

CD14 and development of atopic disease at 2 years of age in children with atopic or non-atopic mothers

U. Holmlund, A. Höglind*, A.-K. Larsson*, C. Nilsson† and E. Sverremark Ekström

Unit of Clinical Allergy Research, Department of Medicine, Karolinska Hospital and Institutet, Stockholm, *Department of Immunology, Wenner-Gren Institute, Stockholm University, Stockholm and †Department of Pediatrics, Sachs' Children's Hospital, Karolinska Institutet, Stockholm, Sweden

Summary

Background CD14, a myeloid cell marker and LPS receptor has been acclaimed to play a role in development and manifestation of atopic allergy, as the gene encoding CD14 is located in a chromosomal region linked to total IgE levels and atopic disease.

Objective To investigate the levels of soluble (s) and membrane bound (m) CD14 in cord blood and at 2 years of age from children with atopic or non-atopic mothers and relate these parameters to atopy development at 2 years of age.

Methods Blood samples were collected at delivery (cord blood) and at 2 years of age among infants with atopic ($n=41$) and non-atopic ($n=32$) mothers. Blood samples were also obtained from mothers at the same occasions. Levels of sCD14 and total IgE were measured in plasma, and percentages of CD14⁺ cells were measured in cord and peripheral blood mononuclear cells.

Results We observed significant differences in sCD14 levels in cord blood, where children with atopic mothers had the highest levels. The same pattern could be observed in the mothers at delivery. At 2 years of age no significant differences in sCD14 levels were observed between children with atopic mothers and children with non-atopic mothers and no association between sCD14 and atopic disease was found. Further, we observed large differences in sCD14 and mCD14 with respect to age, where newborns displayed a higher frequency of CD14⁺ cells compared with the 2-year-olds and the mothers. The reverse was observed for sCD14, with significantly lower values in cord blood than those seen in the 2-year-olds and mothers.

Conclusion Based on our findings, we suggest that CD14 could be involved in the regulation of IgE production, but that it might also be important for the maturation and development of the neonatal immune system.

Keywords atopy, children, IgE, sCD14

Submitted 28 June 2002; revised 6 December 2002; accepted 31 December 2002

Introduction

CD14 is a 55-kDa glycosylphosphatidylinositol-linked glycoprotein expressed on the surface of cells of myeloid origin, preferentially monocytes and macrophages, and found in plasma in its soluble form (sCD14) [1]. CD14 serves as a pattern recognition receptor (PRR), which identifies pathogen, associated molecular patterns (PAMPs) [2], e.g. LPS/endotoxin from the outer membrane of gram-negative bacteria [3], lipoteichoic acids from gram-positive bacteria and mycobacterial glycolipids [4]. Due to the lack of an intracellular signalling domain, CD14 has to come in contact with other molecules for activation to occur. One of the most important molecules for CD14-mediated cellular responses following LPS activation is the Toll-like receptor (TLR) 4 [5, 6]. The binding of CD14 to TLR4 results in release of cytokines, preferentially of the

proinflammatory type, such as IL-6, IL-8, IL-12 and TNF- α [7, 8]. The gene encoding CD14 is located in the chromosomal region 5q31–33, a region that has been linked to total IgE levels as well as atopic disease [9, 10]. Different isoforms of sCD14 exist, generated through different pathways [11, 12]. In healthy individuals sCD14 is present in plasma at a concentration of approximately 2 $\mu\text{g/mL}$ [13]. Importantly, cells that lack membrane bound (m) CD14 become responsive to PAMPs in the presence of sCD14 [8, 14]. In addition to serving as an LPS receptor, sCD14 has been shown to regulate the activation and function of B cells [15] and activated T cells [16]. Furthermore, high concentrations of sCD14 have been detected in breast milk [17, 18], and CD14 has also been implicated as a receptor for apoptotic cells [19]. Thus, the CD14 molecule might be involved in immune regulation, and control levels and/or type of immune responses elicited.

The hygiene hypothesis is by many thought to explain the increase in allergic disease seen during the last decades [20, 21]. Several studies have shown that children growing up on farms with livestock are less allergic than other children [22–24]. Further, endotoxin levels in house dust have been correlated to less

Correspondence: U. Holmlund, Department of Medicine, Unit of Clinical Allergy Research, L2:04 Karolinska Hospital, 171 76 Stockholm, Sweden.
E-mail: ulrika.holmlund@ks.se

allergen sensitization in children [25, 26]. Thus, LPS might be involved in protection towards allergic sensitization, possibly through mechanisms that activate Th1 and/or suppress Th2 type of immune responses.

An inverse association between plasma levels of sCD14 and total IgE has been observed in atopic individuals [27, 28]. It was demonstrated that a polymorphism in the promoter region of the CD14 gene (C to T at position -159), was associated with higher levels of sCD14 and lower levels of IgE. This might suggest a role for the CD14 molecule in manifestation of allergic disease, but so far little is known about the importance of CD14 for the development of atopic disease early in life. To gain insight into this, we investigated the relationship between soluble and membrane bound CD14 in children with atopic or non-atopic mothers as well as atopic disease at 2 years of age. Our results indicate that low sCD14 levels are not a good predictor for development of atopic disease at an early age. Neonates with atopic mothers have increased levels of sCD14 in plasma compared with neonates with non-atopic mothers. The sCD14 levels in CB were also well correlated with the mothers. However, at 2 years of age, sCD14 could not be associated with either having an atopic mother or atopic disease in the child. Interestingly, both sCD14 and mCD14 varied with age, suggesting a role for CD14 in the development and maturation of the immune system.

Materials and methods

Study subjects and diagnosis of atopy

Pregnant women were recruited from maternity wards in Stockholm, Sweden. Based on their clinical history (allergic bronchial asthma and/or allergic rhinoconjunctivitis towards animal dander and/or towards pollen) together with skin prick test (SPT) results, they were divided into two groups, atopic mothers ($n = 41$) or non-atopic mothers ($n = 32$, Table 1). SPT was performed according to the manufacturer's instructions (ALK, Copenhagen, Denmark). A panel of representative inhalation allergens (cat, dog, horse, rabbit, birch, timothy grass, mugwort, *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, *Cladosporium herbarum* and *Alternaria*) was used to test the mothers. The children (41 with atopic mothers and 32 with non-atopic mothers) were SPT tested against the same inhalant allergens as their mothers except for *Dermatophagoides pteronyssinus* and the following food allergens: milk (3% fat, standard milk), egg (weight/volume 1/100), peanut (1/20) and soy (Sojasemp, Semper AB, Stockholm, Sweden). Histamine

(10 mg/mL) served as a positive reference and the allergen diluent was used as the negative control. The SPT was regarded as positive if the weal reaction was greater than or equal to 3 mm in diameter after 15 min. Only those mothers among whom a positive or a negative SPT confirmed the clinical history were invited to participate in the study. All pregnancies were term pregnancies (> 37 weeks) and there were no differences in age of the mothers in the two groups (Table 1). The fathers were also SPT tested; none of the fathers of children with non-atopic mothers were atopic, and in the group of children with atopic mothers the fathers were either atopic ($n = 19$) or non-atopic ($n = 22$). Approval from the ethics committee in Stockholm, Sweden has been granted. All families gave their informed consent to the study.

Clinical evaluation in children

The children were followed prospectively from birth to 2 years of age and were clinically evaluated by one and the same paediatrician. The presence of allergic diseases was defined in each child. The following definitions of the various allergic diseases were used:

- Atopic eczema dermatitis syndrome (AEDS) was defined according to Hanifin and Rajka [29].
- Probable asthma was defined as three or more episodes of wheezing, or any episode of wheezing if combined with a family history of allergic disease or other allergic symptoms in the child [30].
- Allergic rhino-conjunctivitis was diagnosed if rhinitis and/or conjunctivitis appeared at least twice after exposure to a particular allergen and were unrelated to infection.
- Food allergy/acute urticaria were diagnosed as acute onset of symptoms as skin reactions, wheezing, vomiting or diarrhoea on more than one occasion after ingestion or contact with a particular food or allergen.

Allergic classification

The child was classified as atopic if clinical symptoms were combined with one or more positive SPT (≥ 3 mm).

Collection of plasma samples and separation of CBMC and PBMC

Cord blood (CB) samples were aspirated from the umbilical cord vein into heparinized Vacutainer tubes after careful wiping of the cord with alcohol. Plasma was obtained by centrifugation and stored at -20°C until analysed. The APT test [31] was performed to rule out contamination of CB with maternal blood. Cord blood mononuclear cells (CBMC) were isolated

Table 1. Demographic data of atopic and nonatopic mothers and their children

Group*	Cases (n)	Maternal age at delivery (years)	Total IgE† mother (kU/L)	Gestation (weeks)	Mode of delivery (vaginal/ Caesarian)	Boy/girl (n)	Birth weight (g)	Total IgE CB (kU/L)	Total IgE‡ 2-year-olds (kU/L)
Atopic mothers	41	30 (22–38)§	110 (2.5–1312)§	40 (37–43)§	33/8	14/27	3600 (2455–4800)§	0.3 (0.1–16.4)§	23.1 (2–880)§
Non-atopic mothers	32	32 (23–40)	12.7 (2–229)	40 (38–43)	28/4	17/15	3645 (3300–4290)	0.1 (0.1–3.6)	8.7 (2.3–108)

*The diagnosis of atopy was based on positive Phadiatop (Pharmacia CAP System FEIA; Pharmacia & Upjohn Diagnostics AB) and positive skin prick test together with clinical symptoms. †Plasma samples taken at delivery. ‡Twenty-two children with maternal atopic heredity and 21 with no maternal atopic heredity. §Median (range).

by Ficoll-Paque (Pharmacia-Upjohn, Stockholm, Sweden) gradient centrifugation performed twice [32]. Since peripheral blood leucocytes are activated in pregnant women with an increased expression of mCD14 [33], the peripheral blood mononuclear cells (PBMC) were obtained from the mothers when they were non-pregnant (2 years after delivery). PBMC and plasma from the mothers (at delivery and after 2 years) and the 2-year-olds were collected as for the CB, except that the Ficoll-Paque centrifugation was performed only once. The CBMC or PBMC were diluted to 10^7 cells/mL in tissue-culture media (TCM = RPMI 1640-Hepes supplemented with Gentamicin (25 µg/mL), L-glutamine (2 mM) and 10% heat-inactivated fetal calf plasma, all from Life Technologies, Glasgow, UK) containing 10% dimethyl sulphoxide (Sigma, Stockholm, Sweden). Isolated cells were frozen gradually 1 °C/minute to -70 °C in a freezing container (Nalgene Cryo 1 °C, Nalge Company, Rochester, NY, USA) and thereafter stored in liquid nitrogen. There were no differences in time between sampling and freezing of the isolated CBMC or PBMC among the two groups.

Determination of total plasma IgE levels

Maternal (taken at the time of delivery) and 2-year-olds' plasma samples were analysed for total IgE levels with the Pharmacia CAP System IgE FEIA (detection limit 2 kU/L, Pharmacia Diagnostics AB, Uppsala, Sweden). Total IgE levels in cord blood plasma were detected by an ultra-sensitive CAP-FEIA (detection limit 0.1 kU/L), modified by an extended standard curve but otherwise in accordance with the recommendation of the manufacturer (Pharmacia Diagnostics AB).

Soluble CD14 ELISA

sCD14 levels were determined in maternal plasma samples taken at the time of delivery (atopic $n = 41$, non-atopic $n = 32$) and after 2 years (atopic $n = 36$, non-atopic $n = 31$, the women who were pregnant again at that occasion were not included), in cord blood (children with atopic mothers $n = 41$, children with non-atopic mothers $n = 32$) samples and in plasma from the children at 2 years of age (children with atopic mothers $n = 22$, children with non-atopic mothers $n = 21$; only children who at the beginning of the study had become 2 years of age were included), using the sandwich enzyme linked immunoassay technique (ELISA, R & D Systems, Abingdon, UK). The ELISAs were performed according to the manufacturer's instructions. Briefly, the plasma samples and a standard were diluted and added to the microtitre wells pre-coated with a murine monoclonal antibody specific for sCD14. All the samples were run in duplicates and incubated for 3 hours, followed by washings to remove unbound material. Thereafter, a polyclonal anti-CD14 antibody, conjugated with horseradish peroxidase, was added for 1 hour. After washing, the substrate solution was added and the development was stopped after 30 min with sulphuric acid. The optical density was determined using a microplate reader (SLT-Labinstruments, Salzburg, Austria) set at 450 nm, and the detection limit was 0.13 ng/mL.

Flow cytometry

Analysis of mCD14 was performed on a part of the study group as a sufficient amount of cells was not available from all children. Maternal PBMC taken 2 years after delivery (atopic mothers $n = 14$, non-atopic mothers $n = 9$), and PBMC from

their children at birth and at 2 years of age were analysed by flow cytometry. After thawing the cells in TCM containing 20% FCS, CBMC or PBMC were washed two times in phosphate buffered saline (PBS) supplemented with 0.02% NaN₃, and thereafter incubated for 15 min at 4 °C with human plasma (diluted 5x) to block Fc receptors (at a total number of 0.5×10^6 cells). Staining was performed for 30 min, 4 °C, using a titrated amount of mAb diluted in PBS in a total volume of 50 µL. PE-conjugated anti-human CD14 mouse IgG_{2a} kappa (M5E2, PharMingen, San Diego, USA) was used to detect mCD14 and PE-conjugated mouse IgG_{2a} kappa (DAK-GO5, Dako A/S, Glostrup, Denmark) was used as a negative isotype control. After washing, 10 000 cells/sample were analysed using a FACScan flow cytometer (Becton Dickinson, Franklin Lakes, NJ, USA) and CellQuest software (Becton Dickinson). Gating was performed on the living mononuclear cell population of CBMC or PBMC based on the forward and side scatter properties.

Statistical analysis

The Mann-Whitney *U*-test was used to test for differences between groups. Differences between paired samples of neonates (CB) and 2-year-olds, as well as between mothers and neonates, were tested with Wilcoxon matched pairs test. For correlation tests, the Spearman rank correlation coefficient (r_s) was calculated. The difference was considered significant if $P < 0.05$.

Results

Analysis of IgE levels in plasma

Total plasma IgE levels differed significantly between atopic and non-atopic mothers ($P < 0.001$, Table 1) and in cord blood from their children ($P < 0.01$). At 2 years of age no differences in total plasma IgE between children with atopic mothers and children with non-atopic mothers could be seen, although the IgE levels in the 2-year-olds were increased compared with the cord blood levels (Table 1). Significant correlations were observed between maternal total plasma IgE and cord blood IgE in the group with children having atopic mothers ($r_s = 0.6$, $P < 0.001$). No significant correlations were found between the mothers and 2-year-olds in either of the groups (not shown).

sCD14 in relation to age in children with atopic or non-atopic mothers

To investigate how sCD14 is related to atopy, sCD14 levels in cord blood from 73 neonates and their mothers were measured. CB plasma from children with atopic mothers displayed significantly higher levels of sCD14 ($n = 41$, median 720 ng/mL) than CB from children with non-atopic mothers ($n = 32$, median 590 ng/mL) ($P < 0.001$, Fig. 1a). In plasma from 43 of these children (children with atopic mothers $n = 22$, children with non-atopic mothers $n = 21$) sCD14 levels were measured at the age of 2 years. At this time point, the sCD14 levels had increased, but in contrast to results with cord blood no significant difference between children with atopic mothers (median 1590 ng/mL) and children with non-atopic mothers (median 1650 ng/mL) could be observed (Fig. 1a). In plasma samples from the mothers taken at the time of delivery, the atopic

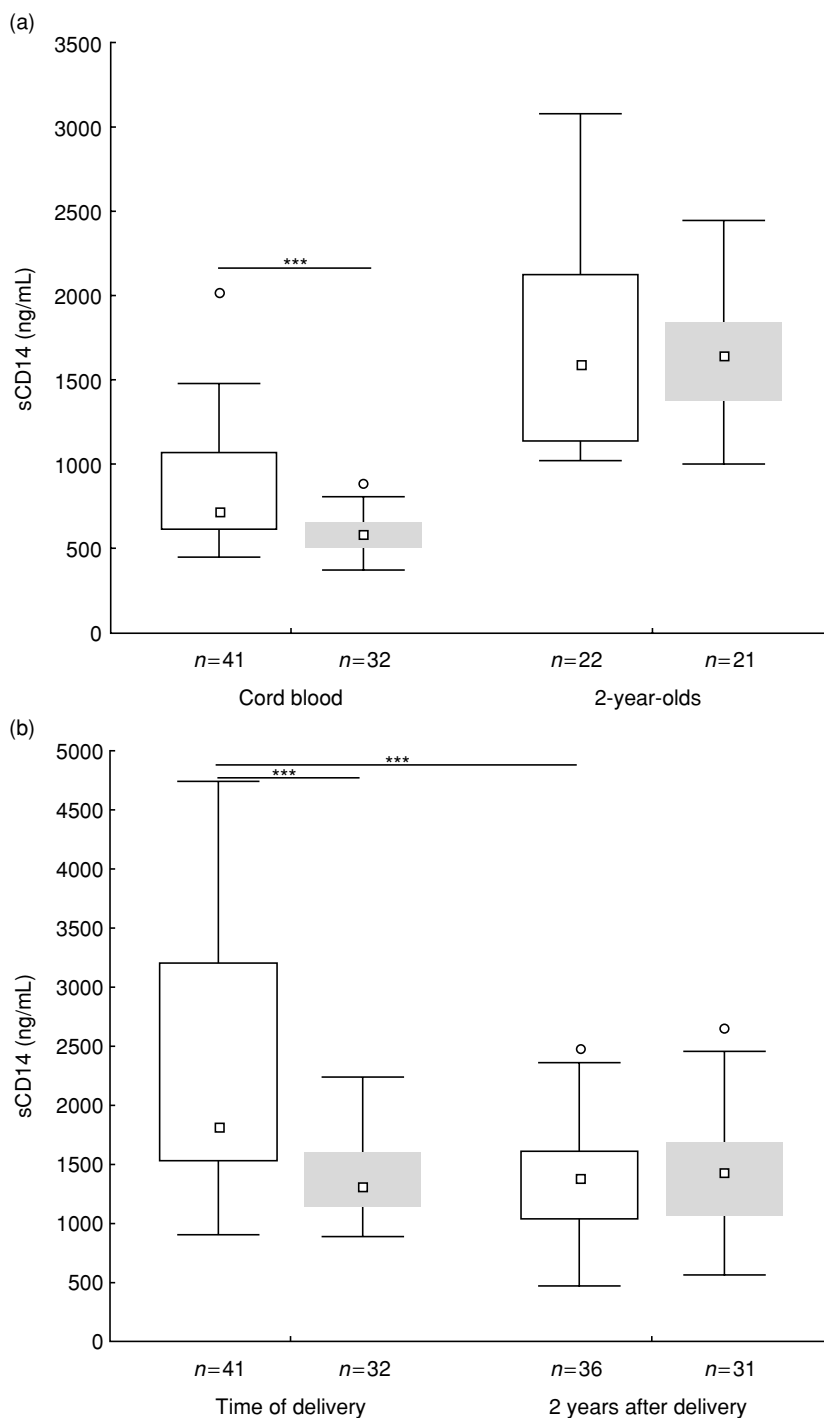


Fig. 1. sCD14 levels in children with atopic and non-atopic mothers. sCD14 was measured in (a) CB from 73 neonates, with atopic (white boxes) or non-atopic (grey boxes) mothers, in the children at 2 years of age, and (b) from their mothers (atopic, white boxes; non-atopic, grey boxes) in plasma taken at the time of delivery and in plasma taken 2 years later (the women who were pregnant again at that occasion were not included) with ELISA (R & D systems) as described in 'Materials and methods'. Results are illustrated using the box plot model. Boxes cover the middle 50% of the data values, between the 25th and 75th percentiles, the central square being the median. Lines extend out to non-outlier max and non-outlier min. ° = Outliers. *** $P < 0.001$. Note the different scales on the y-axes.

mothers had higher sCD14 levels (median 1820 ng/mL) than the non-atopic mothers (median 1310 ng/mL) ($P < 0.001$ Fig. 1b). Two years after delivery (when the women were non-pregnant), the sCD14 levels were significantly lower ($P < 0.001$) than levels during pregnancy in the atopic mothers (median 1380 ng/mL), while the levels in non-atopic mothers (median 1420 ng/mL) were approximately the same during and after pregnancy (Fig. 1b). When we compared the sCD14 levels in cord blood with sCD14 levels in maternal plasma, a correlation was found in the group with atopic mothers ($r_s = 0.7$, $P < 0.001$, Fig. 2a); however, this correlation could only be observed between

sCD14 levels in maternal plasma samples taken at the time of delivery and CB sCD14 levels. Interestingly, we observed a positive correlation between sCD14 and IgE in maternal plasma taken at delivery ($r_s = 0.5$, $P < 0.001$) if the atopic and non-atopic mothers were pooled together ($n = 73$) (Fig. 2b).

sCD14 in relation to development of atopic disease at 2 years of age

Forty-three children were followed to 2 years of age regarding atopy development. Positive SPT together with atopic

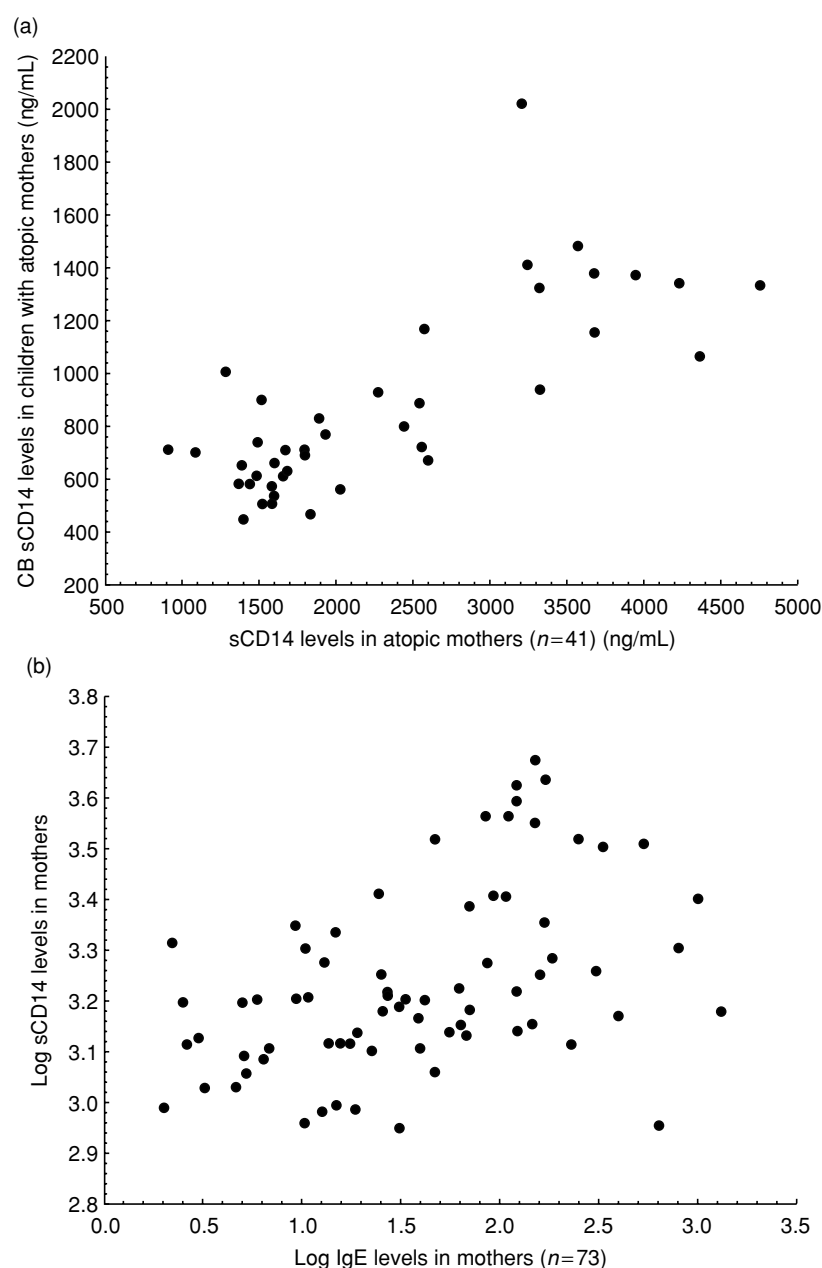


Fig. 2. Maternal sCD14 correlates with CB sCD14 and maternal IgE levels. (a) A strong correlation was observed between maternal plasma sCD14 levels at the time of delivery and cord blood sCD14 levels when the mothers were atopic, $r_s = 0.7$, $P < 0.001$. (b) A positive correlation was observed between maternal plasma sCD14 levels taken at the time of delivery and IgE levels ($r_s = 0.5$, $P < 0.001$) if atopic and non-atopic mothers were pooled together. IgE levels in maternal plasma were measured with a CAP system IgE FEIA (Pharmacia Diagnostics AB). sCD14 levels were measured with ELISA (R & D systems) as described in 'Materials and methods'. Note the different scales on the y-axes.

symptoms (probable asthma and/or AEDS) were significantly higher in the group of children with atopic mothers (8 out of 22) compared with the group with non-atopic mothers (2 out of 21) ($P < 0.05$, Table 2).

To investigate whether sCD14 plasma levels were associated with atopic disease at 2 years of age we grouped the children according to SPT result and atopic symptoms independent of whether the mother was atopic or non-atopic. Children with positive SPT together with atopic symptoms (probable asthma and/or AEDS) ($n = 10$) were compared with SPT negative children with no symptoms of atopic disease ($n = 17$). No association between sCD14 and development of atopic disease at 2 years of age could be seen (Fig. 3).

Membrane CD14 expression in relation to age in children with atopic or non-atopic mothers

Cytofluorometric analyses were conducted to compare the proportion of CD14⁺ cells in CBMC and PBMC in 2-year-olds and their mothers. The data revealed a significantly higher proportion of CD14⁺ cells in the CBMC (median 16%, Fig. 4a) compared with PBMC from the 2-year-olds (median 7%, $P < 0.001$, Fig. 4a) and their corresponding mothers (median 8%, $P < 0.05$, Fig. 4a). Comparisons between the 2-year-olds and their mothers showed a significantly lower proportion of CD14⁺ cells in the children ($P < 0.01$). Percentages of CD14⁺ cells were significantly correlated between the mothers and the 2-year-olds ($r_s = 0.5$, $P < 0.01$, Fig. 4b). No correlations were

Table 2. Development of atopic disease at 2 years of age

Group*	Cases (n)	SPT+† and allergic symptoms‡ (n)	SPT ⁻ without allergic symptoms (n)	SPT ⁺ or allergic symptoms (n)
Children with atopic mothers	22	8	5	9
Children with non-atopic mothers	21	2	12	7

*The diagnosis of atopy was based on positive skin prick test together with clinical symptoms. †Milk, egg, peanut, soy or cat.

‡AEDS, asthma or both AEDS and asthma.

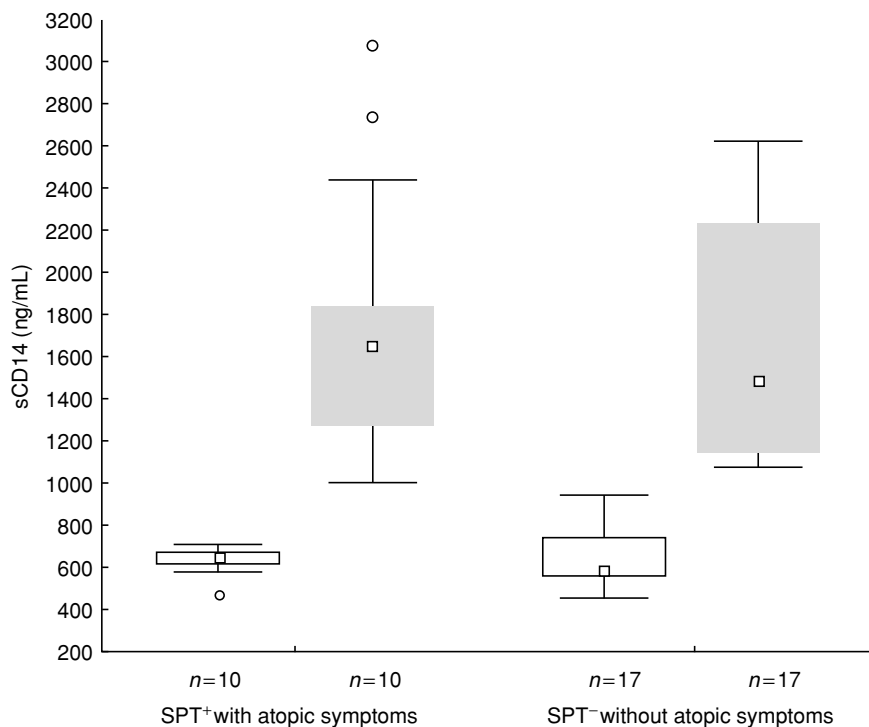


Fig. 3. sCD14 levels in relation to development of atopic disease at 2 years of age. Ten children were SPT positive and had atopic symptoms, while 17 children were non-atopic (SPT negative, without atopic symptoms) at the age of 2 years. sCD14 levels were measured with ELISA (R & D systems) as described in 'Materials and methods'. CB, white boxes; 2-year olds, grey boxes. °= outliers. The extreme outliers are not shown. For explanation of the box-plot model see the legend to Fig. 1.

observed between CBMC and 2-year-olds or between CBMC and the mothers (data not shown).

As shown in Fig. 5, no significant differences in the percentage of CD14⁺ cells between the groups were observed in cord blood, 2-year-olds or in the mothers. We could neither detect a difference in mean fluorescence intensity of CD14 in children with atopic mothers compared with children with non-atopic mothers (not shown). Further, no association between percentage of CD14⁺ cells and development of atopic disease at the age of 2 could be observed (data not shown).

Discussion

In this study we investigated the levels of sCD14 and mCD14 in cord blood and at 2 years of age from children with atopic or non-atopic mothers and related these parameters with maternal atopic disease and atopy development at 2 years of age. We made the observation that neonates with atopic mothers had higher sCD14 levels compared with neonates with non-atopic mothers. The same was true for their mothers, but only in samples taken at the time of delivery, where the atopic mothers

had significantly higher sCD14 levels. However, at 2 years of age no significant difference in sCD14 plasma levels was observed between children with atopic or non-atopic mothers, and no correlation between sCD14 and plasma IgE or association with atopic disease could be seen.

Earlier studies have shown a linkage between serum IgE levels and the chromosomal region in which CD14 is located [27, 28], with IgE and sCD14 being inversely correlated. Interestingly, a positive correlation between total IgE levels and sCD14 was seen in our study when the children with atopic mothers and children with non-atopic mothers were grouped together, both in cord blood and in their mothers. The discrepancies between our results and those by Baldini et al. and Vercelli et al. [27, 28] could be due to the fact that the studies are performed on different populations and that the children in these other studies are older (from 11 years) than the children in our study. Further, Baldini et al. [27] observed higher sCD14 and lower IgE levels in TT homozygotes compared with carriers of both the CC and CT genotypes. However, this difference was only observed in children SPT-positive for local aeroallergens. Our study population was not evaluated for this polymorphism and our 2-year-old children were found to be allergic primarily

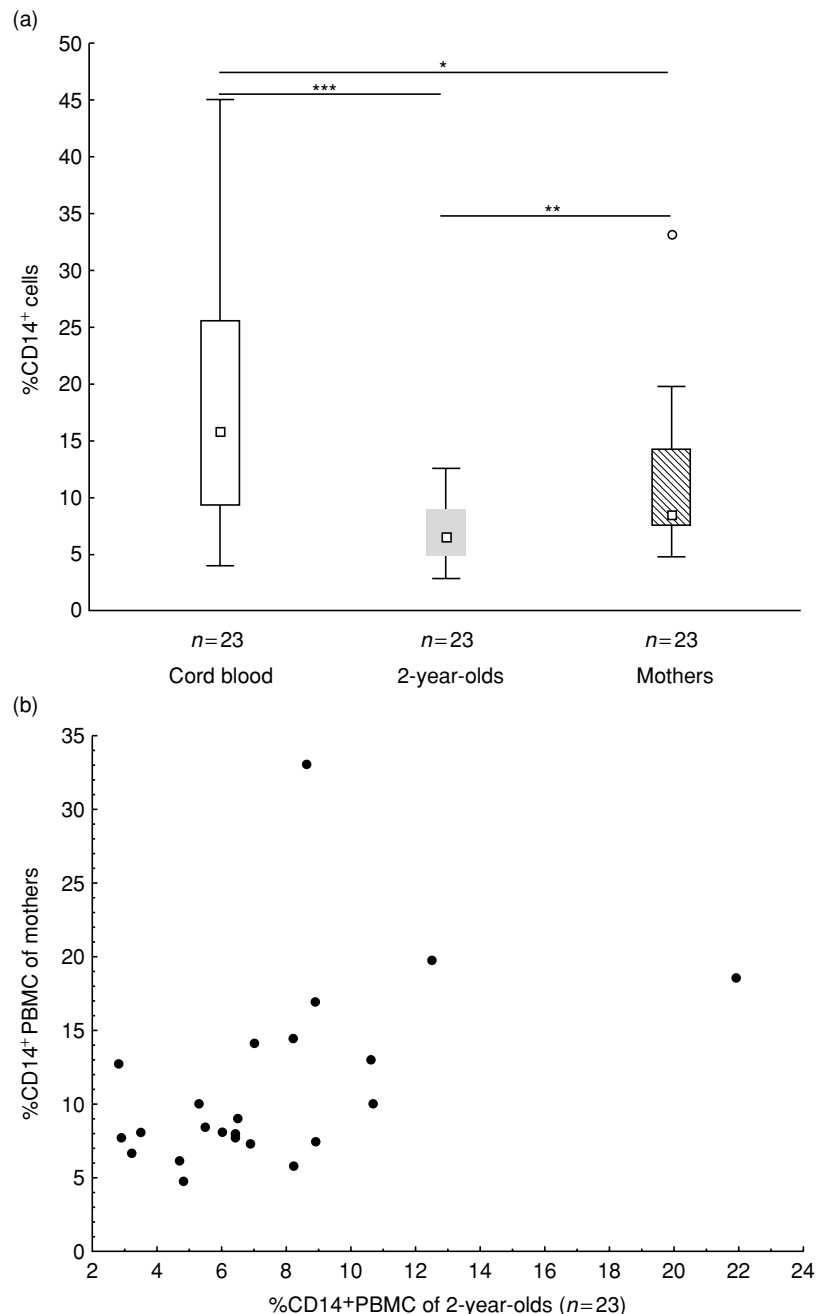


Fig. 4. Membrane CD14 expression with respect to age. (a) Percentages of CD14⁺ cells in CBMC and PBMC of 2-year-olds and their mothers. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. (b) A correlation was observed between mothers and 2-year-olds regarding percentage CD14⁺ cells ($r_s = 0.5$, $P < 0.01$). CB, white boxes; 2-year-olds, grey boxes; mothers, striped boxes. 10 000 cells/sample were analysed using a FACScan flow cytometer (Becton Dickinson). ° = outliers. The extreme outliers are not shown. For explanation of the box-plot model see the legend to Fig. 1.

against food allergens. Further, our results regarding sCD14 at 2 years of age are in agreement with other findings in a recent study by Jones et al. [18].

We also observed a significant correlation between sCD14 levels in CB and maternal blood at the time of delivery. This correlation could be due to passage over the placenta, either of sCD14 itself, or of factors involved in the regulation of sCD14, although this needs to be experimentally demonstrated. No correlation could be observed between CB sCD14 levels and sCD14 levels in maternal plasma from mothers when they were not pregnant; this further supports the theory of either a passage of sCD14 or regulatory factors involved over the placenta. The absence of this correlation in the group with non-atopic mothers could be explained by the fact that the non-atopic

mothers had significantly lower levels of sCD14 in plasma compared with the atopic mothers.

Notably, the relatively high sCD14 levels in plasma from atopic mothers decreased significantly out of pregnancy, while the levels in non-atopic mothers were similar at delivery and after pregnancy. Previous studies have shown that the maternal immune system is activated during the third trimester [33]. This could explain why the sCD14 levels were significantly elevated in the atopic mothers during pregnancy, but not why the same pregnancy-related effect was not observed in non-atopic women. There was no difference in infection frequency between atopic and non-atopic women, making infections an unlikely explanation for these findings. However, we could speculate that the elevated sCD14 levels seen during

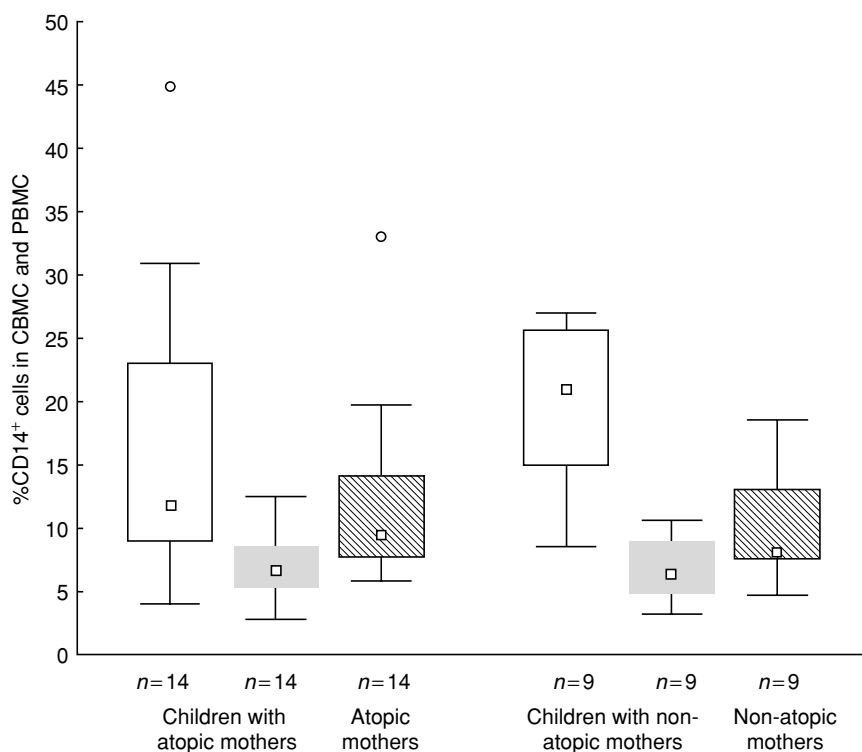


Fig. 5. No difference in percentages of CD14⁺ cells could be seen between children with atopic and children with non-atopic mothers. 10 000 cells/sample were analysed using a FACScan flow cytometer (Becton Dickinson). CB, white boxes; 2-year-olds, grey boxes; Mothers, striped boxes. ° = outliers. The extreme outliers are not shown. For explanation of the box-plot model see the legend to Fig. 1.

pregnancy in atopic women could be due to activation of the immune system due to their atopic disease.

In contrast to previous findings [34] the group of children with atopic mothers gave birth to a higher percentage of girls compared with the group with non-atopic mothers. This is pure coincidence and should not interfere with the results as no difference in sCD14 levels could be observed between males and females either in CB, at 2 years or in adults (our unpublished observation).

Age-related differences are known to exist for many molecules involved in immune responses [35]. We showed that plasma levels of sCD14 were significantly lower in neonates compared with the 2-year-olds and the mothers. In contrast to our findings for sCD14, significantly higher percentages of CD14⁺ cells were found in CBMC than in the PBMC from 2-year-olds and mothers. The high percentage of CD14⁺ cells further suggests that factors involved in the regulation of sCD14 are suppressed or not yet produced in sufficient amounts in the infant, and would be in agreement with the low sCD14 levels observed in the CB plasma. Both IL-4 and IL-13 [36] have been shown to down-regulate sCD14 at the transcriptional level. If the general dogma of a Th2-skewed fetus holds true [37], these findings would correspond well with our observed low CB sCD14 levels. Percentages of circulating CD14⁺ cells were significantly correlated between mothers and 2-year-olds, suggesting a strong maternal influence, possibly genetic, on the cellular distribution in the peripheral blood of the child. There were no differences in percentage of CD14⁺ cells between the groups irrespective of the child's atopic status and if the mother was atopic or not, suggesting that the significantly higher proportion of CD14⁺ cells in the CBMC might be a developmental phenomenon or be caused by labour-associated activation of the fetal immune system [38].

Despite the low levels of sCD14, which were observed in CB, the child will encounter very high levels of sCD14 through breast milk [14, 17, 18]. The function of this is not known, but might involve regulation of the early gastro-intestinal colonization, suggested to play a role in the generation of the atopic phenotype [39]. Further, this sCD14 could be of importance in the early development of the immune system, serving to activate B [15] and/or T cells [16]. In a recent study by Jones et al. it was shown that reduced sCD14 in amniotic fluid and breast milk are associated with development of atopy and eczema in children [18].

Taken together, our results highlight the complex interplay between the innate and the adaptive immune response. CD14 might have a role in the regulation of IgE production, but we suggest that CD14 is also important for the maturation and initial development of the immune system.

Acknowledgements

We gratefully acknowledge the critical and valuable comments from Professor Marita Troye-Blomberg, Stockholm University, Professor Annika Scheynius, Karolinska Institutet and Associate Professor Gunnar Lilja, Sachs' Childrens Hospital. We also thank Ann Sjölund, Anna-Stina Ander, Monica Nordlund and all the staff at delivery units in Stockholm for excellent technical assistance. This work was financially supported by grants from the Swedish Asthma and Allergy Associations Research Foundation, the Swedish Foundation for Health Care Sciences and Allergy Research, the Swedish Medical Research Council (project no. 16x-7924), the Hesselman's Foundation, Magnus Bergwall's Foundation, Konsul Th C Bergh's Foundation, Tore Nilson's Foundation and Apotekare Hedberg's Foundation.

References

- 1 Stelter F. Structure/function relationships of CD14. *Chem Immunol* 2000; 74:25–41.
- 2 Möller G. Receptors for innate pathogen defence in insects are normal activation receptors for specific immune responses in mammals. *Scand J Immunol* 1999; 50:341–7.
- 3 Haziot A, Ferrero E, Köntgen F et al. Resistance to endotoxin shock and reduced dissemination of gram-negative bacteria in CD14-deficient mice. *Immunity* 1996; 4:407–14.
- 4 Pugin J, Heumann ID, Tomasz A et al. CD14 is a pattern recognition receptor. *Immunity* 1994; 1:509–16.
- 5 Muta T, Takeshige K. Essential roles of CD14 and lipopolysaccharide-binding protein for activation of toll-like receptor (TLR) 2 as well as TLR4 reconstitution of TLR2- and TLR4-activation by distinguishable ligands in LPS preparations. *Eur J Biochem* 2001; 268:4580–9.
- 6 Poltorak A, He X, Smirnova I et al. Defective LPS signaling in C3H/HeJ and C57BL/10ScCr mice: mutations in Tlr4 gene. *Science* 1998; 282:2085–8.
- 7 Morrison DC, Ryan JL. Endotoxins and disease mechanisms. *Annu Rev Med* 1987; 38:417–32.
- 8 Verhasselt V, Buelens C, Willems F, De Groote D, Haeflener-Cavaillon N, Goldman M. Bacterial lipopolysaccharide stimulates the production of cytokines and the expression of costimulatory molecules by human peripheral blood dendritic cells: evidence for a soluble CD14-dependent pathway. *J Immunol* 1997; 158:2919–25.
- 9 Marsh DG, Neely JD, Breazeale DR et al. Linkage analysis of IL4 and other chromosome 5q31.1 markers and total serum immunoglobulin E concentrations. *Science* 1994; 264:1152–6.
- 10 Postma DS, Bleecker ER, Amelung PJ et al. Genetic susceptibility to asthma – bronchial hyperresponsiveness coinherit with a major gene for atopy. *N Engl J Med* 1995; 333:894–900.
- 11 Bufler P, Stiegler G, Schuchmann M et al. Soluble lipopolysaccharide receptor (CD14) is released via two different mechanisms from human monocytes and CD14 transfectants. *Eur J Immunol* 1995; 25:604–10.
- 12 Durieux JJ, Vita N, Popescu O et al. The two soluble forms of the lipopolysaccharide receptor, CD14: characterization and release by normal human monocytes. *Eur J Immunol* 1994; 24:2006–12.
- 13 Scherberich JE, Nockher WA. CD14++ monocytes, CD14+/CD16+ subset and soluble CD14 as biological markers of inflammatory systemic diseases and monitoring immunosuppressive therapy. *Clin Chem Laboratory Med* 1999; 37:209–13.
- 14 Filipp D, Alizadeh-Khiavi K, Richardson C et al. Soluble CD14 enriched in colostrum and milk induces B cell growth and differentiation. *Proc Natl Acad Sci USA* 2001; 98:603–8.
- 15 Arias MA, Rey Norez JE, Vita N et al. Cutting edge: human B cell function is regulated by interaction with soluble CD14: opposite effects on IgG1 and IgE production. *J Immunol* 2000; 164:3480–5.
- 16 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.
- 17 Labeta MO, Vidal K, Rey Norez JE et al. Innate recognition of bacteria in human milk is mediated by a milk-derived highly expressed pattern recognition receptor, soluble CD14. *J Exp Med* 2000; 191:1807–12.
- 18 Jones CA, Holloway JA, Popplewell 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.
- 19 Devitt A, Moffatt OD, Raykundalia C, Capra JD, Simmons DL, Gregory CD. Human CD14 mediates recognition and phagocytosis of apoptotic cells. *Nature* 1998; 392:505–9.
- 20 Strachan DP. Hay fever, hygiene and household size. *BMJ* 1989; 299:1259–60.
- 21 Strannegård Ö, Strannegård IL. The causes of the increasing prevalence of allergy: is atopy a microbial deprivation disorder? *Allergy* 2001; 56:91–102.
- 22 Riedler J, Braun-Fahrlander C, Eder W et al. Exposure to farming in early life and development of asthma and allergy: a cross-sectional survey. *Lancet* 2001; 358:1129–33.
- 23 Von Ehrenstein OS, Von Mutius E, Illi S, Baumann L, Böhm O, von Kries R. Reduced risk of hay fever and asthma among children of farmers. *Clin Exp Allergy* 2000; 30:187–93.
- 24 von Mutius E, Braun-Fahrlander C, Schierl R et al. Exposure to endotoxin or other bacterial components might protect against the development of atopy. *Clin Exp Allergy* 2000; 30:1230–4.
- 25 Gereda JE, Leung DYM, 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.
- 26 Braun-Fahrlander C, Riedler J, Herz U et al. Environmental exposure to endotoxin and its relation to asthma in school-age children. *N Engl J Med* 2002; 347:869–77.
- 27 Baldini M, Lohman IC, Halonen M, Erickson RP, Holt PG, Martinez FD. A polymorphism* in the 5' flanking region of the CD14 gene is associated with circulating soluble CD14 levels and with total serum immunoglobulin E. *Am J Respir Cell Mol Biol* 1999; 20:976–83.
- 28 Vercelli D, Baldini M, Martinez F. The monocyte/IgE connection: may polymorphisms in the CD14 gene teach us about IgE regulation? *Int Arch Allergy Immunol* 2001; 124:20–4.
- 29 Hanifin J, Rajka G. Diagnostic features of atopic dermatitis. *Acta Derm Venereol (Stockh)* 1980; 92:44–7.
- 30 Alm JS, Lilja G, Pershagen G, Scheynius A. Early BCG vaccination and development of atopy. *Lancet* 1997; 350:400–3.
- 31 Ogur G, Gül D, Özen S et al. Application of the 'Apt test' in prenatal diagnosis to evaluate the fetal origin of blood obtained by cordocentesis: results of 30 pregnancies. *Prenat Diagn* 1997; 17:879–82.
- 32 Gabrielsson S, Söderlund A, Nilsson C, Lilja G, Nordlund M, Troye-Blomberg M. Influence of atopic heredity on IL-4-, IL-12- and IFN-gamma-producing cells in in vitro activated cord blood mononuclear cells. *Clin Exp Immunol* 2001; 126:390–6.
- 33 Sacks GP, Studena K, Sargent IL, Redman CWG. Normal pregnancy and preeclampsia both produce inflammatory changes in peripheral blood leukocytes akin to those of sepsis. *Am J Obstet Gynecol* 1998; 179:80–6.
- 34 Lilja G, Dannaeus A, Fälth-Magnusson K et al. Immune response of the atopic woman and foetus: effects of high- and low-dose food allergen intake during late pregnancy. *Clin Allergy* 1988; 18:131–42.
- 35 Straub RH, Cutolo M, Zietz B, Scholmerich J. The process of aging changes the interplay of the immune, endocrine and nervous systems. *Mech Ageing Dev* 2001; 122:1591–611.
- 36 Cosentino G, Soprana E, Thienes CP, Siccardi AG, Viale G, Vercelli D. IL-13 down-regulates CD14 expression and TNF-alpha secretion in normal human monocytes. *J Immunol* 1995; 155:3145–51.
- 37 Raghupathy R, Makhseed M, Azizieh F, Omu A, Gupta M, Farhat R. Cytokine production by maternal lymphocytes during normal human pregnancy and in unexplained recurrent spontaneous abortion. *Hum Reprod* 2000; 15:713–8.
- 38 Steinborn A, Sohn C, Sayehli C et al. Spontaneous labour at term is associated with fetal monocyte activation. *Clin Exp Immunol* 1999; 117:147–52.
- 39 Kalliomäki M, Kirjavainen P, Eerola E, Kero P, Salminen S, Isolauri E. Distinct patterns of neonatal gut microflora in infants in whom atopy was and was not developing. *J Allergy Clin Immunol* 2001; 107:129–34.