

Expression of the T-cell markers CD2 and CD28 in healthy and atopic children during the first 18 months of life

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Atopy may be associated with a reduced T-cell function early in life, particularly regarding maturation of Th1 responses. The T-cell surface molecules CD2 and CD28 are involved in important T-cell activation pathways. Stimulation via the CD2 receptor increases the responsiveness to interleukin (IL)-12, which is a potent inducer of Th1 responses, whereas CD28 stimulation is critical for Th2 differentiation. Our aim was to prospectively study the expression of the cell-surface markers CD2 and CD28 on T-cells in relation to development of atopic disease. Children ($n = 172$) were followed from birth to 18 months and the cumulative history of atopic disease was recorded. Blood samples were obtained at birth and at 18 months, and in a subgroup of 78 infants also at 3, 6 and 12 months. Flow cytometry was used to analyze the T-cell markers CD2 and CD28, the latter also within the subsets of T-helper ($CD4+$) and T-cytotoxic ($CD8+$) cells. At 18 months, 31 children had and 118 did not have atopic symptoms. At this age, skin prick test (SPT) positive children with atopic symptoms with or without an atopic family history (AFH) showed a lower expression of CD2 mode fluorescence intensity (FI) as well as a lower proportion of $CD2+$ cells, as compared with non-sensitized children with neither atopic symptoms nor AFH. This was accompanied by a higher expression of CD28 FI on $CD2+CD8+CD28+$ cells. No significant differences were seen at time points before 18 months, although the proportion of $CD2+$ tended to be low also earlier in life. In conclusion, the observed reduced expression of CD2 in atopic infants may support previous findings that atopy is associated with a reduced CD2 function. The high CD28 FI in SPT positive children with atopic symptoms may possibly be a consequence of a TH2-skewed immune system.

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The process of T-cell maturation in children is poorly understood. Earlier T-cell studies (reviewed in 1), e.g. assessment of sheep erythrocyte rosette-forming T-cell numbers, phytohemagglutinin (PHA) responsiveness, interferon- γ (IFN- γ) production, and anti-CD3 responsiveness in atopic children (2), have led to the hypothesis that atopic children show a delayed immunologic maturation after birth. The surface marker CD2, which is the receptor for sheep erythrocytes, enhances the interaction between the major histocompatibility complex (MHC)

and the T-cell receptor via binding to leukocyte function-associated antigen-3 (LFA-3) on the antigen-presenting cell (3). An enhanced interaction promotes T helper 1 (Th1)-like immunity (4), and stimulation via CD2 enhances the responsiveness (proliferation and IFN- γ production) of activated T cells to interleukin (IL)-12 (5). In a previous study, we reported that atopy was associated with a low proportion of $CD2+$ cells at 18 months of age (2).

A primary signal through TCR/CD3 complexes and a second signal through costimulatory

molecules are required for the conversion of resting peripheral blood T lymphocytes into functionally active and responsive effector T cells (6–8). CD28 is expressed on a majority of T cells, and signals through CD28 enhance IL-2-dependent T-cell proliferation, augmenting T-cell effector functions, such as cytokine production and the generation of cytotoxicity (6). In addition to the effect on IL-2 production, CD28 costimulation influences naive CD4⁺ T-cell differentiation towards Th1 and Th2 cells (reviewed in 6). Most studies have suggested that blockade of CD28 at the time of initial T-cell activation prevents development of Th2 but not Th1 responses (reviewed in 6), although contradictory findings have been reported (9). Furthermore, Th2 responses are impaired in CD28 deficient mice, whereas Th1 responses are normal (10, 11).

The aim of this study was to investigate the expression of the T-cell surface markers CD2 and CD28 during the first 18 months of life in prospectively followed children who did or did not develop atopic disease. Previously we have reported on CD2 data from a subgroup of 54 children (2) at 18 months of age. We are now reporting CD2 data from the entire cohort of 172 children at 18 months. In addition, flow cytometry data on CD2 and CD28 at birth, 3, 6 and 12 months are reported, as well as data on CD28 at 18 months of age.

Materials and methods

Subjects

Pregnant mothers at seven maternity-care centers in Linköping were informed of the study and 172 families accepted to participate. All children were born at the University Hospital in Linköping. At 3, 6, 12 and 18 months the parents were asked to complete a mailed questionnaire related to possible allergic symptoms in their children. At 18 months, a medical examination was done by one of us (HZ), focusing on signs of atopic disease. In addition, skin prick tests (SPT) were performed, and a venous blood sample was obtained after application of percutaneous anesthesia. A family history of atopy (AFH) in the immediate family, i.e. parents and siblings of the baby, was obtained through two interviews, once at the beginning of the study and once for rechecking the history at the 18 months follow-up visit. Double (single/no) atopic heredity implied that at least two (one/no) parents and/or siblings had a convincing history of atopic disease. Venous blood samples were drawn at birth and at 18 months (range 16.6–21.0 months). In order

to reduce the number of blood samples, for both ethical and financial reasons, and to prevent drop outs, venous blood samples were drawn at 3 (range: 2.5–4.4 months), 6 (5.3–7.3 months) and 12 months (11.3–13.5 months) only from 78 children, in addition to the samples at birth and 18 months. Forty-eight of these children had double AFH and 30 had no AFH. Thus, blood samples were obtained from 119 infants at birth, and from 66, 71, 69 and 137 children, respectively, at 3, 6, 12 and 18 months. Missing samples were due to technical reasons, e.g. birth during weekends when research laboratory facilities were unavailable, clotting of the cord blood ($n = 17$), difficulties with drawing the blood sample, and technical failure in the laboratory (including computer saving problems on data list mode files).

Eleven samples were discarded (including two cord blood samples), as they were obtained from three children with diseases that possibly could have interfered with the study (IgA deficiency, congenital hypothyreosis and mental retardation due to suspected mitochondrial disease). Thirteen cord blood samples were excluded due to exceeding the tested time limit before staining or analysing and another two cord blood samples were from two drop outs. Moreover, six cord blood samples were excluded from analysis due to perinatal complications (including suspect neonatal sepsis, jaundice due to AB0 blood group incompatibility, neonatal respiratory distress and/or <37 or >42 gestational weeks). All the cord blood samples used in the statistical analysis were thus from healthy, full-term babies with normal vaginal deliveries, except for three cases of cesarean section of which two were planned due to maternal reasons and one was acute due to risk of rupture of the uterus. The children delivered by cesarean section were full-term, as well as in good health.

Diagnostic criteria

Atopic dermatitis was defined as proposed by Hanifin and Rajka (12), employing the modified criteria for young infants (13). Asthma was defined as three or more episodes of bronchial obstruction, each time verified by a physician. Allergic rhinoconjunctivitis was defined as rhinitis and/or conjunctivitis appearing at least twice after exposure to a particular allergen and not related to infection. Gastro-intestinal allergy implied vomiting and/or diarrhea on at least two separate occasions after intake of a certain offending food. Urticaria was defined as allergic if appearing at least twice within 1 h after exposure to a particular antigen.

The classification of the children with regard to atopic disease was done at the 18-month follow-up based on the results of the physical examination, interview with one or both parents, and the answers given in the questionnaires. In unclear cases, the records from well-baby clinics, primary care units, private pediatricians and the Department of Pediatrics were examined. The results of the laboratory data were not known at the time of the classification.

Skin prick tests

SPTs were performed in duplicate at 18 months, using fresh hen's egg white and cow's milk and standardized extracts of cat, birch, house dust mite (*Dermatophagoides farinae*), timothy grass (Solu-Prick SQTM, ALK, Hørsholm, Denmark), and histamine hydrochloride, 10 mg/ml as a positive control, and albumin reagent as a negative control. The size wheal was marked with a filter pen after 15 min and transferred onto a micropore tape for measuring. The SPTs were regarded as positive if the mean wheal diameter was at least 3 mm.

Flow cytometry

Twenty-five microliters of blood was incubated for 10 min at room temperature with 5 µl of murine mAbs to CD2 [T11; fluorescein isothiocyanate (FITC) labeled; Coulter Co., Hialeah, CA, USA], CD8 [T8; energy coupled dye (ECD: phycoerythrin, Texas Red, conjugate) labeled; (Coulter Co.)], CD4 [T4; ECD labeled; (Coulter Co.)], and 10 µl of CD28 [LEU28; PE labeled Becton Dickinson (BD), Stockholm, Sweden], CD3 [T3; ECD labeled; KEBO, Stockholm, Sweden], CD14 (FITC labeled; Dako, Copenhagen, Denmark), and CD45 [phycoerythrin (PE) labeled, Dako], as well as isotype, fluorochrome, and company matched-control antibodies, including 5 µl of mIgG1-ECD (Coulter Co.), mIgG2b-ECD, mIgG1-FITC (Coulter Co.), mIgG1-FITC/mIgG2a-RPE (Dako) and 10 µl of mIgG1 – PE (BD). The samples were distributed into tubes containing the combinations of CD45/CD14/CD3, CD8/CD2/CD28, CD4/CD28 and their corresponding isotypic controls. The erythrocytes were lysed with 1 ml of Ortho-mune Lysing Reagent[®] (Ortho Diagnostic Systems Inc., Raritan, NJ, USA) for 15 min at room temperature followed by centrifugation at 400 g for 5 min at 4°C. The pellet was resuspended in 1 ml of phosphate-buffered saline (PBS) containing 0.08% human serum albumin (Apoteksbolaget, Stockholm, Sweden) and 0.1%

paraformaldehyde (Sigma-Aldrich, Stockholm, Sweden). The cord blood samples were stained within 24 h (median: 12.3 h, range: 2.0–24 h) and then analyzed within 6 h. The 3-, 6-, 12- and 18-month samples were stained within 3.5 h and then analyzed within 4 h.

Flow cytometry was performed on an EPICS Profile cytometer (Coulter Co.). Flow cytometry was standardized with IMMUNOCHECK[®] (Coulter Co.) and STANDARD-BRITE[®] beads (Coulter Co.). The particle count and the white blood cell count (CD45+ cells) were obtained directly on the cytometer. A lymphocyte gate was set according to characteristic forward and side scatter. From each sample, flow data from 7500 lymphocytes were collected. Each sample was saved in an ungated version to list mode files, and analysis was performed using Flowmate[®] software (Dako A/S, Glostrup, Denmark). The percentage positive cells and fluorescence intensity (FI) were analyzed on a four-decade log scale. The FI correlates directly with the number of receptor molecules on the cell surface. Thus, variations in expression may reflect a functional difference.

To exclude contaminating erythrocytes, debris, and monocytes, samples with the combination CD3/CD14/CD45 were stepwise gated. CD45+ cells were gated in single histograms and further analysis was performed in a dot plot histogram with CD45 and CD14. The latter CD14+ cells (monocytes) were then excluded from the gate, which was further analyzed in a dot plot with a CD45 high/side-scatter low population (lymphocytes). Further analysis of the characterized lymphocytes was performed in a histogram, gating the CD3-positive cells. The final gate thus included a population of CD3+CD14-CD45+ cells, i.e. leukocytes expressing the pan T-cell marker. To establish a lymphocyte gate allowing analyses in the tube with CD2, backgating of CD3+ cells in the CD45/CD14/CD3 mAb sample was carried out. The lymphocyte gate showed > 96.0% CD3+ cells of the CD3+CD14-CD45+ cells (98.5 ± 1.0) (mean $\pm$ SD, $n = 10$). In addition, a leukocyte gate was established using CD45 mAb.

In the samples with CD2 mAbs, the established lymphocyte and leukocyte gate was set on forward and side scatter and the proportion of lymphocytes of leukocytes was obtained. The lymphocytes were further gated for CD2+ cells in a single histogram. The final gate thus included CD2+ lymphocytes representing T cells, and possibly some natural killer (NK) cells. The fraction of CD2+ lymphocytes in the lymphocyte gate, of all CD2+ cells in the sample, was > 94% (96.0 ± 1.4) (mean $\pm$ SD,

Table 1. Numbers ($10^9/l$) and proportions of CD2+, CD28+, CD8+CD28+, CD2+CD8+CD28 and CD4+CD28+ lymphocytes during the first 18 months of life

T-cell marker expression	Age (months)									
	0		3		6		12		18	
	n		n		n		n		n	
CD2+ ly ($10^9/l$)	2.6 ± 1.0	71	4.1 ± 1.3	55	4.1 ± 1.3	60	3.5 ± 1.4	63	3.2 ± 1.4	133
% CD2+ly/ly	61 ± 10	73	64 ± 7	57	66 ± 8	63	66 ± 7	64	66 ± 7	133
CD2 FI on CD2+ ly	10.6 ± 2.8	71	8.0 ± 1.4	57	7.9 ± 1.3	62	7.6 ± 1.3	64	8.2 ± 1.4	133
CD28+ ly ($10^9/l$)	2.1 ± 0.8	71	3.7 ± 1.5	55	3.1 ± 1.3	58	3.2 ± 1.2	63	2.8 ± 1.2	133
% CD28+ly/ly	50 ± 12	73	58 ± 8	57	60 ± 8	63	62 ± 10	64	59 ± 8	133
CD28 FI on CD28+ ly	9.0 ± 2.3	73	8.2 ± 1.2	57	7.4 ± 1.4	63	7.3 ± 1.6	64	8.0 ± 1.9	132
CD8+CD28+ ly ($10^9/l$)	0.5 ± 0.2	71	1.0 ± 0.8	55	0.8 ± 0.3	58	0.8 ± 0.5	63	0.8 ± 0.5	132
% CD8+CD28+ly/CD8+ ly	67 ± 14	73	85 ± 13	57	87 ± 11	63	89 ± 14	63	87 ± 13	130
CD28 FI on CD8+CD28+ ly	5.4 ± 1.6	73	5.6 ± 1.6	58	5.8 ± 1.3	63	6.4 ± 2.0	63	6.8 ± 2.5	133
CD2+CD8+CD28+ ly ($10^9/l$)	0.5 ± 0.4	71	0.9 ± 0.3	54	0.9 ± 0.4	60	0.9 ± 0.5	62	0.8 ± 0.5	133
% CD2+CD8+CD28+ly/CD2+ ly	21 ± 9	73	22 ± 6	56	23 ± 9	63	25 ± 8	62	26 ± 9	131
CD28 FI on CD2+CD8+CD28+ ly	5.4 ± 1.6	73	5.6 ± 1.5	60	5.8 ± 1.3	60	6.4 ± 2.0	62	7.0 ± 2.5	129
CD4+CD28+ ly ($10^9/l$)	1.3 ± 0.6	70	2.5 ± 1.1	60	2.5 ± 0.8	62	2.1 ± 0.8	62	1.6 ± 0.6	129
% CD4+CD28+ly/CD4+ ly	98 ± 2	71	99 ± 1	62	99 ± 1	67	99 ± 4	62	99 ± 0.5	129
CD28 FI on CD4+CD28+ ly	11.4 ± 1.2	71	10.2 ± 2.0	62	9.7 ± 1.8	67	99 ± 2.1	62	11.2 ± 1.7	129

The fluorescence intensities (FI) on CD2 and CD28 are also given. Mean ± SD; n: number.

n = 10). The lymphocyte gate was then further gated in a dot plot with CD8 and CD28. Thereafter, the gate of CD2 positive cells was transferred to the CD8/CD28 dot plot for analysis of CD2+CD8+ CD28+ cells. The tubes containing CD4/CD28 were analyzed in dot plots in a similar way.

The results were expressed as the proportion of positive cell subsets, see Table 1, and the absolute numbers (10^9 cells/l) were calculated. CD3 data have previously been published (14). For each population, the corresponding FI was obtained. The CD2 and CD28 FI of the samples were not normally distributed. Thus, CD2 and CD28 mode FI were used.

Statistics

The unpaired *t*-test was used for normally distributed data. The Mann–Whitney *U*-test was used for data that was not normally distributed. The Student's paired *t*-test was used for comparison of data over time (0–3 months, 3–6 months, 6–12 months, 12–18 months and 0–18 months). Correlations over time were analyzed with Pearson's correlation. $p < 0.05$ was considered to be significant. The Kruskal–Wallis ANOVA by ranks was performed to compare data from children with different atopic family history (single, double and no). Calculations were performed using a statistical package: STATISTICA™ 5.1 for Windows (StatSoft®, Tulsa, OK, USA).

Ethics

The study was approved by the regional Ethics Committee for Human Research at the Linköping University Hospital.

Results

Clinical diagnosis and allergic sensitization

Atopic disease developed in 31 children within the first 18 months of life and 118 children were non-atopic. In addition, 21 children fulfilled most, but not all the criteria for atopic disease, and were excluded from comparisons between atopic and non-atopic children. Two children were excluded as the families did not comply to follow-up during the entire study period. Thus, 170 of 172 children participated through out the study. Another two children did not attend the follow up at 18 months and their mothers were thus interviewed over the telephone. They were both healthy according to questionnaires and the interviews. All of the atopic children had eczema, four of them also had asthma, and two had urticaria.

SPTs were performed at 18 months in 160 children. A positive SPT was verified in 13 (42%) of the atopic children, in 2 (2%) of the non-atopic children and in 3 (14%) of the children who did not fulfill all the criteria for atopic disease. The 18 children were SPT positive to egg, and two of them also to cow's milk and one to cat allergen.

When relating the studied variables to atopy, we compared atopic children (cumulative history of atopic disease within the first 18 months of life) and non-atopic children (without atopic symptoms during the first 18 months of life), and atopic SPT-positive children with or without a positive AFH were compared with non-atopic children with a negative SPT and no AFH.

Age-dependent changes in CD2 and CD28 expression

Data of all children are shown in Table 1. In addition, individually age-dependent comparisons were performed. Only significant changes are reported below. Cross-references to the data shown in Table 1 are noted.

The absolute number of CD2+ lymphocytes (line 1, Table 1) increased from birth up to 3 months of age, was unchanged from 3 to 6 months, and then followed by a decrease up to 18 months of age. In contrast, the CD2 FI (line 3) decreased from birth to 6 months of age, but then increased from 12 to 18 months of age. The number (line 1) and proportion (line 2) of CD2+ lymphocytes were higher at 18 months than at birth, whereas the CD2FI (line 3) was lower.

The number of CD28+ lymphocytes, in both the CD4+ (line 13) and CD8+ subsets (line 7), increased from birth to 3 months of age, followed by a decrease from 3 to 18 months age. It was, however, still higher at 18 months than at birth. The proportion of CD28+ lymphocytes (line 5) increased over the first 6 months of life, and was then stable during the study period. Similarly, the proportion of CD8+CD28+ lymphocytes of CD8+ lymphocytes (line 8) increased the first 3 months, and then remained stable. Almost all CD4+ lymphocytes expressed CD28 both at birth and at 18 months of age (line 14). The CD28 FI on CD28+ lymphocytes (line 6) was similar during the first 3 months of life and then decreased from 3 to 6 months. It was still lower at 18 months than at birth. The CD28 FI on CD28+CD4+ lymphocytes (line 15) decreased during the first 6 months of life, was then stable from 6 to 12 months of age, followed by an increase from 12 to 18 months of age. The CD28 FI was similar at 18 months and at birth. In contrast, the CD28 FI on CD8+CD28+ lymphocytes (line 9) was higher at 18 months than at birth.

The proportion of CD2+ lymphocytes at 3 months correlated with the proportion of CD2+ lymphocytes at 6, 12 and 18 months (Pearson's correlation, $r = 0.37\text{--}0.46$, $p < 0.01\text{--}p < 0.001$), and the CD2 FI on CD2+ lympho-

cytes at 6 months correlated with the CD2 FI on CD2+ lymphocytes at 12 and 18 months of age ($r = 0.39$, $p < 0.01$ and $r = 0.37$, $p < 0.01$, respectively). The CD28 FI on CD8+CD28+ lymphocytes at 18 months of age did not correlate to this variable at the lower ages (data not shown).

CD2 and CD28 expression in relation to atopic disease

The proportion of CD2+ lymphocytes was lower in the atopic children ($n = 27$) than in the non-atopic children ($n = 90$), [$63\% \pm 7.1$ vs. $66\% \pm 7.2$ (mean $\pm$ SD), $p = 0.03$] at 18 months of age. Furthermore, the proportion of CD2+ lymphocytes in SPT positive children with atopic symptoms tended to be lower at 3 months of age and was lower at the age of 18 months than in non-sensitized children with neither atopic symptoms nor AFH (Fig. 1). The former children also had a lower CD2 FI at 18 months than children without signs of atopy [7.5 ($6.6\text{--}7.5$) vs. 8.0 ($7.5\text{--}9.9$), (median (range)), $p = 0.03$].

The number, the proportion nor the CD28 expression of CD28+ lymphocytes were related to atopic symptoms alone at any age (data not shown). At 18 months of age, however, SPT-positive children with atopic symptoms showed a higher CD28 FI on CD2+ CD8+CD28+ lymphocytes than non-sensitized children with neither atopic symptoms nor AFH (Fig. 2a). The CD28 FI on CD28+CD4+ lymphocytes was not related to atopic symptoms and sensitization (Fig. 2b). Thus, the difference in CD28 FI seems to be related to the CD8+ and not the CD4+ cell population.

The CD2 and CD28 expression, including the subpopulations reported in Table 1, was similar in children with single, double or no AFH (data not shown).

Discussion

Our findings of an increasing number and proportion of CD2+ lymphocytes with age support previous cross-sectional studies (15, 16). Furthermore, it is well known that neonatal cells stimulated with PHA display a decreased proliferation, as well as a reduced production of cytokines compared with adults (1,17). PHA activates lymphocytes via CD2 and the T-cell antigen receptor (18). In contrast to increasing numbers and proportions of CD2 with age, we found that the CD2 FI on CD2+ lymphocytes decreased during the first 6 months of age, and then increased from 12 to 18 months of age.

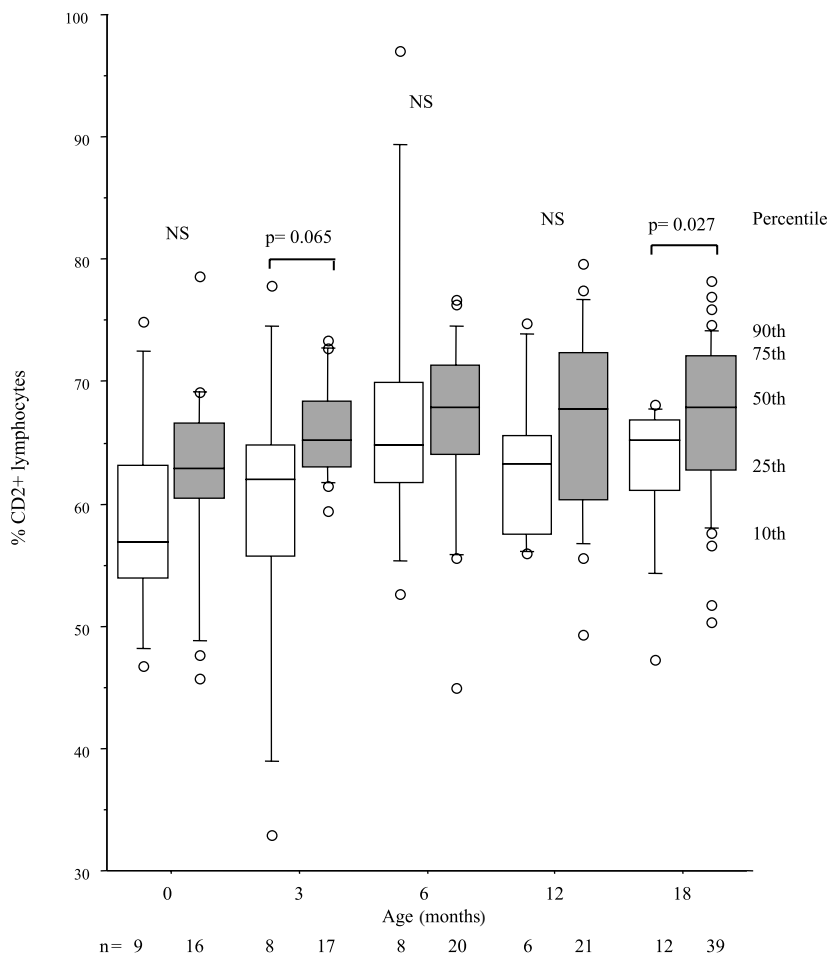


Fig. 1. Proportion of CD2+ lymphocytes at birth, 3, 6, 12 and 18 months of age in atopic skin prick test (SPT)-positive children with or without an atopic family history (AFH) (empty box plots) and non-atopic SPT-negative children with no AFH. The 10th, 25th, 50th, 75th and 90th percentiles are indicated, as well as outliers. $p < 0.05$ was considered to be significant.

Age-dependent changes in CD2 FI have previously not been reported. Altogether, it is likely that CD2 function increases with age.

Atopic children have lower numbers of lymphocytes forming rosettes with sheep erythrocytes and a reduced responsiveness to PHA, as compared with non-atopic children (reviewed in 1). Furthermore, peripheral blood mononuclear cells (PBMC) from atopic 12-year-old children express less IL-12R β_2 mRNA than non-atopic children after stimulation via the CD2 pathway (PHA) (19). We have previously observed that atopy is associated with a low proportion of CD2+ lymphocytes (2), accompanied by a reduced PHA responsiveness at 18 months of age and now confirm this observation in a considerably larger group of children, as well as a similar trend at 3 months of age. In addition, a lower CD2 fluorescence intensity of atopic children with positive SPT at 18 months is observed. The non-significant differences at lower ages may be due to a type II error, as the median and quartiles were lower in the atopic than in the non-atopic children at all ages

(Fig. 1). Furthermore, the proportion of CD2+ly at 3 months correlated with the proportion of CD2+ly at 6, 12 and 18 months, and the CD2 FI on CD2+ lymphocytes at 6 months correlated with the CD2 FI on CD2+ lymphocytes at 12 and 18 months of age. Collectively, this supports our hypothesis of a decreased CD2 function in atopic children. This is in agreement with findings of a lower cell proliferation after PHA stimulation at birth in children before the onset of atopic disease (20). Thus, a reduced CD2 function may be a primary defect.

In contrast, Lilja and co-workers who followed 60 children prospectively, reported a higher absolute number of CD2+ cells at birth, but not at 2 and 6 months of age in atopic ($n = 11$) than in non-atopic ($n = 49$) children. In agreement with our study, the proportion of CD2+ cells was similar, however (21). Lilja did not study CD2 after the age of 6 months. CD2 is the receptor for sheep erythrocytes (18). Lower numbers of lymphocytes forming rosettes with sheep erythrocytes has been reported at birth in children with atopic heredity (22).

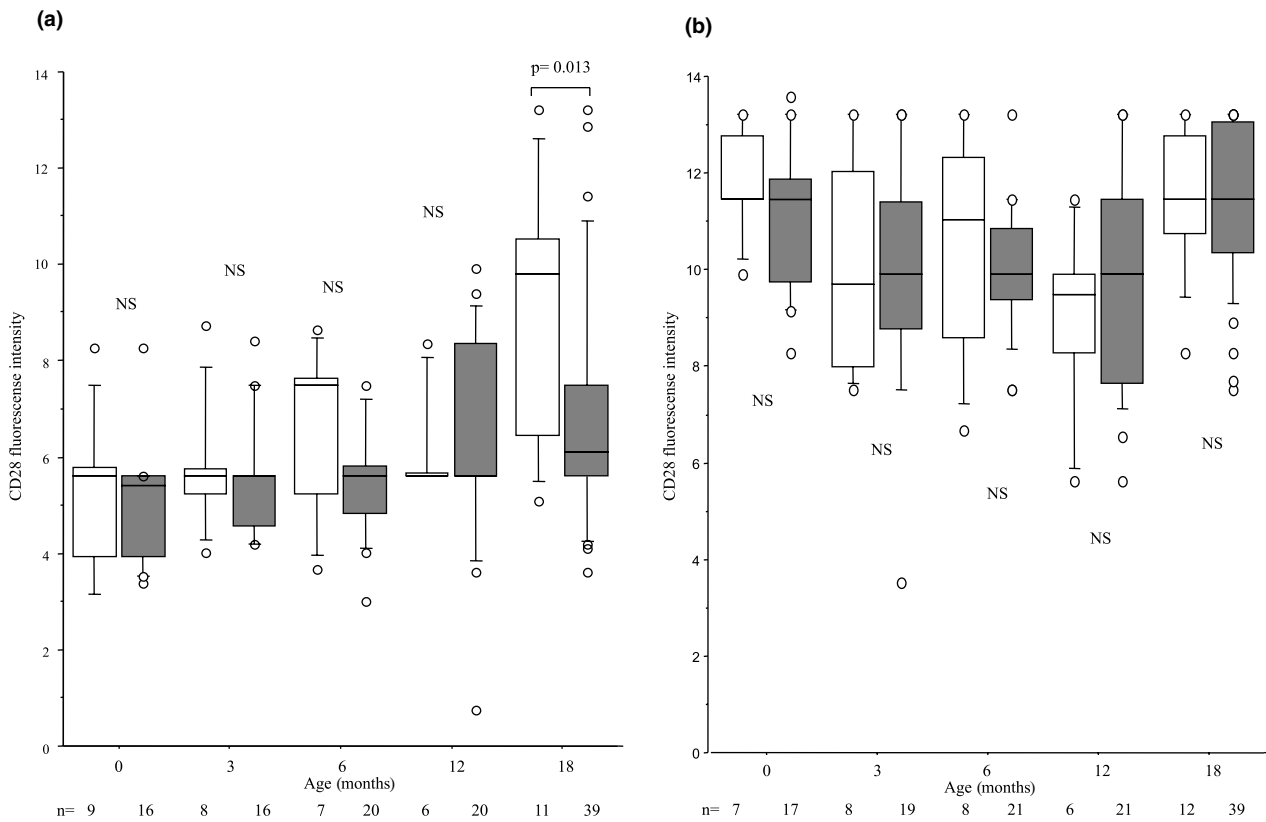


Fig. 2. (a) The expression of CD28 (measured as mode fluorescence intensity) on CD2+CD8+CD28+ lymphocytes at birth, 3, 6, 12 and 18 months of age in atopic skin prick test (SPT)-positive children with or without an atopic family history (AFH) (empty box plots) and non-atopic SPT-negative children with no AFH. The 10th, 25th, 50th, 75th and 90th percentiles are indicated, as well as outliers. $p < 0.05$ was considered to be significant. (b) The expression of CD28 (measured as mode fluorescence intensity) on CD4+CD28+CD28+ lymphocytes at birth, 3, 6, 12 and 18 months of age in atopic skin prick test (SPT)-positive children with or without an atopic family history (AFH) (empty box plots) and non-atopic SPT-negative children with no AFH. The 10th, 25th, 50th, 75th and 90th percentiles are indicated, as well as outliers. $p < 0.05$ was considered to be significant.

A reduced CD2 function in atopic children could have important consequences, as CD2 receptor stimulation enhances the responsiveness (proliferation and IFN- γ production) of activated T cells to IL-12 (5). We have recently observed that stimulation of PBMC with monoclonal antibodies to CD2 resulted in an up-regulation of IL-12R β_2 mRNA expression in healthy adults (unpublished observations). Moreover, CD2 enhances the interaction between the MHC and the T-cell receptor via binding to LFA-3 on the antigen-presenting cell (3), and thereby Th1-like immunity is promoted (4). In addition, Th1 cells express higher levels of CD2 mRNA than Th2 cells (23). Thus, it is tempting to speculate that a low CD2 expression could lead to a failure to down-regulate Th2 responses.

Virtually all CD4+ cells expressed CD28 from birth to 18 months, while the proportion of CD8+ cells expressing CD28 was lower despite an increase in the first 3 months of life. Thus, the age-dependent changes in CD28 expression dur-

ing the first 18 months mainly took place within the CD8 cell subset, possibly reflecting the normal antigen response and T-cell maturation.

No previous study has followed the changes in CD28 expression in a large group of healthy children during the first 18 months of life. Earlier studies have reported results from a wide age span or smaller numbers of children and are thus difficult to compare with ours. Hassan and co-workers (24) reported a similar CD28 fluorescence intensity on naive and memory CD4+ cord blood cells and adult cells, which may support our findings. Another study reported a decreasing proportion of CD8+CD28+ lymphocytes comparing newborns (aged 1–4 days) with children in an age group range from 2–11 years and with adults (25). After the age of 50, the number of CD8+CD28+ T cells dramatically decreases (26). CD8+CD28- T cells are reported to be antigen experienced and more functionally differentiated cells than the CD8+CD28+ T cells, with a high capacity for IFN γ production (26).

At 18 months of age, sensitized children with atopic symptoms showed a higher CD28 FI than non-sensitized children without atopic symptoms. These findings may be a consequence of a Th2 dominance. Several studies show that CD28 stimulation is critical for Th2 differentiation, although contradictory findings have been reported (9). During antigen stimulation of T cells from transgenic mice expressing a TCR specific for ovalbumin, increasing CD28 ligation resulted in increased production of IL-4 and IL-5 (7). Human naive cord blood CD4⁺ T cells are not primed to produce Th2 cytokines in the absence of CD28 costimulation, and consequently default to the Th1 subset, independent of the presence of exogenous cytokines (8). Stimulation via CD28 elicits Th2 differentiation of naive CD4⁺ T cells particularly efficiently in the context of low TCR signaling (27). In addition, CD28-deficient mice have preserved Th1-mediated cytotoxic T lymphocyte and cellular immunity, accompanied by low basal serum levels of the Th2-dependent IgG₁ isotype and high levels of the Th1-dependent IgG_{2a} isotype (10). In another model, using transgenic mice expressing a TCR specific for ovalbumin, CD28-deficient mice displayed reduced IL-4 and proliferative responses to ovalbumin, while IFN- γ production was normal (11).

Surprisingly, we did not find any differences in CD28 expression within the CD4⁺ cell subset between atopic and non-atopic children. This may be due to the fact that we studied resting peripheral cells and other investigators performed studies on activated cells. However, the ability of CD8⁺ T cells to regulate the production of IgE is a well-recognized example of T-cell mediated suppression (reviewed in 28), although the mechanisms are unclear. Furthermore, a study of adult venom allergic patients, reacting to hymenoptera stings, and controls showed a higher proportion of CD28⁺ cells within the CD8⁺, but not the CD4⁺ subset of the patients compared with controls (29). Interestingly, CD8⁺CD28⁻ T cells are good producers of IFN- γ (26) and have been suggested to have immunoregulatory properties (30). Moreover, IL-4 prevents loss of CD28 expression on CD8⁺ T cells (31). Thus, the Th2 dominance observed in atopic disease may cause a slower generation of CD8⁺CD28⁻ cells. Furthermore, it is tempting to speculate that the increased expression of CD28 on CD8⁺ T cells in atopic children may reflect an impaired immunoregulatory function of the CD8⁺ T-cell subset.

Although we have not studied cytokine responses prospectively in these children, other studies have shown delayed maturation of Th1

responses and consolidation of Th2 responses in atopic children. Prescott and coworkers reported lower PHA-induced production of IFN- γ by cord blood mononuclear cells from children who were atopic at age 2 than in non-atopic infants (32), suggesting an inability to increase IFN- γ during the early postnatal period as being a crucial defect in atopic development. They also showed an age-dependent decline in non-atopic Th2 responses to inhalant allergens, and a reciprocal pattern in those who developed atopy at the age of 2 years. Another study (33) reported that children developing atopy by the age of 12 months had lower house dust mite-induced IFN- γ at birth compared with non-atopic children. By the age of 12 months, atopic children showed a Th2 cytokine profile, accompanied by an impaired allergen-induced production of the anti-inflammatory cytokine IL-10 (33).

In conclusion, the observed reduced expression of CD28 in atopic infants may support previous findings that atopy is associated with a reduced CD28-function. The high CD28 FI in atopic children may possibly be a consequence of a Th2-skewed immune system.

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