a *CHD7* mutation. Complications other than immunodeficiency were mild in our case, which assisted successful CBT. In addition, none of the previous reported cases received conditioning regimen, whereas our case received reduced-intensity conditioning to reinforce engraftment of donor cells.

A previous report demonstrated that in patients with DGS who underwent BMT, reconstitution had predominantly occurred through the expansion of the donors' mature T-cell pool. Circulating T cells exhibited a memory phenotype with a restricted repertoire and were devoid of TRECs.[8] In contrast, we observed naive phenotype T cells and a fairly diverse T-cell receptor repertoire even 2 years after CBT. Because cord blood contains more naive T cells than the bone marrow and the T-cell repertoire in cord blood is diverse, CBT might be superior than BMT for the reconstitution of naive T cells compared.[9]

Recent studies showed that newborn screening for severe combined immunodeficiency can efficiently detect infants with complete DGS before they suffer from opportunistic infections.[10,11]

We suggest that CBT with reduced-intensity conditioning is a therapeutic option for complete DGS, which can particularly be useful for uninfected patients who are identified through newborn screening. Further reports should be accumulated to confirm the effectiveness of our procedure as a curative treatment for patients with complete DGS.

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

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 patients with atopic dermatitis (AD) during disease initiation in the first years of life.

To study early AD development, blood was obtained from 29 infants and toddlers aged 0 to 3 years (mean, 14.5 months; SCORAD score: range, 21-84; mean, 54) with moderate-to-severe AD, 13 children aged 3 to 6 years with infancy-onset and persistent disease (mean, 57.1 months; SCORAD score: range, 36-73; mean, 53), and their respective age-matched control subjects (demographic data are shown in Table E1 in this article's Online Repository at www.jacionline.org).

T-cell surface markers and intracellular flow cytometric panels were generated (see the Methods section in this article's Online Repository at www.jacionline.org) to measure polar

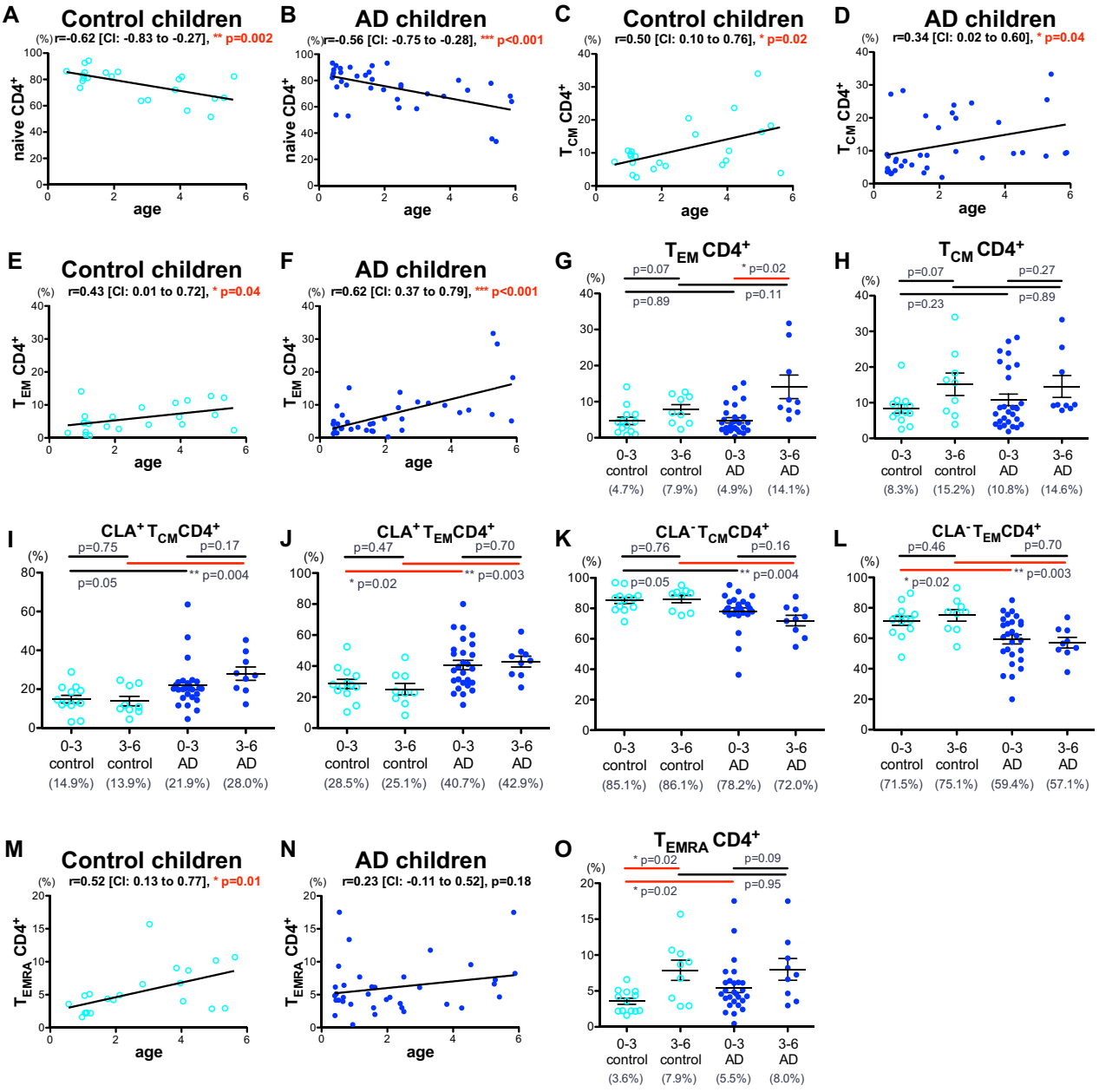

FIG 1. T-cell memory subsets in pediatric patients with AD and control subjects. **A-F,** Significant decreases in naive cell counts (Fig 1, *A* and *B*) and increases in T_{CM}/T_{EM} cell subset counts (Fig 1, *C-F*) were observed in patients with AD and control subjects over time (CI values appear in parentheses). **G** and **H,** Contrary to T_{CM} cells, T_{EM} cell counts increased significantly between the 0- to 3-year-old and 3- to 6-year-old AD cohorts. **I** and **J,** The skin-homing/CLA^+ T_{CM} cell subset was significantly higher in the 3- to 6-year-old group with AD compared with control subjects (Fig 1, *I*), whereas the CLA^+ T_{EM} cell subset was already significantly higher in the 0- to 3-year-old AD cohort (Fig 1, *J*). **K** and **L,** CLA^- T_{CM} cell counts were significantly lower in the 3- to 6-year-old group with AD compared with those in control subjects (Fig 1, *K*), whereas CLA^- T_{EM} cell subset number were already significantly lower in the 0- to 3 year-old AD cohort (Fig 1, *L*). **M-O,** T_{EMRA} cell counts increased significantly over time only in control subjects (Fig 1, *M* and *N*), whereas a higher baseline value was recorded in the 0- to 3-year-old group with AD (Fig 1, *O*). Bar plots represent means ± SEMs.

differentiation and activated $CD4^+/CD8^+$ T-cell frequencies in central memory T ($T_{CM}/CCR7^+CD45RO^+$) cell and effector memory T ($T_{EM}/CCR7^-CD45RO^+$) cell subsets in skin-homing/cutaneous lymphocyte antigen $(CLA)^+$ and CLA^- subsets. Circulating CLA^+ T cells constitute blood biomarkers for skin inflammation.[2] T_{CM} and T_{EM} cell populations were further characterized by mid (inducible costimulator [ICOS]) and late (HLA-DR) activation markers. Data were analyzed

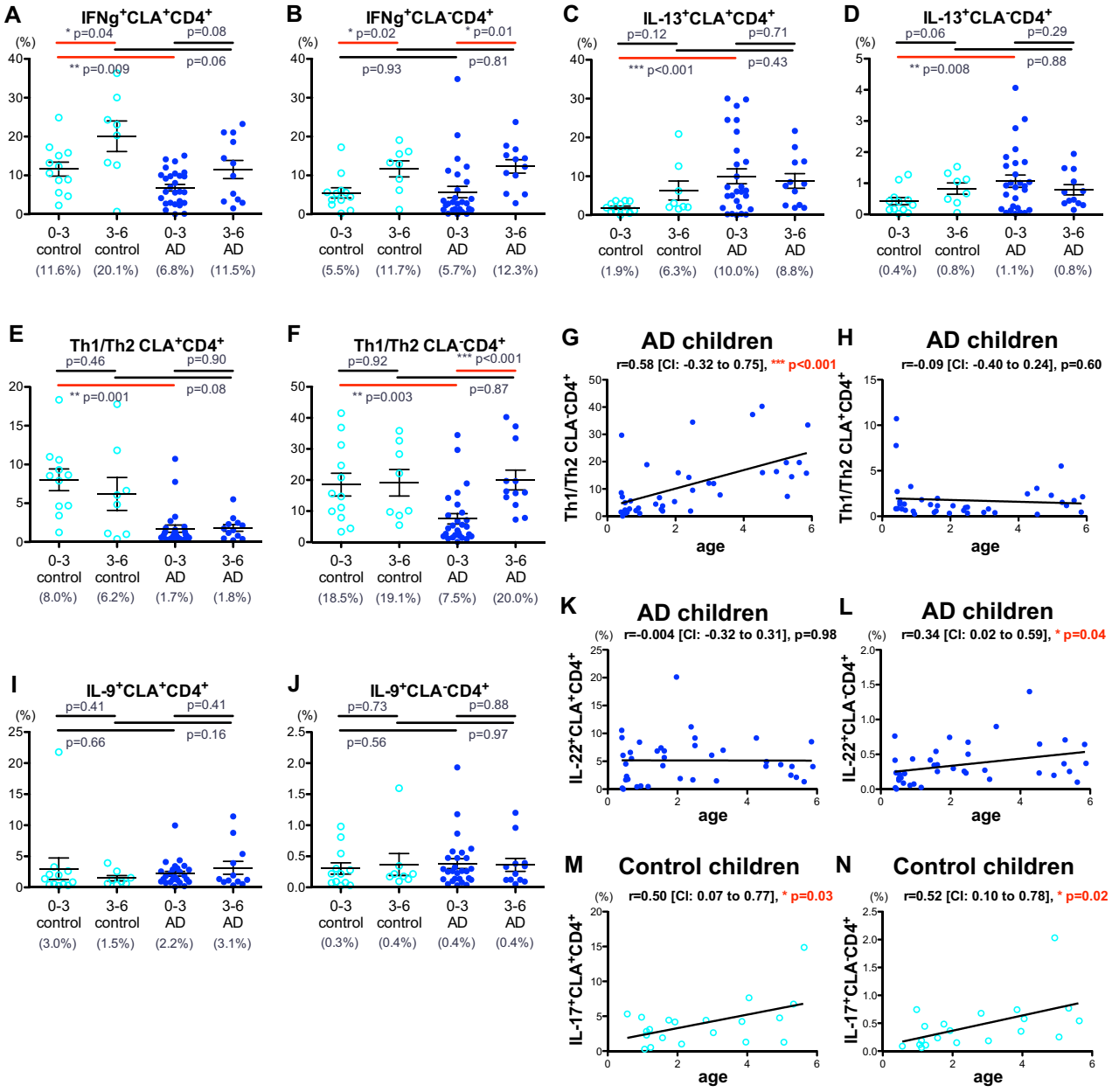

FIG 2. CLA^+/CLA^- polar subset frequencies and correlations with age. **A-D,** Significantly lower IFN-γ^+CLA^+ (Fig 2, *A*) but not IFN-γ^+CLA^- (Fig 2, *B*) percentages were demonstrated between the 0- to 3-year-old AD and control groups, whereas IL-13^+ levels were higher in both the CLA^+ (Fig 2, *C*) and CLA^- (Fig 2, *D*) subsets. **E-H,** A significantly lower T_H1/T_H2 ratio was observed in the 0- to 3-year-old group with AD in both CLA^+ (Fig 2, *E*) and CLA^- (Fig 2, *F*) subsets and increased with age only in the CLA^- compartment (Fig 2, *G* and *H*) of pediatric patients with AD (CI values appear in parentheses). **I-J,** No IL-9 differences were observed between pediatric patients with AD and control subjects. **K-N,** Significant IL-22^+CLA^- and IL-$17^+CLA^-/CLA^+$ increases with age were observed in the control subjects and patients with AD, respectively. Bar plots represent means ± SEMs.

with the Student *t* test and Pearson correlation coefficient to correlate variables. *P* values of less than .05 were considered significant.

At birth, most T cells are naive. Antigen exposure induces T cells to differentiate into T_{EM}, T_{CM}, and terminally differentiated effector memory (T_{EMRA}/$CCR7^-CD45RO^-$) cells.[3] In both control subjects and patients with AD, $CD4^+$ naive cell counts progressively decreased (control subjects: $r = -0.62$, $P = .002$; patients with AD: $r = -0.56$, $P < .001$; Fig 1, *A* and *B*) parallel to increases in T_{CM}/T_{EM} cell subsets ($P < .05$; Fig 1, *C-F*). Uniquely in patients with AD, numbers of T_{EM} (but not T_{CM}) cells, which have rapid effector function, were higher in 3- to

6-year-old patients with AD versus those in 0- to 3-year-old patients with AD (14.1% vs 4.9%, $P = .02$; Fig 1, *G* and *H*). More CLA^+ T_{CM} cells were present in 3- to 6-year-old patients with AD (but not 0- to 3-year-old patients with AD) versus control subjects (28.0% vs 13.9%, $P = .004$; Fig 1, *I*). In contrast, the CLA^+/T_{EM} cell subset was higher in both the 0- to 3-year-old (40.7% vs 28.5%, $P = .02$; Fig 1, *J*) and 3- to 6-year-old (42.9% vs 25.1%, $P = .003$; Fig 1, *J*) groups. Opposite trends were observed in the CLA^- populations (Fig 1, *K* and *L*). Differences were not observed between the 2 AD cohorts (0-3/3-6 year olds; $P > .16$; Fig 1, *I-L*). Numbers of T_{EMRA} cells, the most differentiated effector memory subset, positively correlated with age only in control subjects ($r = 0.5$, $P = .01$; Fig 1, *M* and *N*), likely based on their earlier differentiation in patients with AD (0-3 years old: 5.5% vs 3.6%, $P = .02$; Fig 1, *O*). 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 patients with AD nor control subjects showed significant increases in $CD8^+$ T_{CM}/T_{EM} cell counts ($P > .14$; see Fig E1 in this article's Online Repository at www.jacionline.org), supporting our recent demonstration that the T_H1/T_H2 imbalance in early AD is $CD4^+$ selective, with $CD8^+$ T cells playing a lesser role in initial AD development.[4]

We recently reported that T_{CM}/T_{EM} ICOS activation, which also stimulates T_H2 expansion and IgE switching, is greater in children with AD.[4] Indeed, ICOS activation was greater in younger (0- to 3 years old) children with AD, particularly in CLA^- cells (T_{CM} cells: 7.3% vs 4.1%, $P = .02$; T_{EM} cells: 18.6% vs 9.6%, $P = .02$; see Fig E2, *A* and *B*, in this article's Online Repository at www.jacionline.org), with CLA^+ differences more obvious in the 3- to 6-year-old group (see Fig E2, *C* and *D*). Chronological decreases of memory subset activation were observed in both groups but reached significance only in control subjects ($P < .05$; see Fig E2, *E-L*). Ongoing T_{CM} ICOS activation in patients with early AD indicates maintenance of a large lymph node T-cell reservoir, whereas early high numbers of T_{EM} cells are probably crucial for disease initiation. No differences in HLA-DR chronic activation were seen between control subjects and patients with AD ($P > .2$, data not shown). However, the age-related increases exclusively in patients with AD (T_{CM} cells: CLA^+ $r = 0.4$, $P = .02$ and CLA^- $r = 0.47$, $P = .004$; see Fig E2, *M-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 T_{EM} cells ($P > .13$, data not shown).

Because activation of memory cells precedes T-cell differentiation, we next studied polar subsets. The smaller skin-homing T_H1 subset in patients with AD versus control subjects[5] was particularly prominent in patients with AD aged 0 to 3 years (6.8% vs 11.6%, $P = .009$; Fig 2, *A*). As previously reported,[6] significant increases in IFN-γ^+CLA^+ cells were observed in older versus younger control subjects (20.1% vs 11.6%, $P = .04$; Fig 2, *A*) but not in patients with AD ($P = 0.08$; Fig 2, *A*). These changes were not seen in the CLA^- subset (Fig 2, *B*), emphasizing the role of inappropriate T_H1 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%, $P < .001$; Fig 2, *C*) and CLA^- (1.1% vs 0.4%, $P = .008$; Fig 2, *D*) subsets, without further increases in patients with AD aged 3 to 6 years ($P > .29$; Fig 2, *C* and *D*), supporting the frequent concurrence of noncutaneous atopic manifestations beginning early in AD. These results underscore the role of primary CLA^+ T_H1/T_H2 cytokine dysregulation in AD initiation, which is further demonstrated by a low T_H1/T_H2 ratio in patients with AD aged 0 to 3 years versus control subjects (CLA^+: 1.7% vs 8%, $P = .001$ and CLA^-: 7.5% vs 18.5%, $P = .003$; Fig 2, *E* and *F*). Children with AD demonstrated "normalization" of the T_H1/T_H2 ratio with age only in the CLA^- subset ($r = 0.58$, $P < .001$; Fig 2, *G* and *H*), potentially correlating temporally with resolution of associated atopic disorders (eg, food allergies).[7]

Early T_H2 activation might be a driver of the subsequent atopic march because the 0- to 3-year-old AD cohort had low rates of associated atopic conditions unlike the 3- to 6-year-old group, in which most patients had atopic associations (38% vs 92%, $P = .002$; see Table E1). This discrepancy in atopic disorder occurrence supports the notion that early cutaneous sensitization predisposes to other atopic diseases.

IL-9 was linked to peanut allergy in older children.[8] However, CLA^+/CLA^- IL-9 levels in our young children with AD, 10 of whom had IgE sensitization to peanuts (vs 1 control subject), were similar to those seen in control subjects, without a difference between age groups ($P > .16$; Fig 2, *I* and *J*, and see Fig E3, *A-D*, in this article's Online Repository at www.jacionline.org). The possibility that T_H9 cells reside in the skin of patients with early AD but later migrate to blood to mediate systemic allergic responses deserves further study. IL-22 levels positively correlated with age only in the CLA^- compartment of children with AD ($r = 0.34$, $P = .04$; Fig 2, *K* and *L*, and see Fig E3, *E* and *F*), whereas IL-17^+ exclusively correlated with age in control subjects (CLA^+: $r = 0.5$, $P = .03$; CLA^-: $r = 0.52$, $P = .02$; Fig 2, *M* and *N*, and see Fig E3, *G* and *H*). T_H22 is involved in host defenses, and T_H17 is involved in antimicrobial production and neutrophil chemotaxis.[9] Failure to increase CLA^+ T_H17 and T_H22 subsets in early AD might contribute to cutaneous immune compromise. Absence of increased IL-22^+ and IL-17^+ blood levels result from high T_H2 signals or, as hypothesized for T_H9, reflect migration of these subsets into lesional skin. Finally, early T_H17, T_H9, and T_H22 cell frequencies were much higher in CLA^+ than CLA^- subsets in both patients with AD and control subjects (Fig 2, *I* and *J*, and see Fig E3, *I-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 control subjects in the very young AD cohort (0-3 years old), suggesting that future studies elucidating immune subset fluctuations and evaluating AD developmental mechanisms should focus on this population.

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Human amniotic fluid antibodies protect the neonate against respiratory syncytial virus infection

To the Editor:

Respiratory syncytial virus (RSV) is a leading cause of acute lower respiratory tract infections, hospitalizations, and mortality in young children. Nearly all children are infected at least once with RSV by age 2 years.[1] Early RSV infection plays an important role in the pathogenesis of recurrent wheeze in the first year of life and possibly asthma development later in life.[2,3] In a single year, an estimated 34 million episodes of RSV-associated acute lower respiratory tract infections may occur in children younger than 5 years.[4]

Despite the high incidence of RSV infections during early childhood, RSV infection in the first weeks of life is uncommon.[5] Newborns are likely to be protected against RSV infection by maternal RSV-neutralizing antibodies (nAbs).[6] Previous studies have demonstrated that serum RSV nAbs correlate with protection against RSV infection.[7] These RSV nAbs are frequently present at protective levels in the serum of infants in the first 6 months after birth.[8] Nevertheless, the highest burden of RSV infections is in infants from age approximately 4 weeks to 6 months.[1,4,5] Thus, serum RSV nAbs do not fully explain why healthy term babies are resistant to RSV infection during neonatal age. Because serum RSV nAbs only partly reflect the RSV-protective capacity at the site of infection, which is the respiratory mucosa, better local correlates of humoral immune protection are needed. Indeed, nasal RSV-specific antibodies were recently shown to better correlate than serum RSV-specific antibodies with RSV protection in adults.[9] Amniotic fluid (AF) contains maternal antibodies and is in direct contact with the respiratory tract of the fetus. However, nothing is known on the presence of RSV nAbs in AF.

Therefore, we investigated the presence of RSV nAbs in a panel of 28 AF samples from healthy term infants and hypothesized that (1) RSV nAbs are present in human AF and that (2) the presence of these antibodies in the lungs contributes to protection against RSV infection. First, we measured by ELISA the total levels of IgG (mean, 96 μg/mL [range, 21-589 μg/mL]) and IgA (mean, 41 μg/mL [range, 4-361 μg/mL]) in AF (Fig 1, *A*, and see Fig E1, *A*, in this article's Online Repository at www.jacionline.org). Further details of the methods used in this study can be found in the Methods section in this article's Online Repository at www.jacionline.org. All the AF samples contained RSV-specific IgG (mean, 2.8 μg/mL [range, 0.5-10.5 μg/mL]) and lower levels of RSV-specific IgA (mean, 0.1 μg/mL [range, 0-1 μg/mL]) (Fig 1, *B*, and see Fig E1, *B*). AF RSV-specific IgG also bound to RSV-infected HEp-2 cells in a fluorescence-activated cell sorting–based antibody-binding assay, but not to uninfected cells (see Fig E2 in this article's Online Repository at www.jacionline.org). In an RSV neutralization assay with palivizumab (human IgG_1 against the RSV F protein) used as control antibody (Fig 1, *C*), we detected RSV-neutralizing activity in all AF samples (Fig 1, *D*). However, all RSV-neutralizing activity was removed when AF was preincubated with prefusion, but not postfusion, RSV F protein (Fig 1, *E*, and see Fig E1, *C-F*), suggesting specificity of RSV nAbs in AF for prefusion RSV F protein surfaces. AF RSV-neutralizing activity was mainly IgG-dependent, as demonstrated by an IgG depletion assay (Fig 1, *F*, and see Fig E3, *A* and *B*, in this article's Online Repository at www.jacionline.org), and to a lesser extent presumably on other immunoglobulin isotypes (ie, IgA) and nonimmunoglobulin AF constituents (ie, cytokines and oligosaccharides). We analyzed matched cord blood samples of 20 donors for total IgG and RSV-specific IgG concentrations as well as for RSV-neutralizing activity (see Fig E4 in this article's Online

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METHODS

Isolation of PBMCs and multiparameter flow cytometric blood analyses

PBMCs were obtained by means of gradient centrifugation with Ficoll-Paque Plus (Amersham Biosciences, Pittsburgh, Pa). Blood was placed under the Ficoll gradient. After spinning, PBMCs were collected at the interface between the plasma and the Ficoll gradient and washed with PBS before staining for flow cytometric analysis. For T-cell surface staining, PBMCs were washed and incubated for 30 minutes on ice with fluorochrome-conjugated mAbs to cell-surface molecules (CD3-phycoerythrin [PE] Alex Fluor 610, CD8-V-500, CD4-Qdot800, CD45RO-PECy7, CLA–fluorescein isothiocyanate, ICOS-PerCpCy5.5, CCR7 Alexa Fluor 700, HLA-DR–allophycocyanin-H7, and Live/dead-Blue Dye). Then the cells were fixed in ice with 4% paraformaldehyde (BD Biosciences, San Jose, Calif) for 20 minutes.

For intracellular cytokine staining, whole blood was activated with 25 ng/mL phorbol 12-myristate 13-acetate and 2 μg/mL ionomycin in the presence of 10 μg/mL brefeldin A (protein transporter inhibitor) for 4 hours at 37°C. All reagents were purchased from Sigma-Aldrich (St Louis, Mo). Nonactivated control subjects were only treated with brefeldin A. After this incubation, EDTA (2 mmol/L; Invitrogen, Grand Island, NY) was added for 15 minutes at room temperature (RT) to stop activation. The blood was then incubated with FACS lysing solution for 10 minutes at RT and washed with PBS twice. Then the cells were incubated for 20 minutes with fluorochrome-conjugated mAbs to cell-surface molecules (CD3-PE Alex Fluor 610, CD4-Qdot800, and CLA–fluorescein isothiocyanate). Subsequently, the cells were permeabilized (Perm/Wash, BD Biosciences) and incubated for 20 minutes 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, San Diego, Calif). All incubations for cell-surface and intracellular staining were done at RT. Appropriate FMO-isotype controls were used.

After incubation, cells were washed and suspended in PBS. This was followed by acquisition with a BD LSR II flow cytometer (BD Biosciences) and analysis with FlowJo software (TreeStar, Ashland, Ore).

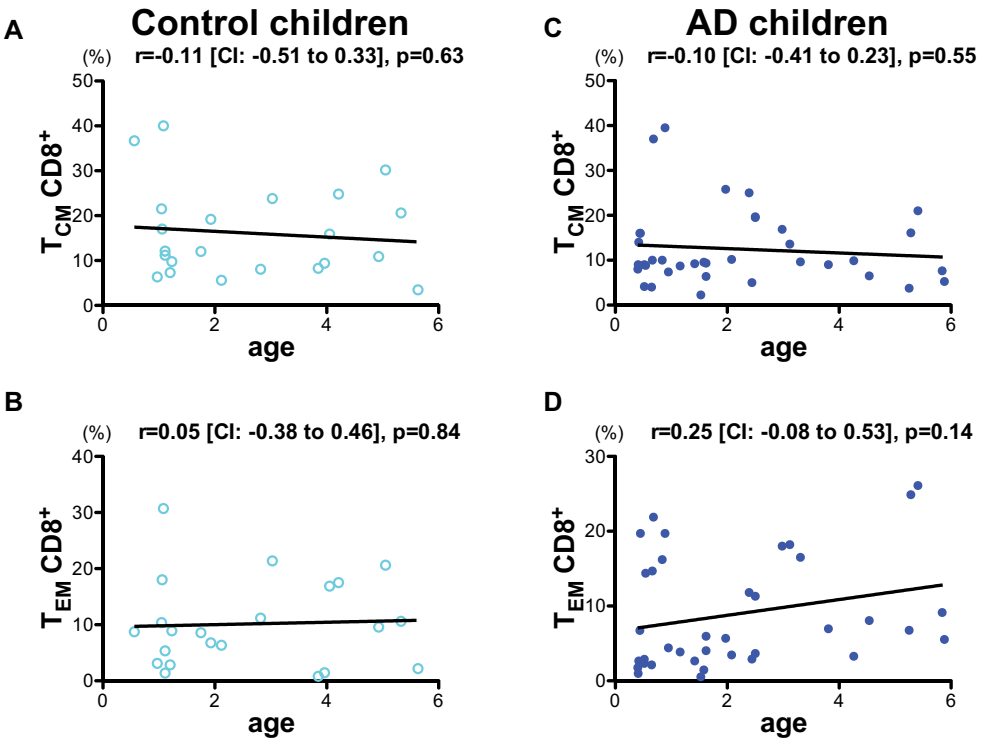

FIG E1. T_{CM}/T_{EM} $CD8^+$ cell subset correlations versus age in patients with AD and control subjects. **A-D,** T_{CM}/T_{EM} $CD8^+$ cell subsets were not correlated with age in either the AD or control groups (CI values appear in parentheses).

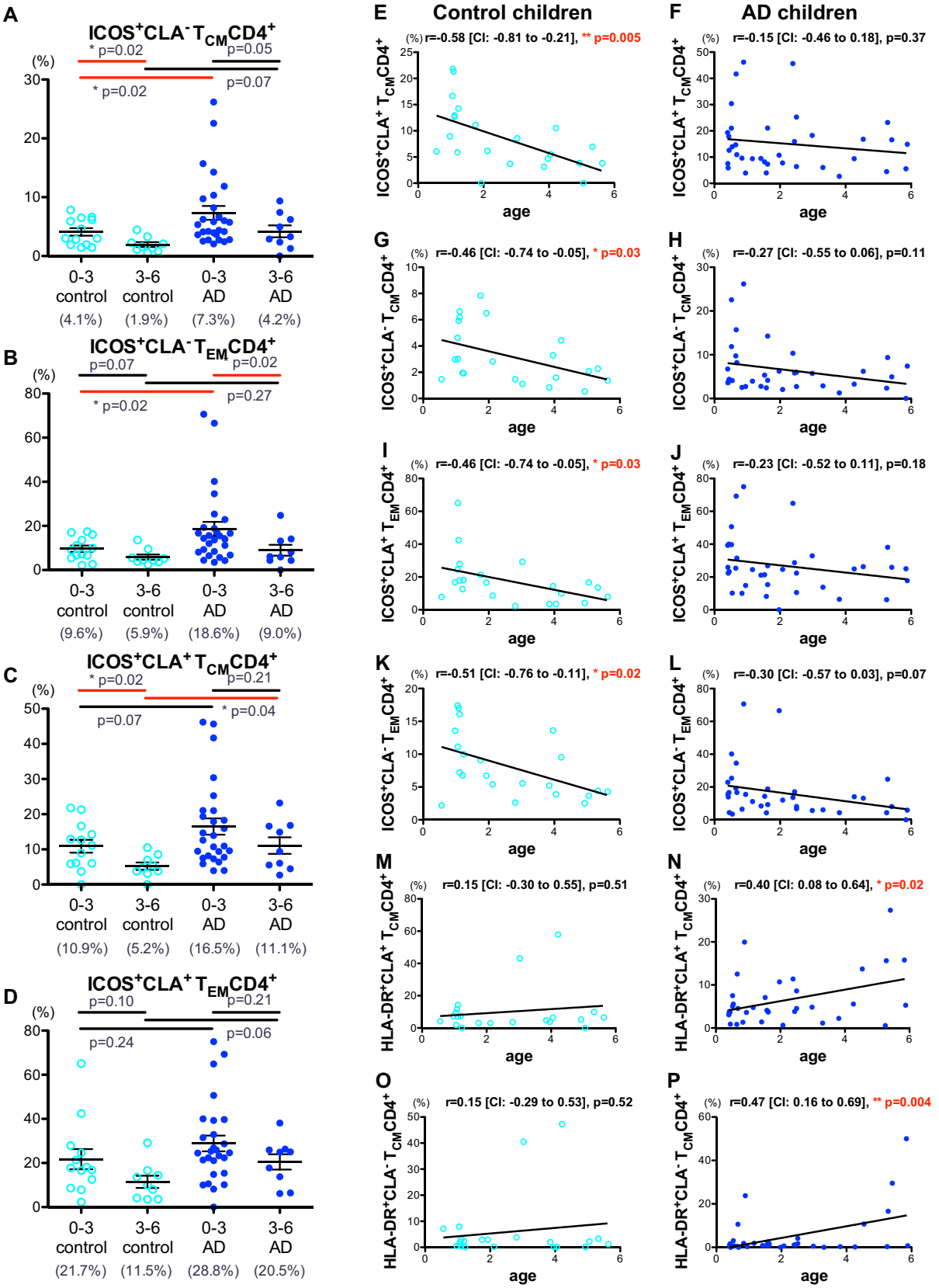

FIG E2. CLA^+/CLA^- ICOS-activated T_{CM}/T_{EM} cell frequencies and correlations versus age in patients with AD and control subjects. **A-D,** The youngest (0-3 years) AD group had higher T_{CM} (Fig E2, *A*) and T_{EM} (Fig E2, *B*) $ICOS^+CLA^-$ activation compared with control subjects, whereas CLA^+ differences were more prominent between the 3- to 6-year-old groups (Fig E2, *C* and *D*). **E-P,** ICOS activation decreased over time exclusively in control subjects (Fig E2, *E-L*), whereas HLA-DR chronic activation increased selectively in patients with AD (Fig E2, *M-P*; CI values appear in parentheses). Bar plots represent means ± SEMs.

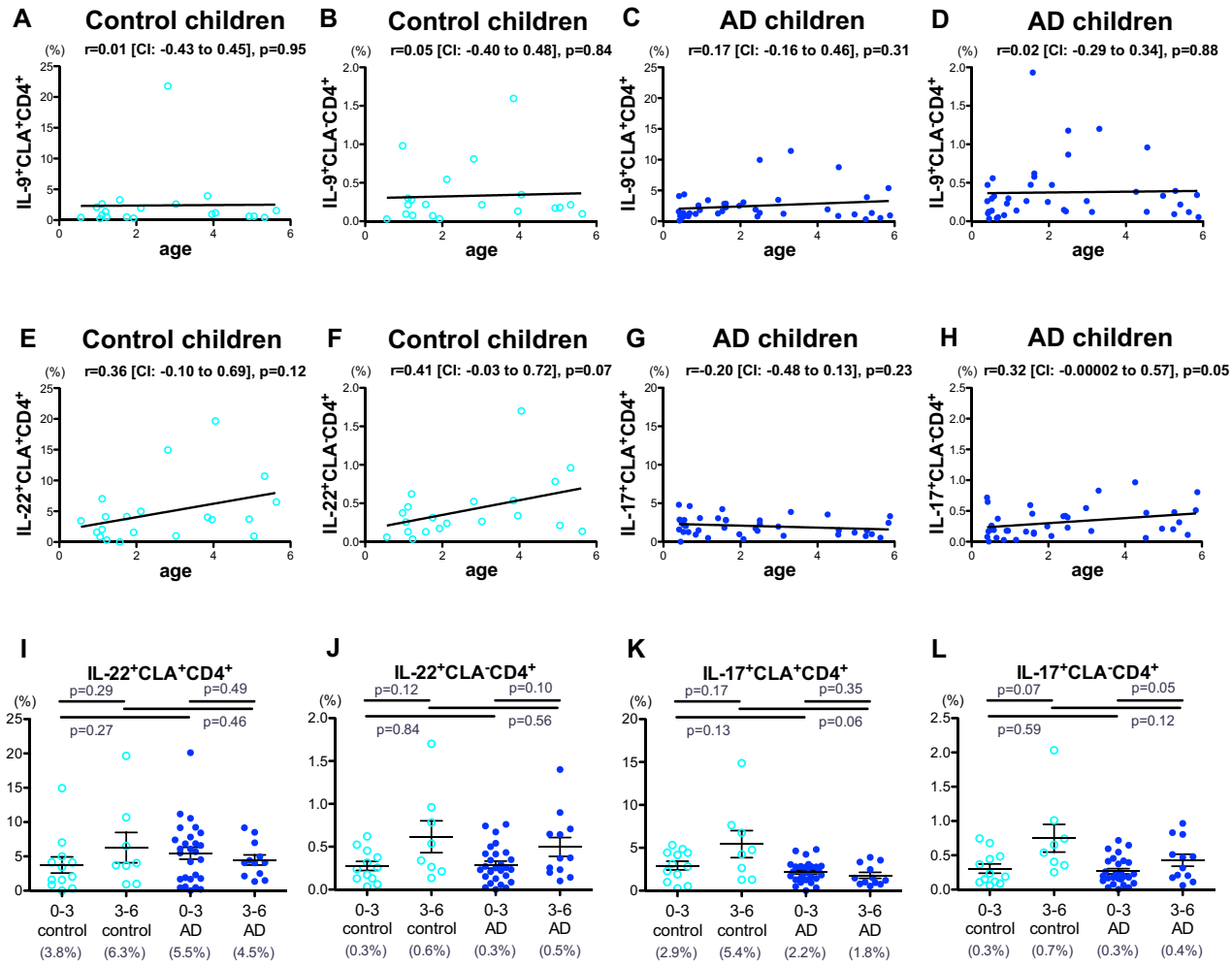

FIG E3. CLA^+/CLA^- T_H9, T_H22, and T_H17 cell subset frequencies and correlations versus age in patients with AD and control subjects. **A-D,** Nonsignificant correlations were found between T_H9 cell frequencies and age in either control subjects or patients with AD (CI values appear in parentheses). **E-H,** Nonsignificant correlations were found between T_H22 (Fig E3, *E* and *F*) and T_H17 (Fig E3, *G* and *H*) cell counts with age in control subjects and pediatric patients with AD, respectively. **I-L,** CLA^+/CLA^- T_H22/T_H17 cell frequencies among the different groups. Bar plots represent means ± SEMs.

TABLE E1. Epidemiologic, laboratory, and clinical data for children with AD and control subjects

	Patients with AD aged 0-3 y	Control subjects aged 0-3 y	*P* value	Patients with AD aged 3-6 y	Control subjects aged 3-6 y	*P* value
Age (mo)	14.5 ± 1.8 (4-35)	16.8 ± 1.9 (6-33)	.5	57.1 ± 3.1 (37-70)	53.4 ± 3.3 (36-67)	.4
Sex (n)			.3			.7
Female	11	8		6	3	
Male	18	6		7	6	
Ethnicity (n)			.1			.9
Hispanic	3	0		1	1	
Asian	5	0		4	1	
African American	5	1		3	2	
White	16	13		5	5	
SCORAD score	54.1 ± 2.6 (21-84)	NA	—	53.0 ± 3.6 (36-73)	NA	—
Duration of AD (mo)	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)	.01	825.4 ± 382.1 (95-2,248)	83.7 ± 31.8 (3.2-159)	.05
Other atopy	11	0	<.0001	12	0	<.0001

Data are presented as means ± SEMs unless otherwise specified.
NA, Not applicable.
*Reference range: 0 to 100 kU/L.