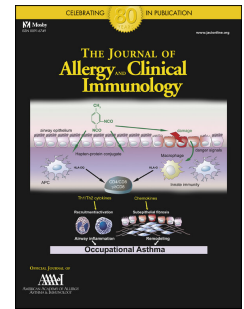


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**Accelerated T-cell activation and differentiation of polar subsets
characterizes early atopic dermatitis development**

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Disclosures: The authors have declared that they have no conflict of interest.

Capsule Summary

The development of early pediatric AD is characterized by ongoing peripheral immune activation, delayed Th1 differentiation and a disproportionally expanded Th2 axis.

Keywords: Atopic dermatitis, pediatric, Central memory T-cells, Effector memory T-cells, ICOS, HLA-DR, CLA, Th1, Th2, Th9, Th17, Th22.

To the Editor:

Infancy is a crucial period for immune development. Cellular immune responses are immature at birth, with decreased natural killer cell activity and regulatory responses.¹ Little is known about T-cell activation and subset differentiation in AD during disease initiation in the first years of life.

To study early AD development, blood was obtained from 29 moderate-to-severe AD infants and toddlers aged 0-3 years (mean 14.5 months; SCORing of AD [SCORAD] range 21-84, mean 54), 13 children aged 3-6 years with infantile onset and persistent disease (mean 57.1 months; SCORAD range 36-73, mean 53), and their respective age-matched controls (demographic data, Table E1).

T-cell surface markers and intracellular flow cytometry panels were generated (see Methods in online repository/OR) to measure polar differentiation and activated CD4⁺/CD8⁺ T-cell frequencies in central (Tcm/CCR7⁺CD45RO⁺) and effector memory (Tem/CCR7⁻CD45RO⁺) subsets in skin homing/cutaneous lymphocyte antigen positive (CLA⁺) and CLA⁻ subsets. Circulating CLA⁺ T-cells constitute blood biomarkers for skin inflammation.² Tcm and Tem populations were further characterized by mid (inducible co-stimulator/ICOS) and late (HLA-DR) activation markers. Data were analyzed using *Student's t-test* and *Pearson correlation coefficient* to correlate variables. *P*<0.05 was considered significant.

At birth, most T-cells are naïve. Antigen exposure induces T-cells to differentiate into Tem, Tcm and terminally differentiated effector memory cells (T_{EMRA}/CCR7⁻CD45RO⁻).³ In both controls and AD, CD4⁺ naïve cells

progressively decreased (controls: $r=-0.62$, $P=0.002$ and AD: $r=-0.56$, $P<0.001$; Fig 1A-B), parallel to increases in Tcm/Tem subsets ($P<0.05$; Fig 1C-F). Uniquely in AD, Tem (but not Tcm), which has rapid effector function, was higher in AD 3-6 year olds (y/o) vs. 0-3 y/o (14.1% vs. 4.9%, $P=0.02$; Fig 1G-H). More CLA⁺ Tcm were in AD 3-6 y/o (but not AD 0-3 y/o) vs. controls (28.0% vs. 13.9%, $P=0.004$; Fig 1I). In contrast, the CLA⁺/Tem subset was higher in both AD 0-3 y/o (40.7% vs. 28.5%, $P=0.02$; Fig 1J) and 3-6 y/o (42.9% vs. 25.1%, $P=0.003$; Fig 1J) groups. Opposite trends were observed in the CLA⁻ populations (Fig 1K-L). Differences were not observed between the two AD cohorts (0-3/3-6 y/o) ($P>0.16$; Fig 1I-L). T_{EMRA}, the most differentiated effector memory subset, positively correlated with age only in controls ($r=0.5$, $P=0.01$; Fig 1M-N), likely based on their earlier differentiation in AD (0-3 years: 5.5% vs. 3.6%, $P=0.02$; Fig 1O). These data demonstrate increased effector T-cell differentiation at AD initiation exclusively in the skin-homing compartment.

Past studies showed fewer CD8⁺ than CD4⁺ T-cells in infants.¹ Consistently, neither AD patients nor controls showed significant increases in CD8⁺ Tcm/Tem cells ($P>0.14$; Fig E1A-D), supporting our recent demonstration that the Th1/Th2 imbalance in early AD is CD4⁺-selective, with CD8⁺ T-cells playing a lesser role in initial AD development.⁴

We recently reported that Tcm/Tem ICOS activation, which also stimulates Th2 expansion and IgE switching, is higher in AD children.⁴ Indeed, ICOS activation was higher in the younger (0-3 y/o) AD children, particularly in CLA⁻ cells (Tcm: 7.3% vs. 4.1%, $P=0.02$; Tem: 18.6% vs. 9.6%, $P=0.02$; Fig E2A-B),

with CLA⁺ differences more obvious in the 3-6 y/o group (Fig E2C-D). Chronological decreases of memory subset activation were observed in both groups but reached significance only in controls ($P<0.05$; Fig E2E-L). Ongoing Tcm ICOS activation in early AD indicates maintenance of a large lymph node T-cell reservoir, while early high levels of Tem cells are probably crucial for disease initiation. No differences in HLA-DR chronic activation were seen between controls and AD ($P>0.2$; not shown). However, the age-related increases exclusively in AD (Tcm: CLA⁺ $r=0.4$, $P=0.02$ and CLA⁻ $r=0.47$, $P=0.004$; Fig. E2M-P) suggest a later contribution of HLA-DR to AD. Skin residence could explain why chronological HLA-DR increases in blood were not significant for Tem cells ($P>0.13$; not shown).

Because activation of memory cells precedes T-cell differentiation, we next studied polar subsets. The smaller skin-homing Th1 subset in AD vs. controls⁵ was particularly prominent in AD 0-3 y/o (6.8% vs. 11.6%, $P=0.009$; Fig 2A). As previously reported,⁶ significant increases in IFN- γ ⁺CLA⁺ were observed in older vs. younger controls (20.1% vs. 11.6%, $P=0.04$; Fig 2A), but not in AD ($P=0.08$; Fig 2A). These changes were not seen in the CLA⁻ subset (Fig 2B), emphasizing the role of inappropriate Th1 developmental delay exclusively among the skin-homing compartment in early AD. Conversely, IL-13⁺ levels were significantly higher in the younger AD group in both CLA⁺ (10.0% vs. 1.9%, $P<0.001$; Fig 2C) and CLA⁻ (1.1% vs. 0.4%, $P=0.008$; Fig 2D) subsets, without further increases in AD 3-6 y/o ($P>0.29$; Fig 2C-D), supporting the frequent concurrence of non-cutaneous atopic manifestations beginning early in AD.

These results underscore the role of primary CLA⁺ Th1/Th2 cytokine dysregulation in AD initiation, which is further demonstrated by a low Th1/Th2 ratio in AD 0-3 y/o vs. controls (CLA⁺: 1.7% vs. 8%, $P=0.001$ and CLA⁻: 7.5% vs. 18.5%, $P=0.003$; Fig 2E-F). AD children demonstrated “normalization” of the Th1/Th2 ratio with age only in the CLA⁻ subset ($r=0.58$, $P<0.001$; Fig 2G-H), potentially correlating temporally with resolution of associated atopic disorders (e.g., food allergies).⁷

Early Th2 activation may be a driver of the subsequent atopic march as the 0-3 y/o AD cohort had low rates of associated atopic conditions, unlike the 3-6 y/o group in whom most patients showed atopic associations (38% vs. 92%, $P=0.002$; Table E1). This discrepancy in atopic disorders occurrence supports the notion that early cutaneous sensitization predisposes to other atopic diseases.

IL-9 was linked to peanut allergy in older children⁸. However, CLA⁺/CLA⁻ IL-9 levels in our young AD children, 10 of whom had IgE-sensitization to peanuts (vs. 1 control), were similar to controls without difference between age groups ($P>0.16$; Figs 2I-J and E3A-D). The possibility that Th9 cells reside in early AD skin but later migrate to blood to mediate systemic allergic responses deserves further study. IL-22 positively correlated with age only in the CLA⁻ compartment of AD children ($r=0.34$, $P=0.04$; Figs 2K-L, E3E-F), while IL-17⁺ exclusively correlated with age in controls (CLA⁺: $r=0.5$, $P=0.03$; CLA⁻: $r=0.52$, $P=0.02$; Figs 2M-N, E3G-H). Th22 is involved in host defenses and Th17 in antimicrobial production and neutrophil chemotaxis.⁹ Failure to increase CLA⁺ Th17 and Th22 subsets in early AD might contribute to cutaneous immune

compromise. Absence of increased IL-22⁺ and IL-17⁺ blood levels result from high Th2 signals or, as hypothesized for Th9, reflect migration of these subsets into lesional skin. Finally, early Th17, Th9 and Th22 frequencies were much higher in CLA⁺ than CLA⁻ subsets in both AD and controls (Figs 2I-J and E3I-L), suggesting that cutaneous antigenic exposure drives early subset development.

In conclusion, pediatric AD development is characterized by early excessive T-cell activation and imbalanced subset differentiation. Effector memory subsets and polarized T-cell frequencies are most distinct from controls in the very young AD cohort (0-3 y/o), suggesting that future studies elucidating immune subset fluctuations and evaluating AD developmental mechanisms should focus on this population.

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References

1. Gasparoni A, Ciardelli L, Avanzini A, Castellazzi AM, Carini R, Rondini G, et al. Age-related changes in intracellular TH1/TH2 cytokine production, immunoproliferative T lymphocyte response and natural killer cell activity in newborns, children and adults. *Biol Neonate* 2003; 84:297-303.
2. Ferran M, Romeu ER, Rincon C, Sagrista M, Gimenez Arnau AM, Celada A, et al. Circulating CLA⁺ T lymphocytes as peripheral cell biomarkers in T-cell-mediated skin diseases. *Exp Dermatol* 2013; 22:439-42.
3. Cossarizza A, Ortolani C, Paganelli R, Barbieri D, Monti D, Sansoni P, et al. CD45 isoforms expression on CD4⁺ and CD8⁺ T cells throughout life, from newborns to centenarians: implications for T cell memory. *Mech Ageing Dev* 1996; 86:173-95.
4. Czarnowicki T, Esaki H, Gonzalez J, Malajian D, Shemer A, Noda S, et al. Early pediatric atopic dermatitis shows only a cutaneous lymphocyte antigen (CLA) 1 T(H)2/T(H)1 cell imbalance, whereas adults acquire CLA1(+) T(H)22/T(C)22 cell subsets. *Journal of Allergy and Clinical Immunology* 2015; 136:941-51.
5. Czarnowicki T, Gonzalez J, Shemer A, Malajian D, Xu H, Zheng XZ, et al. Severe atopic dermatitis is characterized by selective expansion of circulating T(H)2/T(C)2 and T(H)22/T(C)22, but not T(H)17/T(C)17, cells within the skin-homing T-cell population. *Journal of Allergy and Clinical Immunology* 2015; 136:104-15.

- 228 6. Kawamoto N, Kaneko H, Takemura M, Seishima M, Sakurai S, Fukao T,
229 et al. Age-related changes in intracellular cytokine profiles and Th2
230 dominance in allergic children. *Pediatr Allergy Immunol* 2006; 17:125-33.
- 231 7. Sicherer SH, Sampson HA. Food allergy: Epidemiology, pathogenesis,
232 diagnosis, and treatment. *J Allergy Clin Immunol* 2014; 133:291-307; quiz
233 8.
- 234 8. Brough HA, Cousins DJ, Munteanu A, Wong YF, Sudra A, Makinson K, et
235 al. IL-9 is a key component of memory TH cell peanut-specific responses
236 from children with peanut allergy. *J Allergy Clin Immunol* 2014; 134:1329-
237 38.
- 238 9. Korn T, Bettelli E, Oukka M, Kuchroo VK. IL-17 and Th17 Cells. *Annu Rev*
239 *Immunol* 2009; 27:485-517.
- 240
- 241

Figure Legends**Figure 1. T-cell memory subsets in pediatric AD patients and controls.**

Significant decreases in naïve cells (**A-B**) and increases in Tcm/Tem subsets (**C-F**) were observed in AD and controls over time (CI values appear in parentheses). **G-H**) Contrary to Tcm, Tem increased significantly between the 0-3 y/o and 3-6 y/o AD cohorts. **I**) The skin-homing/CLA⁺ Tcm subset was significantly higher in the 3-6 y/o AD group compared to controls, while (**J**) the CLA⁺ Tem subset was already significantly higher in the 0-3 y/o AD cohort. **K**) CLA⁻ Tcm cells were significantly lower in the 3-6 y/o AD group compared to controls, while (**L**) the CLA⁻ Tem subset was already significantly lower in the 0-3 y/o AD cohort. **M-N**) T_{EMRA} cells increased significantly over time only in controls, while (**O**) a higher baseline value was recorded in the AD 0-3 y/o group. Bar plots represent means ± SEMs. SEM, standard error of the mean; CI, Confidence Interval; Tcm, central memory T-cells; Tem, effector memory T-cells; CLA, cutaneous lymphocyte antigen.

Figure 2. CLA⁺/CLA⁻ polar subset frequencies and correlations with age.

Significantly lower IFN- γ ⁺CLA⁺ (**A**) but not IFN- γ ⁺CLA⁻ (**B**) percentages were demonstrated between the 0-3 y/o AD and control groups, while IL-13⁺ levels were higher in both CLA⁺ (**C**) and CLA⁻ (**D**) subsets. A significantly lower Th1/Th2 ratio was observed in the 0-3 y/o AD group in both CLA⁺ (**E**) and CLA⁻ (**F**) subsets and increased with age only in the CLA⁻ compartment (**G-H**) of pediatric AD (CI values appear in parentheses). No IL-9 differences were observed

265 between pediatric AD and controls **(I)**. Significant IL-22⁺CLA⁻ and IL-17⁺CLA⁻
266 /CLA⁺ increases with age were observed in the controls and AD, respectively **(K-**
267 **N)**. Bar plots represent means $\pm$ SEMs. SEM, standard error of the mean; CI
268 values appear in parentheses. CI, Confidence Interval; CLA, cutaneous
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Figure 1

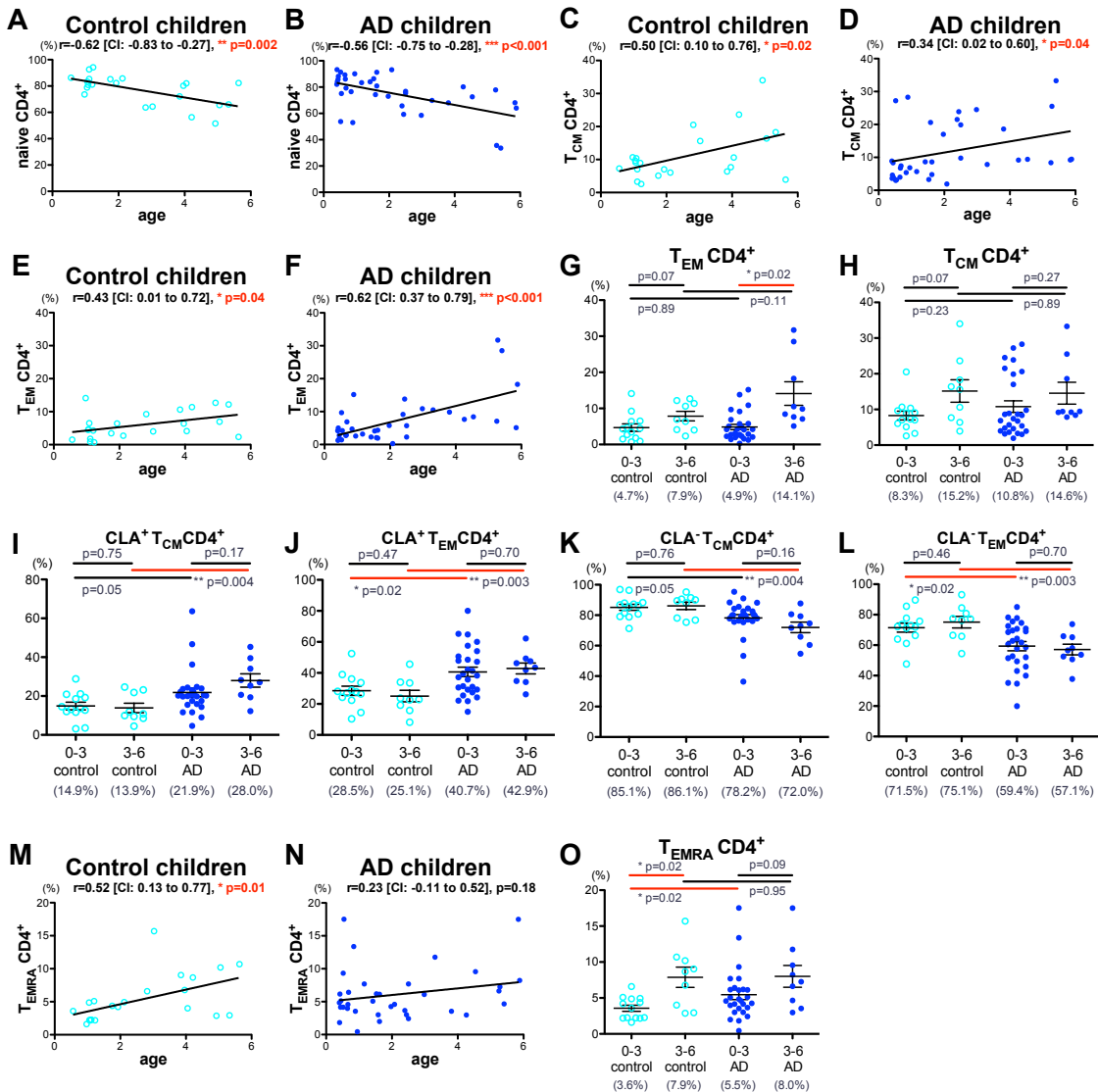
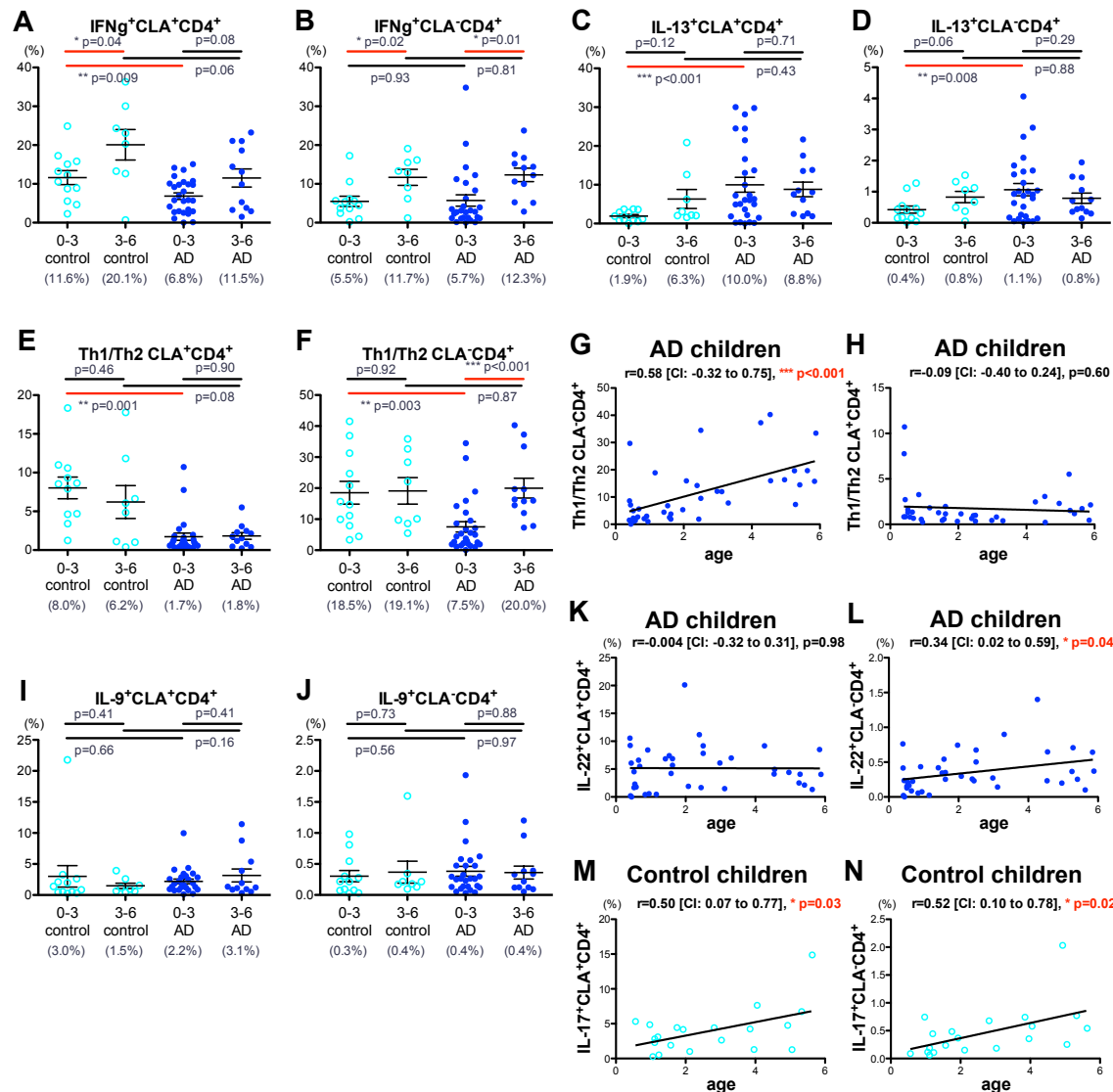


Figure 2



1 **Materials and methods**

2 **Isolation of Peripheral blood mononuclear cells and multiparameter flow** 3 **cytometry blood analyses**

4 Peripheral blood mononuclear cells (PBMCs) were obtained by gradient
5 centrifugation with Ficoll-Paque Plus (Amersham Biosciences, Pittsburgh, Pa).
6 The blood was laid under the Ficoll gradient; after spinning, PBMCs were
7 collected at the interface between the plasma and the Ficoll gradient and washed
8 with PBS before staining for flow cytometry analysis. For T-cell surface staining,
9 PBMCs were washed and incubated for 30 minutes in ice with fluorochrome-
10 conjugated monoclonal antibodies (mAbs) to cell surface molecules (CD3-Pe AF
11 610, CD8-V-500, CD4-Qdot800, CD45RO-PeCy7, CLA-FITC, ICOS-
12 PerCpCy5.5, CCR7-Alexa Fluor 700, HLA-DR-APC-H7, Live/dead-Blue Dye).
13 Then, the cells were fixed in ice with 4% paraformaldehyde (BD Biosciences CA)
14 for 20 minutes.

15 For intracellular cytokine staining, whole blood was activated with 25
16 ng/mL of phorbol 12-myristate 13-acetate (PMA) and 2 µg/mL of ionomycin in the
17 presence of 10 µg/mL brefeldin A (protein transporter inhibitor) for 4 hours at
18 37°C. All reagents were purchased from Sigma Aldrich (St. Louis, MO). Non-
19 activated controls were only treated with brefeldin A. After this incubation, EDTA
20 (2 Mm, Invitrogen, NY) was added for 15 minutes at room temperature (RT) to
21 stop activation. The blood was then incubated with FACS lysing solution for 10
22 minutes at RT and washed with PBS twice. Then, the cells were incubated for 20
23 minutes with fluorochrome-conjugated mAbs to cell surface molecules (CD3-Pe

AF 610, CD4-Qdot800, CLA-FITC). Subsequently, the cells were permeabilized (Perm/Wash; BD Biosciences, CA) and incubated for 20 min with fluorochrome conjugated mAbs (IL-13-PerCpCy5.5, IL-22-PeCy7, IL-9-Pe and IFN- γ - Alexa Fluor 700) in 5% mouse serum (eBioscience, CA). All incubations for cell surface and intracellular staining were done at room temperature. Appropriate FMO-isotype controls were used. Following incubations, cells were washed and suspended in PBS. This was followed by acquisition with a BD LSR II flow cytometer (BD Biosciences) and analysis by flow-Jo software (Treestar, Ore).

Table E1. Epidemiologic, laboratory, and clinical data for children with AD and controls

	Children with AD aged 0 to 3	Children Controls aged 0 to 3	<i>P</i> value	Children with AD aged 3 to 6	Children Controls aged 3 to 6	<i>P</i> value
Age (months)	14.5 ± 1.8 (4-35)	16.8 ± 1.9 (6-33)	0.5	57.1 ± 3.1 (37-70)	53.4 ± 3.3 (36-67)	0.4
Sex, no.			0.3			0.7
Female	11	8		6	3	
Male	18	6		7	6	
Ethnicity, no.			0.1			0.9
Hispanic	3	0		1	1	
Asian	5	0		4	1	
African American	5	1		3	2	
Caucasian	16	13		5	5	
SCORAD score	54.1 ± 2.6 (21-84)	NA	-	53.0 ± 3.6 (36-73)	NA	-
Duration of AD (months)	11.3 ± 1.5 (3-29)	NA	-	48.9 ± 3.5 (18-62)	NA	-
IgE† (kU/L)	984.4 ± 509.1 (5.3-5,000)	16.8 ± 4.4 (1-43.3)	* 0.01	825.4 ± 382.1 (95-2,248)	83.7 ± 31.8 (3.2-159)	0.05
Other atopy	11	0	<0.0001	12	0	<0.0001

† Reference range: 0 to 100 kU/L

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

Supplementary Figure Legends

Figure E1. Tcm/Tem CD8⁺ subsets' correlations versus age in AD patients

and controls. A-D) Tcm/Tem CD8⁺ subsets were not correlated with age in either AD or control groups (CI values appear in parentheses). CI, Confidence Interval; Tcm, Central memory T-cells; Tem, Effector memory T-cells.

Figure E2. CLA⁺/CLA⁻ ICOS activated Tcm/Tem cells frequencies and

correlations versus age in AD patients and controls. The youngest (0-3yrs) AD group had higher Tcm **(A)** and Tem **(B)** ICOS⁺CLA⁻ activation compared to controls, while **(C-D)** CLA⁺ differences were more prominent between the 3-6yrs groups. **E-L)** ICOS activation decreased over time exclusively in the controls, while **(M-P)** HLA-DR chronic activation increased selectively in AD (CI values appear in parentheses). Bar plots represent means $\pm$ SEMs. SEM, standard error of the mean; CI, Confidence Interval; Tcm, Central memory T-cells; Tem, Effector memory T-cells; CLA, cutaneous lymphocyte antigen.

Figure E3. CLA⁺/CLA⁻ Th9, Th22 and Th17 subset frequencies and

correlations versus age in AD patients and controls. A-D) Non-significant correlations were found between Th9 and age in either controls or AD (CI values appear in parentheses). **E-F)** Non-significant correlations were found between Th22 and **(G-H)** Th17 with age in controls and pediatric AD, respectively. **I-L)** CLA⁺/CLA⁻ Th22/Th17 frequencies among the different groups. Bar plots

69 represent means $\pm$ SEMs. SEM, standard error of the mean; CI, Confidence

70 Interval; CLA, cutaneous lymphocyte antigen.

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SCORAD score	54.1 ± 2.6 (21-84)	NA	-	53.0 ± 3.6 (36-73)	NA	-
Duration of AD (months)	11.3 ± 1.5 (3-29)	NA	-	48.9 ± 3.5 (18-62)	NA	-
IgE† (kU/L)	984.4 ± 509.1 (5.3-5,000)	16.8 ± 4.4 (1-43.3)	* 0.01	825.4 ± 382.1 (95-2,248)	83.7 ± 31.8 (3.2-159)	0.05
Other atopy	11	0	<0.0001	12	0	<0.0001

Data are presented as means ± SEMs.

† Reference range: 0 to 100 kU/L.

Figure E1

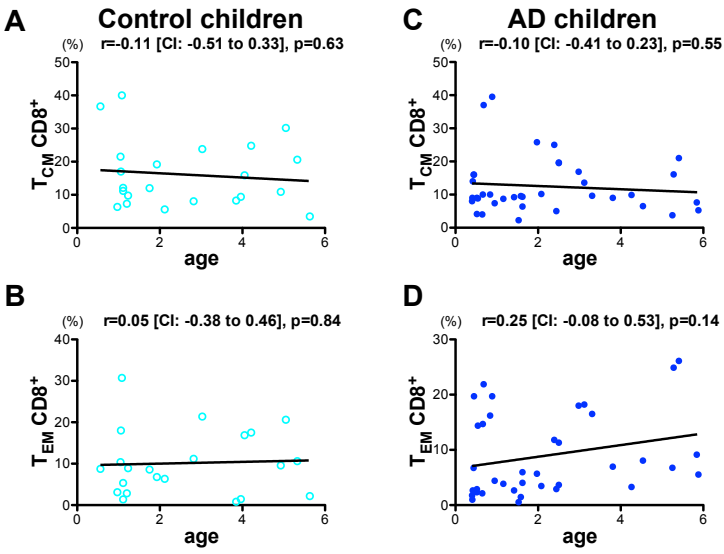


Figure E2

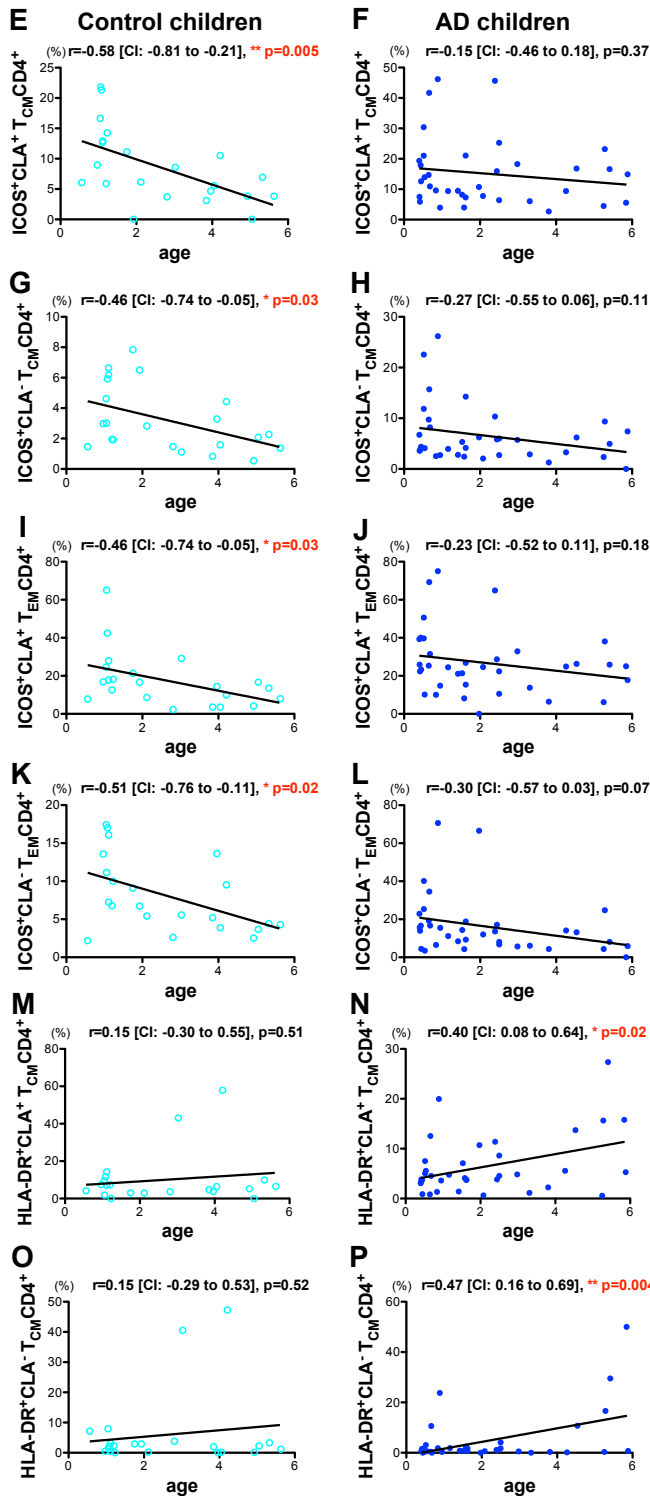
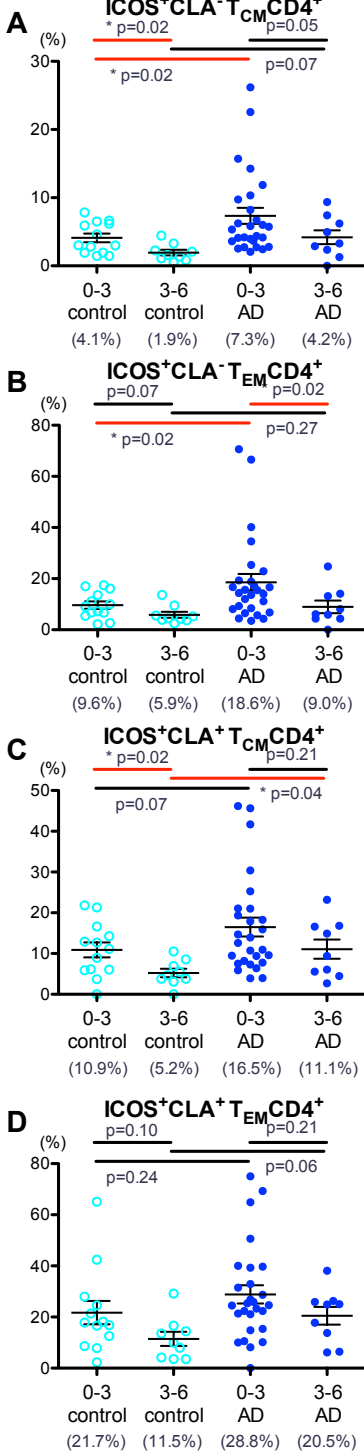


Figure E3

