relative controls. No significant CD4 ICOS expression differences were observed between the AD groups in both CLA⁺/CLA⁻ Tem/Tcm. **B)** Contrary to adults, in which higher CD8 ICOS expression was measured in CLA⁺ Tcm/Tem cells compared to their relative controls, no differences were observed between AD children and controls.

Figure 3. HLA-DR expression in CLA⁺ and CLA⁻ memory T-cell subsets in adult and pediatric AD

A) Comparable levels of HLA-DR expression on T_{CM}/T_{EM} cells between children with and without AD. **B)** AD adults had higher HLA-DR expression compared to controls, particularly among the CLA⁺ and CD8 populations. **C)** Lower CD69 in adults than in children with AD in both CD4 and CD8 subsets.

Figure 5. IL-22, IL-17 and IL-9 frequency in CLA⁺ and CLA⁻ CD4 and CD8 T-cells

A) No significant IL-22 differences were measured between children with and without AD.

B) Th17/Tc17 levels were higher in AD adults versus children, specifically in the CLA⁻

CD4/CD8 subsets. **C)** AD adults had higher Th9 levels in CLA⁺ cells than AD children.

Table 1

Epidemiological, laboratory and clinical data for AD patients and controls

	Adult AD	Adult Controls	<i>P</i> value	Children AD	Children Controls	<i>P</i> value
Age (yrs for adults; mos for children)	42 ± 2.1 (18–74)	39 ± 2.8 (19–66)	0.3	25 ± 1.9 (5–70)	32 ± 1.8 (6–67)	0.34
Gender, no.			0.8			0.51
Female	18	12		11	7	
Male	24	13		8	10	
Ethnicity, no.			0.8			0.055
Asian	3	3		5	0	
Black	6	2		2	1	
White	33	19		12	16	
SCORAD score	64.5 ± 2.45 (32–97)	NA		53.5 ± 14.4 (30–73)	NA	
IgE ^a (Ku/L)	628.6 ± 11.4 (4–50000)	NA		719.8 ± 318.3 (23–1,772)	28.8 ± 13.3 (0–141)	0.082
Eosinophil count ^b (K/mL)	0.45 ± 0.05 (0–1.2)	NA		0.71 ± 0.26 (0.20–2.12)	0.27 ± 0.088 (0–1.12)	0.14

Data presented as mean ± SEM unless otherwise specified. AD, atopic dermatitis; SCORAD, SCORing of AD index; SEM, Standard error of the mean

^aReference range, 0–200 kU/L (adults), 0–100 kU/L (children)^bReference ranges, 0–0.8 K/mL (absolute eosinophil count)