

Reduced levels of soluble CD14 in atopic children

H. A. Zdolsek and M. C. Jenmalm

Department of Molecular and Clinical Medicine, Division of Paediatrics, and Clinical Research Centre, Faculty of Health Sciences, Linköping University, Sweden

Summary

Background A reduced microbial stimulation has been reported as a reason for the increasing prevalence of atopic diseases in industrialized countries. Antigen-presenting cells (APC), responding to microbial signals by pattern recognition receptors such as CD14, have an important role in the development of the Th1/Th2 balance.

Objective We hypothesized that atopic children have a lower expression of CD14 on monocytes and lower soluble CD14 levels (sCD14).

Methods Seventy-six children were followed prospectively from birth and signs of atopic disease were evaluated. The expression of CD14 on monocytes was analysed with flow cytometry at 0, 3, 6, 12 and 18 months. Circulating levels of sCD14 were analysed by ELISA and total IgE was analysed by fluoroenzymo immunoassay at these ages, and in a subgroup, followed up at 7 years.

Results Levels of sCD14 were reduced at 7 years both in children with a current or a cumulative history of atopy compared to non-atopic children with $P = 0.002$ and 0.001 , respectively. Sensitized children with atopic symptoms had lower sCD14 at 3 and 18 months and at 7 years of age than non-atopic non-sensitized children with $P = 0.023$, 0.039 and 0.008 , respectively.

Conclusion The lower levels of sCD14 observed in atopic children may be a consequence of an atopic family heredity and/or atopic disease, but it may also reflect a reduced capacity to respond to microbial signals.

Keywords antigen-presenting cells, atopic disease, CD14(m), childhood, soluble CD14

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Introduction

Immune responses to allergens are regulated by competing T helper (Th)1 and Th2 cell populations (reviewed in [1]) and atopic disease is associated with Th2-skewed responses [1]. A Th2 skewness may also be seen in new-borns [2], including immune responses to allergens [3]. Factors stimulating the immune system, e.g. from microbes, may be needed to redress and regulate the fetal and new-born Th1/Th2 imbalance during infancy and early childhood [4]. One such factor is endotoxin, the lipopolysaccharide (LPS) component of the outer membrane of Gram-negative bacteria. Low levels of endotoxin in house dust was associated with a high prevalence of skin prick test (SPT) reactivity in wheezing American children [5]. Furthermore, we have recently observed that house dust endotoxin levels were inversely related to the development of atopic disease and sensitization in Swedish children followed prospectively [6]. A decreased risk of atopic dermatitis, but increased risk of respiratory infections during the first 6 months of life was noted in

children with high exposure of endotoxin in dust from mother's mattresses [7].

Antigen-presenting cells (APC), responding to microbial signals by evolutionary conserved molecules, the so-called pattern recognition receptors [8], have an important role in the development of the Th1/Th2 balance [9]. One well-characterized pattern recognition receptor is CD14, which is expressed on myeloid cells, primarily on monocytes [10]. It binds lipopolysaccharides (LPS) and other bacterial wall-derived components (reviewed in [11, 12]). Toll-like receptor 4 (TLR4) then transduces a signal into the APC [8], resulting in high secretion of IL-12. This cytokine in turn promotes Th1 responses [13, 14]. Soluble CD14 (sCD14), which is derived from cell surface CD14 (reviewed in [11]), facilitates the interaction of membrane CD14 negative cell populations, such as epithelial cells and dendritic cells, with LPS [15]. However, it is not yet known, whether monocytes and macrophages are the major sources of sCD14, since hepatocytes have also been shown to produce sCD14 [16, 17].

It has been hypothesized that variations in the promoter region of the CD14 gene might alter the expression of CD14 [18], possibly influencing the capacity to respond to microbial stimulation. Further, IL-4 production is inhibited by sCD14 [19], and sCD14 can inhibit IgE production in an LPS-independent fashion [20]. A lower LPS responsiveness in adults has been reported [21] with asthma than in healthy

Correspondence: Helena Aniansson Zdolsek, Department of Molecular and Clinical Medicine/Division of Paediatrics, University Hospital, S-581 85 Linköping, Sweden.
E-mail: helzd@imk.liu.se

subjects. Jones et al. [22] reported that low maternal sCD14 levels in breast milk collected 3 months *post partum* were associated with eczema in the infants by 6 months of age.

We hypothesized that atopic children have a lower expression of CD14 on monocytes and lower circulating levels of APC-derived sCD14. The aim of this study was therefore to investigate the expression of the surface antigen marker CD14 on monocytes during the first 18 months in relation to development of atopic disease. In a sub-group of children followed up at 7 years, circulating sCD14 and total IgE levels were also analysed.

Materials and methods

Subjects

The children in this study were selected from a prospective study in Linköping originally including 170 children followed from birth to 18 months of age, as described previously [23]. A family history of atopy (AFH) in the immediate family, i.e. parents and siblings of the baby, was obtained through two interviews, the first at the beginning of the study and the second 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.

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.

For this study, we chose 77 (31 atopic and 46 non-atopic) from the original cohort [23] who had a clear cumulative history of atopic disease at 18 months of age. In addition, the non-atopic children included had no atopic family history (AFH). The data of one atopic child was excluded due to congenital hypothyreosis, which possibly could have inter-

fered with the study. A flow chart over the investigation of the children is shown in Fig. 1.

Thus, 46 non-atopic and 30 atopic children were selected. At 18 months, all atopic children had a cumulative history of atopic dermatitis. Four of them also had asthma, and 2 had urticaria. Four of the atopic children had no AFH, 10 single AFH and 16 double AFH.

At the age of 7 years, 54/76 children were invited to a follow up (see flow chart in Fig. 1). The criteria for selection were: the availability of separated peripheral blood mononuclear cells (PBMC) at 18 months of age, and a clear atopic ($n = 25$) or non-atopic ($n = 29$) cumulative history of atopic disease at this age. These children also participated in a cross sectional study at 18 months [24] and were found to be proper representatives of the cohort and for economical and practical reasons, we chose the same children to be followed up at 7 years.

Venous blood samples were obtained at birth, 3, 6, 12, 18 months, and at 7 years. The blood was drawn into EDTA-containing tubes and into serum collection tubes. An aliquot of the blood was used for flow cytometry performed on fresh blood. Serum and plasma samples were frozen at -20°C . Missing samples were due to birth during weekends when research laboratory facilities were unavailable, difficulties with drawing the blood sample, and technical failure in the laboratory (including computer saving problems of flow cytometry analyses on data list mode files). Four cord blood samples were excluded from analysis due to perinatal complications (including neonatal respiratory distress and/or <37 or >42 gestational weeks).

The 72 cord blood samples used for the statistical analysis were thus from healthy, full-term babies delivered vaginally ($n = 66$) or after caesarean section ($n = 6$) of which four were planned due to maternal reasons and two was acute due to risk of asphyxia of the baby. However, the children delivered with caesarean section were full-term, as well as well-being.

Diagnostic criteria

The classification of the children with regard to cumulative atopic disease was done twice, at 18 months and at 7 years. At 7 years of age, children with atopic symptoms within the last 12 months were defined as having 'current symptoms'.

The following definitions of the various atopic diseases were used:

Allergic rhinoconjunctivitis (ARC) = rhinitis and/or conjunctivitis appearing at least twice after exposure to a particular allergen and not related to infection.

Gastro-intestinal allergy = vomiting and/or diarrhoea on at least two separate occasions after intake of a certain offending food.

Urticaria was regarded as allergic if appearing at least twice within 1 h after exposure to a particular antigen.

At the age of 18 months, atopic dermatitis was defined as proposed by Hanifin and Rajka [25], employing the modified criteria for young infants [26] with one exception: the criterion of having a family history of atopic disease was not used. At the age of 7 years, atopic dermatitis (AD) implied persisting or relapsing itching rash that was intermittent for at least 6 months.

The definition of asthma bronchiale (AB) at 18 months implied three or more episodes of obstructive bronchitis, each

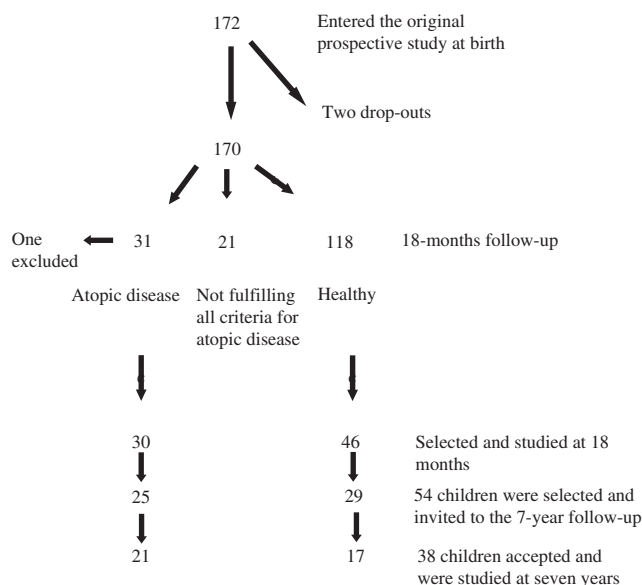


Fig. 1. Flow chart of the original cohort and the children participating in this study.

episode verified by a physician. At 7 years, AB implied at least one episode of obstructive bronchitis after the age of 2 years verified by a physician together with an AB diagnosis in the examined records.

The classification at 18 months of age was based on a medical examination performed by the same physician (H.Z.); an interview with one or both parents and answers given in questionnaires mailed to the families at 3, 6, 12 and 18 months of age.

At the 7-year follow-up, the children were investigated by an experienced research nurse and the parents were asked questions about any on-going atopic symptoms. The research nurse scored the severity of atopic eczema according to the scoring atopic dermatitis (SCORAD) index [27]. A parent answered questionnaires focusing on allergic symptoms after the age of 18 months including similar questions regarding symptoms within the last year. The classification of the children was done by the same physician as at the 18-month follow-up.

Both at the 18-month and the 7-year follow-up, the records from primary care units, private paediatricians and the Paediatric Clinic were examined if the parents answered that their child had been seen by a physician, due to asthma, wheezing, skin problems, urticaria or any possible allergic reaction. The results of the laboratory data (except for the SPT, 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*) and timothy grass, (Solu-Prick SQTM, ALK, Hørsholm, Denmark). Single SPTs at 7 years were performed using standardized extracts of cat, dog, horse, birch, timothy grass, mugwort, house dust mites (*D. farinae* and *pteronysinus*) and mould (*Cladosporium herbarum* and *Alternaria*) (Solu-Prick SQTM, ALK, Hørsholm, Denmark).

Histamine hydrochloride, 10 mg/mL was used as the positive, and albumin reagent as a negative control. The weal size 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 weal diameter was at least 3 mm. Antihistamines were not taken within three days before the SPT was performed.

sCD14 analysis

sCD14 in serum or plasma was detected with ELISA according to the manufacturer's instructions (R&D Systems, Abingdon, UK). Blood samples were assayed at a dilution of 1/200 (cord blood) and 1/500 at other ages. Pilot studies in seven adults showed similar levels of sCD14 in serum and plasma, as well as similar levels of sCD14 after three times of thawing/freezing.

IgE antibody analysis

Total serum IgE levels (kU/L) were analysed with a fluoroenzyme immunoassay, UniCAP[®], according to the recommendations of the manufacturer (Pharmacia Diagnostics, Uppsala, Sweden).

Flow cytometry

Twenty-five microlitres of blood was incubated for 10 min at room temperature with 5 µL of murine mAbs to CD14 (FITC labelled; Dako, Copenhagen, Denmark) and CD45 (phycoerythrin (PE) labelled, Dako), as well as isotype, fluorochrome, and company matched-control antibodies, including IgG1-FITC/IgG2a-RPE (Dako). The samples were distributed into tubes containing the combinations of CD45/CD14, and their corresponding isotypic controls. The method used is described in detail elsewhere [23]. Each sample was saved in an ungated version to list mode files, and analysis was performed using Flowmate[®] software (Dako A/S, Glostrup, Denmark). To exclude contaminating erythrocytes and debris, samples with the combination CD14/CD45 were stepwise gated. CD45⁺ cells (leucocytes) were gated in a single histogram and further analysis was performed in a histogram with CD45⁺CD14⁺ cells. The final gate thus included CD45⁺CD14⁺ cells representing monocytes, and possibly some neutrophil granulocytes.

The results were expressed as the proportion of leucocytes (see Table 2), and the absolute numbers (10⁶ cells/L) were calculated. The corresponding fluorescence intensity (FI) was obtained. The CD14 FI of the samples were not normally distributed on a histogram. Thus, CD14 mode FI and not mean FI was used.

Statistics

As the data were not normally distributed, non-parametric tests were used. Comparison between unpaired groups were analysed with the Mann-Whitney *U*-test. The Wilcoxon paired test was used for comparison of data over time (0–3 months, 3–6 months, 6–12 months, 12–18 months, 0–18 months, 18 months–7 years, birth–7 years). Correlations were analysed with Spearman's rank order correlation coefficient test. The Kruskal-Wallis ANOVA by ranks was performed to compare data from children with different atopic family history (single, double and no), and the Mann-Whitney *U*-test for comparison of data from children with double or no AFH. *P* < 0.05 was considered to be significant. Calculations were performed with a statistical package STATISTICATM 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.

Results

Clinical diagnosis and allergic sensitization

A flow chart of the original cohort earlier described [23] including the children participating in this study is shown in Fig. 1.

Thirty-eight children attended the clinical follow-up at the age of 7 years of whom 21 had a cumulative history of atopy at 18 months. The symptoms of atopic disease and the SPT results of children classified as atopic are shown in Table 1. Seventeen children had not shown any atopic symptoms at 18

Table 1. Symptoms and skin prick test (SPT) results of children classified as atopic, of whom 30 were followed up to 18 months of age and 21 to 7 years of age

Cumulative atopic disease at 18 months, <i>n</i> = 30	30/30 AD, 4/30 AB, 2/30 urticaria
Cumulative atopic disease at 18 months in children followed up to 7 years, <i>n</i> = 21	21/21 AD, 4/21AB, 2/21 urticaria
Cumulative atopic disease at 7 years, <i>n</i> = 21	21/21AD, 8/21AB, 9/21ARC
Current atopic disease reported last 12 months, <i>n</i> = 16/21	12/16AD, 8/16 ARC, 6/16AB, 1/16 urticaria
Symptoms of atopic disease reported between 18 months and seven years, <i>n</i> = 17/21	8/17 AB, 15/17 AD, 9/17 ARC
Cumulative SPT = SPT-positivity at 18 months and/or at 7 years	15/21 SPT ⁺ , 6/21 SPT ⁻
SPT-results at 7 years. 20/21 children were tested.	12/20 SPT ⁺ , 8/20 SPT ⁻

AB, asthma bronchiale; AD, atopic dermatitis; ARC, allergic rhinoconjunctivitis; cumulative atopic disease, cumulative history of atopic disease; current atopic disease, history of atopic symptoms within the last 12 months before the 7-year follow-up. At the 7-year follow-up 7/12 children with AD were classified as having on-going mild atopic eczema according to the SCORAD index.

Table 2. Numbers (10⁶/L) and proportions of CD14⁺ cells in relation to the development of atopic disease within the first 18 months of life (NA, non-atopics, A, atopics)

	Age, months									
	0		3		6		12		18	
	Median (p25–p75)	<i>n</i>	Median (p25–p75)	<i>n</i>	Median (p25–p75)	<i>n</i>	Median (p25–p75)	<i>n</i>	Median (p25–p75)	<i>n</i>
CD14 ⁺ cells (10 ⁶ /L)										
All children ^a	1070 (770–1360)	44	490 (420–670)	33	580 (440–620)	31	450 (370–600)	34	430 (290–520)	63
NA	940 (750–1320)	23	580 (350–670)	20	520 (430–610)	20	440 (340–560)	22	400 (310–500)	39
A	1140 (910–1410)	21	460 (420–510)	13	590 (450–620)	11	500 (380–670)	12	450 (290–533)	24
% CD14 cells/leucocytes										
All children ^b	8 (6–9)	45	5 (5–7)	33	5 (4–6)	32	6 (4–7)	34	5 (4–7)	63
NA	8 (6–9)	23	5 (5–7)	20	5 (4–6)	21	6 (5–6)	22	5 (4–6)	39
A	8 (7–9)	22	6 (5–6)	13	5 (5–6)	11	6 (4–7)	12	6 (5–7)	24
CD14 FI on CD14 ⁺ cells										
All children ^c	106 (87–133)	45	132 (114–155)	33	131 (120–156)	32	131 (126–150)	34	129 (109–134)	63
NA	115 (95–132)	23	132 (115–166)	20	132 (120–152)	21	132 (127–131)	22	120 (109–132)	39
A	105 (75–131)	22	131 (114–132)	13	147 (120–178)	11	135 (125–171)	12	133 (111–142)	24

The fluorescence intensities (FI) on CD14⁺ cells are also noted. No significant differences were seen between atopic and non-atopic children. ^{a,b,c}: Please refer to Results below for definitions.

months, and continued to be symptom free up to 7 years of age. At 7 years of age 2/21 atopic children had no AFH, 7/21 had single AFH and 12/21 had double AFH.

SPTs were performed at 18 months in 73/76 children. Positive tests were seen in 15 children, of whom 13 had a cumulative history of atopic disease.

At 7 years of age, SPTs were performed in 37/38 children of whom 25 were SPT negative and 12 were positive for one or several of the allergens tested. Sixteen children had a history of a positive SPT either at 18 months and/or at 7 years of age and 22 had not.

Age-dependent changes in CD14 membrane (*m*) expression

Data of all children are shown in Table 2. Individually age-dependent comparisons were performed. Only significant changes are reported as increasing or decreasing changes below. Cross-references to the data in Table 2 are noted.

The absolute number of CD14⁺ cells (monocytes) (cross-references ^a in Table 2) decreased from birth to 3 months of age, was unchanged from 3 to 12 months, and was then followed by a decrease to 18 months of age. Furthermore, the proportion of CD14⁺ monocytes (cross-reference ^b) decreased slightly from birth to 3 months of age, but was then similar. In contrast, the CD14 FI (cross-reference ^c) increased

from birth to 3 months of age, and was then similar up to 18 months of age.

CD14(*m*) expression in relation to atopic disease and total IgE levels

Neither the number, nor the proportion nor the CD14 FI expression of CD14⁺ leucocytes was related to atopic symptoms alone at any age (data not shown). Moreover, there was no relation to atopic symptoms together with sensitization (data not shown) or to atopic family history (data not shown). Total IgE did not correlate with CD14(*m*) expression in all children at any age.

sCD14 in relation to atopic disease and total IgE levels

The sCD14 levels increased from birth to 3 months of age, and remained unchanged to 6 months of age in both atopic (with a cumulative history of atopic disease at 18 months) and non-atopic children (Fig. 2). In atopic children, the sCD14 levels were similar between 6 and 12 months, whereas the levels increased in non-atopic children. Between 12 and 18 months, the levels remained unchanged in both atopic and non-atopic children, but then decreased to 7 years of age in both atopic (with a cumulative history of atopic disease at 7 years of age) and non-atopic children. However, the levels of

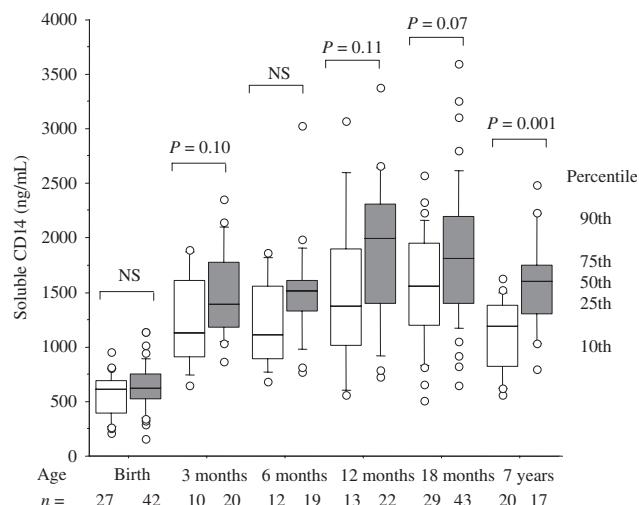


Fig. 2. Levels of serum soluble CD14 at birth, 3, 6, 12 and 18 months, and at 7 years of age in children with a cumulative history of atopy (empty box plots) and non-atopic children with no AFH. The 10th, 25th, 50th, 75th and 90th percentiles as well as outliers, are indicated. NS, non-significant.

sCD14 in all children were still higher at 7 years of age than at birth.

Children with a cumulative history of atopy tended to have lower sCD14 levels at 3, 12 and 18 months of age (Fig. 2), and the sCD14 levels were significantly lower at 7 years of age. In addition, 7-year-old children with current atopic symptoms had lower sCD14 levels than non-atopic children ($P = 0.002$). Moreover, the former had lower levels of sCD14 already at 3 and 12 months of age with $P = 0.04$ and 0.02, respectively, and tended to have lower levels than non-atopic children at birth, 6 and 18 months of age with $P = 0.09$, 0.09 and 0.10, respectively.

Sensitized children with cumulative atopic symptoms had lower sCD14 at 3 and 18 months, and at 7 years of age than non-sensitized non-atopic children, while the sCD14 levels were similar at birth, 6 and 12 months of age (Figs 3a and b). Both cumulative and current atopic airway and skin symptoms were associated with low levels of sCD14 (data not shown).

Total IgE did not correlate with sCD14 in all children at any age.

sCD14 in relation to atopic family history

sCD14 levels increased from birth to 3 months of age, and then decreased from 18 months to 7 years of age both in children with a double AFH and in children without AFH (Fig. 4). However, children without AFH had higher levels of sCD14 at 6 than at 12 months, whereas children with a double AFH had similar levels (Fig. 4).

Children with no, single or double AFH were compared at birth, 18 months and at 7 years of age and children with double and no AFH at 3, 6 and 12 months of age. At birth and at 6 months, the levels were similar, whereas children with a double AFH had lower sCD14 levels than children with no AFH at 3, 12 and 18 months, as well as at 7 years of age (Fig. 4). Moreover, children with double AFH had lower sCD14 than children with single AFH at 7 years of age (Fig. 4).

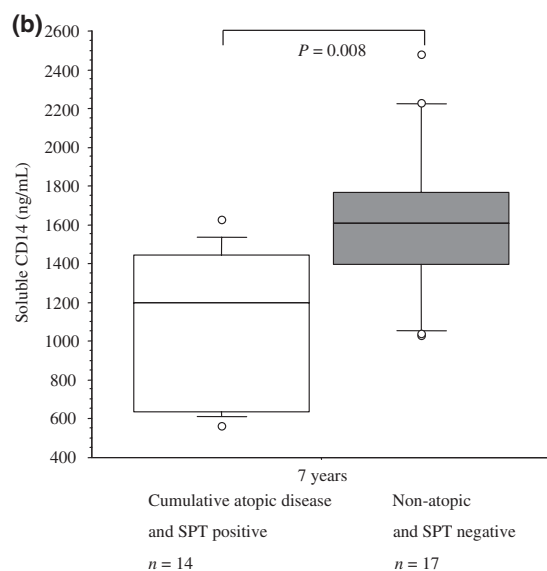
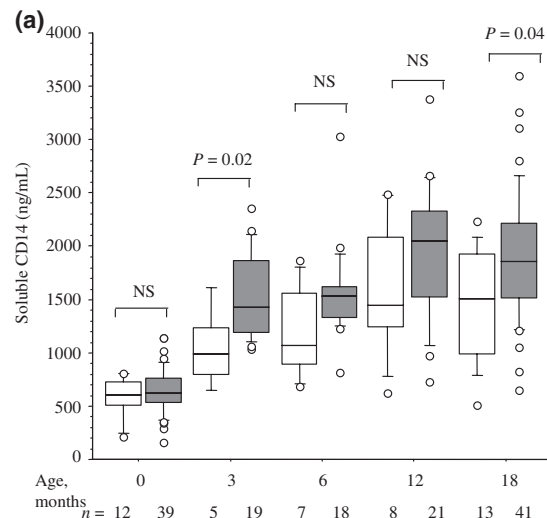


Fig. 3. (a) Levels of serum soluble CD14 at birth, 3, 6, 12 and 18 months in children with a cumulative history of atopy and a positive skin prick test (SPT) at 18 months (empty box plots) and non-atopic children with negative SPTs and no AFH. The 10th, 25th, 50th, 75th and 90th percentiles are indicated, as well as outliers. (b) Levels of serum sCD14 at 7 years of age in children with a cumulative history of atopy and a positive SPT at 18 months and/or at 7 years of age (empty box plots) and non-atopic children with negative SPTs and no AFH. The 10th, 25th, 50th, 75th and 90th percentiles as well as outliers, are indicated. NS, non-significant.

Children with or without maternal history of atopic disease showed similar sCD14-levels at birth, 3, 6 and 12 months. At 18 months however, a maternal family history of atopic disease was associated with a tendency to lower sCD14 levels, and at 7 years with significantly lower sCD14 levels ($n = 17$ vs. 55, $P = 0.07$ at 18 months and $n = 12$ vs. 25, $P = 0.03$ at 7 years). Neither did a paternal history of atopic disease influence sCD14 levels at birth, 3, 6 and 12 months. Lower sCD14 levels were observed in children with a paternal history of atopic disease at 18 months and 7 years vs. children without paternal history of atopic disease ($n = 12$ vs. 60, $P = 0.004$ at 18 months and $n = 7$ vs. 30, $P = 0.01$ at 7 years).

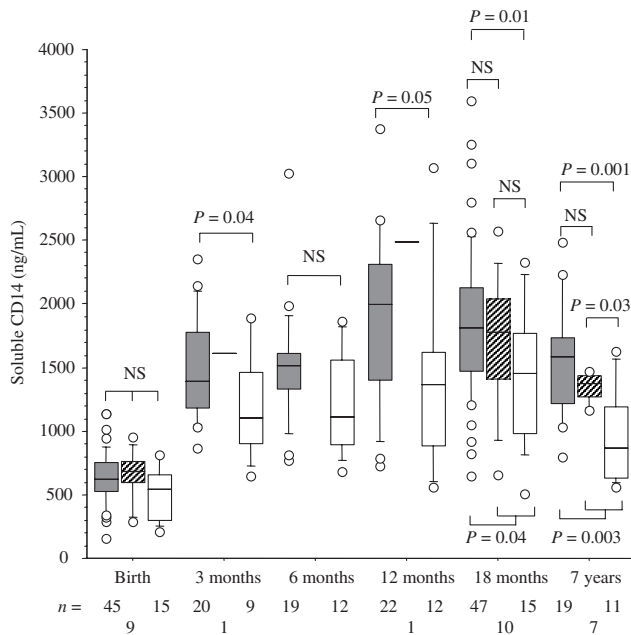


Fig. 4. Levels of serum soluble CD14 at birth, 3, 6, 12 and 18 months, and at 7 years of age in children with double atopic family history (AFH) (empty box plots), single AFH (striped box plots), and children with no AFH (filled box plots). The 10th, 25th, 50th, 75th and 90th percentiles as well as outliers, are indicated. $P < 0.05$ was considered to be significant. NS, non-significant.

Correlations between CD14(m) expression and sCD14

No significant correlations were seen between the proportion/ the number of CD14⁺ cells or the FI on CD14⁺ cells vs. sCD14 levels at any age.

Discussion

The levels of sCD14 increased during the first 18 months in all children, but were lower at the age of 7 years compared to 18 months. However, in contrast to non-atopic children, sCD14 levels did not increase between 6 and 12 months of age in children who developed atopic disease within the first 18 months of life. It is tempting to speculate that these findings may reflect a vulnerable period between 6 and 12 months with an impaired capacity to respond to microbial signals in children who subsequently develop atopy. Infections early in life have been suggested to protect against development of atopic disease, due to the Th2 inhibitory effect of the Th1-skewed host defence responses during infections [28].

Jones et al. [22] reported increasing levels of sCD14 from birth until the age of 5 years in children with high risk of developing atopy. They also found lower levels of sCD14 in adults compared to infants or children older than 4 months of age. Increasing levels of sCD14 with age were seen compared to levels of newborn, 2-years old children and their mothers [29]. Our findings do not disagree with the age-dependent data reported by these two studies. We did not study children at 2 years, 5 years of age or adults, however.

There are few studies investigating sCD14 levels in healthy children, although higher levels of sCD14 in serum of children aged 3–17 years than in adults have been reported [30].

The increase in soluble levels of CD14 in small children may enable increasing responsiveness to microbial products over age. During the lactation period, sCD14 is obtained via breast milk, however [31]. Interestingly, low levels of sCD14 in breast milk were associated with a diagnosis of AD by 6 months of age, irrespective of SPT reactivity [22]. Moreover, lower sCD14 levels in amniotic fluid were associated with subsequent development of AD and sensitization during the first year of life [22].

We found that children with a cumulative history of atopic disease tended to have lower sCD14 levels at 3, 12 and 18 months than non-atopic children. At 7 years of age, children with cumulative as well as current atopic symptoms had significantly lower sCD14 levels. Furthermore, using a more strict classification, sensitized children with a cumulative history of atopic disease had lower sCD14 levels than non-atopic, non-sensitized children, first seen at 3 months of age, and also at 12 months, 18 months and 7 years of age. This is in contrast to the Jones study [22], where the children were followed up to 5 years of age. They found higher plasma sCD14 levels at birth in children without asthma, but with a transient SPT positivity at 2 years, than non-sensitized, non-atopic children and children with asthma and a positive SPT at 2 and 5 years of age. However, levels of plasma sCD14 were similar in atopic and non-atopic children after birth [22]. In their study, only children with a positive atopic family history were followed up. We, however, compared healthy children without atopic family history with children who developed atopic disease. In addition, most of the atopic children in our study had a positive family history. Thus our design differed from the Jones study by comparing groups of children representing both ends of the scale. This may explain the differences to the Jones study.

Holmlund et al. [29] reported similar sCD14 levels at birth and at 2 years in a 2-year prospective study comparing 10 children with a positive SPT and a cumulative history of disease at 2 years with 17 SPT negative children with no symptoms. Their findings at birth were similar to our study. In contrast, we found lower sCD14 levels after birth in children developing atopic disease. The difference between Holmlund's and our data could be due to a smaller number of children followed up in their study compared to ours. The children developing atopic disease in our study also had a strong atopic family history since many of them had not only one but two parents or one parent and a sibling with a history of atopic disease.

In contrast to our study, Holmlund et al. [29] also reported higher sCD14 levels in atopic mothers compared to non-atopic mothers at the time of delivery. Interestingly 8/41 of the atopic mothers (20%) but only 4/32 (12%) of the non-atopic mothers in their study delivered with caesarean section. We hypothesize that a traumatic event such as delivery with a caesarean section could give rise to higher sCD14 levels. This may explain their findings of higher sCD14 in the atopic compared to non-atopic mothers at the time of birth, but similar levels 2 years later. Due to small numbers of children, statistically we were not able to compare data of children having an atopic mother, but no atopic father or sibling with children with no atopic family history at all.

Variations in the promoter region of the CD14 gene, i.e. a C-to-T transition at base pair –159 from the major

transcription start site, might alter the expression of CD14 [18], possibly influencing the capacity to respond to microbial stimulation. Baldini and co-workers reported higher serum sCD14 levels in TT homozygotes than in CC- homozygotes and CT heterozygotes. The TT polymorphism was associated with reduced total IgE levels in SPT⁺ schoolchildren. Moreover, sCD14 correlated with concanavalin A induced production of high IFN- γ and low IL-4 levels of PBMC [18]. In another study, adults with a CC homozygote phenotype had higher numbers of positive SPTs, as well as higher total serum IgE levels and more atopic symptoms than subjects with CT and TT alleles [32].

Despite the above-mentioned studies of the CD14 genotype, CD14(m) expression or sCD14 levels at birth were not related to AFH. The latter findings are in agreement with the study by Jones et al. [22]. They did not study sCD14 levels in relation to AFH after birth, however, as only children with a positive AFH were followed up. We found lower sCD14 levels in children with double AFH than in children with no AFH at 3, 12, and 18 months and at 7 years of age. Moreover, children with double AFH had lower sCD14 levels than children with single AFH at 7 years of age.

The CD14 fluorescence intensity on CD14⁺ cells increased during the first 3 months of age in all children, possibly reflecting the maturation of monocytes. We found similar CD14(m) expression in atopic and non-atopic children, and no correlations between CD14(m) and sCD14. We speculate that this may be due to an equilibrium between the shedding of sCD14 and CD14(m) expression *in vivo* and/or that the levels of sCD14 are related to the amount of CD14 in intracellular compartments of the monocyte. Another interpretation for finding similar expressions of CD14(m), but differences in sCD14-levels between atopic and non-atopic children, may be that another source of sCD14 than monocytes, namely hepatocytes [16, 17], is responsible for the observed differences in sCD14-levels. Since we could not study the CD14 expression of hepatocytes, we have no data to support this hypothesis, however. If a large portion of sCD14 is released by antigen presenting cells in the lung and liver into the bloodstream, this could also explain our absent correlation between CD14(m) measured on circulating monocytes and sCD14.

Thus, we did not find any differences in CD14(m) expression related to atopic disease or atopic family history. There are few other studies to compare with. In agreement to our study, Holmlund et al. [29] found similar CD14(m) on CD14⁺ cells at birth and at 2 years of age in children with atopic compared to non-atopic mothers.

In contrast to our findings, increased levels of serum sCD14 were found in individuals with AD [33], as well as increased levels in sputum after inhalation of LPS in atopic asthmatics [34] compared to controls. Increased sCD14 levels were also reported in bronchoalveolar lavage fluid after allergen provocation of allergic asthmatics [35]. Furthermore, higher levels of sCD14 in serum were observed in children with status asthmaticus than at recovery [36]. It may be speculated that acute atopic reactions induced in these studies, and a high disease activity, are related to increased levels of sCD14, whereas mild atopic symptoms, as in the present study, may be more dependent upon a low capacity to respond to microbial signals. This is supported by findings of a lower

LPS-responsiveness in adults with asthma than in healthy subjects [21].

In conclusion, atopic children had reduced levels of sCD14, which could be observed already at three months of age in infants who subsequently developed atopy and a positive SPT. The lower levels of sCD14 may be a consequence of atopic family heredity and/or atopic disease, but it may also reflect a reduced capacity to respond to microbial signals in atopic children.

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References

- Romagnani S. The role of lymphocytes in allergic disease. *J Allergy Clin Immunol* 2000; 105:399–408.
- Yang LP, Byun DG, Demeure CE, Vezzio N, Delespesse G. Default development of cloned human naive CD4 T cells into interleukin-4- and interleukin-5-producing effector cells. *Eur J Immunol* 1995; 25:3517–20.
- Prescott SL, Macaubas C, Holt BJ et al. Transplacental priming of the human immune system to environmental allergens: universal skewing of initial T cell responses toward the Th2 cytokine profile. *J Immunol* 1998; 160:4730–7.
- Holt PG, Sly PD, Björkstén B. Atopic vs. infectious diseases in childhood: a question of balance? *Pediatr Allergy Immunol* 1997; 8:53–8.
- Gereda JE, Leung DY, Thatayatikom A et al. Relation between house-dust endotoxin exposure, type 1 T cell development, and allergen sensitisation in infants at high risk of asthma. *Lancet* 2000; 355:1680–3.
- Böttcher MF, Björkstén B, Gustafson S, Voor T, Jenmalm MC. Endotoxin levels in Estonian and Swedish house dust and atopy in infancy. *Clin Exp Allergy* 2003; 33:273–6.
- Gehring U, Bolte G, Borte M et al. Exposure to endotoxin decreases the risk of atopic eczema in infancy: a cohort study. *J Allergy Clin Immunol* 2001; 108:847–53.
- Aderem A, Ulevitch RJ. Toll-like receptors in the induction of the innate immune responses. *Nature* 2000; 406:782–7.

- 9 Holt PG, Stumbles PA. Regulation of immunological homeostasis in peripheral tissues by dendritic cells: the respiratory tract as a paradigm. *J Allergy Clin Immunol* 2000; 105:421–9.
- 10 Ziegler-Heitbrock HWL, Ulevitch RJ. CD14: cell surface receptor and differentiation marker. *Immunol Today* 1993; 14:121–5.
- 11 Ulevitch RJ, Tobias S. Receptor-dependent mechanisms of cell stimulation by bacterial endotoxin. *Annu Rev Immunol* 1995; 13:437–57.
- 12 Cleveland MG, Gorham JD, Murphy TL, Tuomanen E, Murphy KM. Lipoteichoic acid preparations of gram-positive bacteria induce interleukin-12 through a CD14-dependent pathway. *Infect Immun* 1996; 64:1906–12.
- 13 Trinchieri G. Interleukin-12: a cytokine produced by antigen-presenting cells with immunoregulatory functions in the generation of T-helper cells type 1 and cytotoxic lymphocytes. *Blood* 1994; 84:4008–27.
- 14 Manetti R, Gerosa F, Giudizi MG et al. Interleukin 12 induces stable priming for interferon γ (IFN- γ) production during differentiation of human T helper (Th) cells and transient IFN- γ production in established Th2 cell clones. *J Exp Med* 1994; 179:1273–83.
- 15 Frey EA, Miller DS, Jahr TG et al. Soluble CD14 participates in the response of cells to lipopolysaccharide. *J Exp Med* 1992; 176:1665–71.
- 16 Su GL, Dorko K, Strom SC, Nussler AK, Wang SC. CD14 expression and production by hepatocytes. *J Hepatol* 1999; 31:435–42.
- 17 Pan Z, Liming Z, Hetherington CJ, Dong-Er Z. Hepatocytes contribute to soluble CD14 production and CD14 expression is differentially regulated in hepatocytes and monocytes. *J Biol Chem* 2000; 275:36430–5.
- 18 Baldini M, Lohman C, Halonen M, Erickson RP, Holt PG, Martinez FD. A polymorphism in the 5' flanking region of the CD14 gene is associated with total serum immunoglobulin E. *Am J Respir Cell Mol Biol* 1999; 20:976–83.
- 19 Rey Norez JE, Bensussan A, Vita N et al. Soluble CD14 acts as a negative regulator of human T cell activation and function. *Eur J Immunol* 1999; 29:265–76.
- 20 Arias MA, Rey Norez JE, Vita N et al. Human B cell function is regulated by interaction with soluble CD14: opposite effects on IgG1 and IgE production. *J Immunol* 2000; 164:3480–5.
- 21 Michel O, Dentener M, Corazza F, Buurman W, Rylander R. Healthy subjects express differences in clinical responses to inhaled lipopolysaccharide that are related with inflammation and with atopy. *J Allergy Clin Immunol* 2001; 107:797–804.
- 22 Jones CA, Holloway JA, Poppelwell EJ et al. Reduced soluble CD14 levels in amniotic fluid and breast milk are associated with the subsequent development of atopy, eczema, or both. *J Allergy Clin Immunol* 2002; 109:858–66.
- 23 Aniansson Zdzolek H, Ernerudh J, Holt PG, Nilsson J, Björkstén B. Expression of the T-cell markers CD3, CD4 and CD8 in healthy and atopic children during the first 18 months of life. *Int Arch Allergy Immunol* 1999; 119:6–12.
- 24 Jenmalm MC, Aniansson Zdzolek H, Holt PG, Björkstén B. Expression and responses to CD2 and CD3 in 18 month old children with and without atopic dermatitis. *Pediatr Allergy Immunol* 2000; 11:175–82.
- 25 Hanifin JM, Rajka G. Diagnostic features of atopic dermatitis. *Acta Dermatol Venerol* 1980; (Suppl. 92):44–7.
- 26 Seymour JL, Keswick BH, Hanifin JM, Jordan WP, Milligan M. Clinical effects of diaper types on the skin of normal infants and infants with atopic dermatitis. *Am Acad Dermatol* 1987; 17:988–97.
- 27 Kunz B, Oranje AP, Labereze L, Stalder JF, Ring J, Taieb A. Clinical validation and guidelines for the SCORAD index: consensus report of the European Task Force on Atopic Dermatitis. *Dermatology* 1997; 195:10–9.
- 28 Strachan DP. Hay fever, hygiene, and household size. *BMJ* 1989; 299:1259–60.
- 29 Holmlund U, Höglind A-K, Larsson A-K, Nilsson C, Sverremark Ekström E. CD14 and development of atopic disease at 2 years of age in children with atopic or non-atopic mothers. *Clin Exp Allergy* 2003; 33:455–63.
- 30 Sack U, Burkhardt U, Borte U, Schädlich H, Berg K, Emmrich F. Age-dependent levels of select immunological mediators in sera of healthy children. *Clin Diagn Lab Immunol* 1998; 5:28–32.
- 31 Labéta MO, Vidal K, Rey Norez JE et al. Innate recognition of bacteria in human milk is mediated by a milk-derived highly expressed recognition receptor, soluble CD14. *J Exp Med* 2000; 191:108–15.
- 32 Koppelman GH, Reijmerink NE, Stine CO et al. Association of a promoter polymorphism of the CD14 gene and atopy. *Am J Respir Crit Care Med* 2001; 163:965–9.
- 33 Wüthrich B, Kägi MK, Joller-Jemelka H. Soluble CD14 but not interleukin-6 is a new marker for clinical activity in atopic dermatitis. *Arch Dermatol Res* 1992; 284:339–42.
- 34 Alexis N, Eldridge M, Reed W, Bromberg P, Peden DB. CD14-dependent airway neutrophil response to inhaled LPS: role of atopy. *J Allergy Clin Immunol* 2001; 107:31–5.
- 35 Virchow JC, Julius P, Matthys H, Kroegel C, Luttmann W. CD14 expression and soluble CD14 after segmental allergen provocation in atopic asthma. *Eur Respir J* 1998; 11:317–23.
- 36 Garty BZ, Monselise Y, Nitzan M. Soluble CD14 in children with status asthmaticus. *Isr Med Assoc J* 2000; 2:104–7.