Early pediatric atopic dermatitis shows only a cutaneous lymphocyte antigen (CLA)$^+$ T_H2/T_H1 cell imbalance, whereas adults acquire CLA$^+$ T_H22/T_C22 cell subsets

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Background: Identifying differences and similarities between cutaneous lymphocyte antigen (CLA)$^+$ polarized T-cell subsets in children versus adults with atopic dermatitis (AD) is critical for directing new treatments toward children.

Objective: We sought to compare activation markers and frequencies of skin-homing (CLA$^+$) versus systemic (CLA$^-$) "polar" CD4 and CD8 T-cell subsets in patients with early pediatric AD, adults with AD, and control subjects.

Methods: Flow cytometry was used to measure CD69/inducible costimulator/HLA-DR frequency in memory cell subsets, as well as IFN-γ, IL-13, IL-9, IL-17, and IL-22 cytokines, defining T_H1/cytotoxic T (T_C) 1, T_H2/T_C2, T_H9/T_C9, T_H17/T_C17, and T_H22/T_C22 populations in CD4 and CD8 cells, respectively. We compared peripheral blood from 19 children less than 5 years old and 42 adults with well-characterized moderate-to-severe AD, as well as age-matched control subjects (17 children and 25 adults).

Results: Selective inducible costimulator activation ($P < .001$) was seen in children. CLA$^+$ T_H2 T cells were markedly expanded in both children and adults with AD compared with those in control subjects, but decreases in CLA$^+$ T_H1 T-cell numbers were greater in children with AD (17% vs 7.4%, $P = .007$). Unlike in adults, no imbalances were detected in CLA$^-$ T cells from pediatric patients with AD nor were there altered frequencies of T_H22 T cells within the CLA$^+$ or CLA$^-$ compartments. Adults with AD had increased frequencies of IL-22–producing CD4 and CD8 T cells within the skin-homing population, compared with controls (9.5% vs 4.5% and 8.6% vs 2.4%, respectively; $P < .001$), as well as increased HLA-DR activation ($P < .01$).

Conclusions: These data suggest that T_H2 activation within skin-homing T cells might drive AD in children and that reduced counterregulation by T_H1 T cells might contribute to excess T_H2 activation. T_H22 "spreading" of AD is not seen in young children and might be influenced by immune development, disease chronicity, or recurrent skin infections.

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Key words: *Atopic dermatitis, T cell, cutaneous lymphocyte antigen, IL-13, IL-22, IFN-γ, inducible costimulator, CD69, HLA-DR*

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Despite being one of the most common pediatric disorders,[1,2] atopic dermatitis (AD) harbors immunologic 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 of age and immune polarization in children can differ from that in 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% to 10%) is significantly lower than that in 3- to 10-year-old children (approximately 15%) and adults (30% to 50%), whereas the percentage of naive T cells (CD45RA$^+$) is highest in newborns (approximately 90%).[8-11]

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Abbreviations used
AD: Atopic dermatitis
CLA: Cutaneous lymphocyte antigen
ICOS: Inducible costimulator
PE: Phycoerythrin
PMA: Phorbol 12-myristate 13-acetate
T_C: Cytotoxic T
T_{CM}: Central memory T
T_{EM}: Effector memory T

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 patients with 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 biopsy specimens to classify T-cell subsets limits the feasibility of intracellular cytokine staining of skin from adults and even more so in children. Therefore studying polarized, activated circulating CLA^+ memory T-cell subsets might provide a surrogate to skin phenotyping.

Previous studies indicate low IFN-γ and high T_H2 signals in cord blood of newborns with AD.[19-21] Subsequent infant and toddler AD studies mostly demonstrate increased T_H2 signals,[22-24] with controversial data regarding T_H1 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] Although we have recently reported a detailed blood phenotype of adult AD,[33] few pediatric studies differentiate cytokine expression in CLA subsets,[30] and virtually none compare adults and children.

Defining the similarities and differences between activated polarized T-cell subsets in adults and patients with early-onset pediatric AD is critical for understanding initial pathogenic immune mechanisms. Furthermore, linking IgE levels with T-cell polarization in patients with 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 patients with AD. We found that although AD onset in children is T_H2 dominated and characterized by short-term activation, adult AD extends to additional T_H subsets, particularly T_H22.

METHODS

Patients' characteristics and blood samples

Blood was obtained (with patients' or parents' informed consent) from 19 children (11 female and 8 male children; age range, 5-70 months; mean age, 25 months) with moderate-to-severe AD, as well as from 17 pediatric control subjects (7 female and 10 male subjects; age range, 6-67 months; mean age, 32 months) and 42 adults (18 female and 24 male subjects; age range, 18-74 years; mean age, 42 years) with moderate-to-severe AD and 25 adult control subjects (12 female and 13 male subjects; age range, 19-66 years; mean age, 39 years) under an institutional review board–approved protocol. No age ($P = .3$), ethnicity ($P = .8$/$P = .055$, children/adults), or sex ($P = .8$/$P = .5$, children/adults) disparities were observed between the groups. Children with AD were within 6 months from AD diagnosis.

AD adult serum IgE levels (normal, <200 kU/L) ranged from 4 to 50,000 kU/L (mean, 6,238 kU/L), and levels in children with AD (normal, <100 kU/L) ranged from 23 to 1,772 kU/L (mean, 526 kU/L). Control children's levels ranged 0 to 43 kU/L (mean, 26 kU/L).

SCORAD scores were used to evaluate disease severity (adults: range, 32-97; mean, 65; children: range, 30-73; mean, 54).

Control subjects had no personal history of inflammatory disease and no family history of atopy. Demographic and laboratory data are summarized in Table I.

Isolation of PBMCs

PBMCs were isolated from whole blood by using Ficoll-Paque Plus (GE Healthcare, Uppsala, 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 the Methods section in this article's Online Repository at www.jacionline.org).

Stimulation of blood cell populations for cytokine responses

Whole blood was incubated with phorbol 12-myristate 13-acetate (PMA; 25 ng/mL) plus ionomycin (2 μg/mL) in the presence of brefeldin A (10 μg/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 the Methods section in this article's Online Repository).

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

PBMCs were stained with fluorochrome-labeled antibodies to cell-surface markers (CD3, CD8, CD4, CD45RO, CCR7, inducible costimulator [ICOS], HLA-DR, and CLA). Stimulated and nonstimulated 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 the Methods section in this article's Online Repository).

Statistical analysis

Data were analyzed by using the Student *t* test to compare variables. IgE values were $\log_{10}$-transformed before analyses. Variables were correlated by using Pearson correlations. A *P* value of less than .05 was considered significant.

RESULTS

Surface staining was used to measure expression of early (CD69), middle (ICOS), and late (HLA-DR) activation markers in central memory (T_{CM}/$CCR7^+CD45RO^+$) and effector memory (T_{EM}/$CCR7^-CD45RO^+$) T cells in skin-homing/CLA^+ and CLA^- subsets to examine immune activation in children versus adults with AD. The $CD25^+CD127^-CCR4^+$ phenotype was used to exclude regulatory T cells from all PBMC analyses. Subsequently, intracellular cytokine staining 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 T_H1/cytotoxic T (T_C) 1, T_H2/T_C2, T_H22/T_C22, T_H17/T_C17, and T_H9/T_C9 subsets among CD4 and CD8 T cells, respectively, in CLA^+ and CLA^- subsets. The gating strategy appears in Fig E1 in this article's Online Repository at www.jacionline.org.

Effector T-cell numbers are uniquely increased in adults with AD

As previously described,[11] our data show that the proportion of naive T cells was higher in both control children versus control adults (CD4: 79% vs 60%, $P < .001$; CD8: 68% vs 56%, $P = .01$; Fig 1) and children versus adults with AD (CD4: 78% vs 59%, $P < .001$; CD8: 77% vs 55%, $P < .001$; Fig 1), whereas T_{CM}/T_{EM} cell subsets were lower in control children and children