

Breastfeeding, soluble CD14 concentration in breast milk and risk of atopic dermatitis and asthma in early childhood: birth cohort study

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Summary

Background Breast milk contains a variety of bioactive substances, among them, soluble CD14 (sCD14), which plays an important role in innate immunity.

Objective We analysed data of a large prospective birth cohort study to examine the determinants of sCD14 in breast milk, and investigated whether breastfeeding practice and sCD14 concentrations in breast milk are determinants of the risk of atopic dermatitis (AD) and asthma in children.

Methods Eight hundred and three mothers and their newborns were included in this analysis. We measured sCD14 concentrations in breast milk samples collected 6 weeks post-partum. During a 2-year follow-up the cumulative incidences of AD and asthma were recorded.

Results Overall, AD was reported for 20.6% of the 2-year-olds and asthma was reported for 19.6%. We found the lowest incidence of physician-reported AD in children of mothers without a history of atopic diseases if breastfed for 6 to less than 9 months. Furthermore, we found an inverse association between duration of breastfeeding and risk of asthma, which was especially evident in children with mothers without a history of atopic disease ($P = 0.01$). These patterns persisted after control for other factors by multivariate analysis methods. The protective effect of breastfeeding seemed to be synergistic with sCD14 concentrations in breast milk (P for trend 0.0005).

Conclusions The results of this prospective birth cohort study suggest that a longer duration of breastfeeding does decrease the risk for asthma in early childhood, especially in children of mothers without a history of atopic disease. The beneficial effects of breastfeeding might be further supported by high levels of sCD14 in breast milk.

Keywords asthma, atopic dermatitis, birth cohort study, breast feeding, soluble CD14

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Introduction

The incidence of atopic diseases such as atopic dermatitis (AD) and asthma is currently increasing dramatically in industrialized countries [1]. A reduced microbial exposure and, as a result, a different development of the T helper type 1 (Th1)/T helper type 2 (Th2) immune response is discussed as one possible explanation for the observed increase of atopic diseases in early childhood [2].

One factor that may partly influence the Th1/Th2 development in early childhood may be soluble CD14 (sCD14) in breast milk. CD14 is an important factor in the development and maturation of the immune system. It is a well-recognized pattern recognition receptor that is expressed primarily on macrophages and B cells, and is part of the lipopolysaccharide (LPS)-recognition complex that binds microbial and other bacterial wall components [3]. It initiates signal transduction

via the Toll-like receptor 4 (TLR 4) resulting in high secretion of IL-12, which promotes a strong Th1 response. In addition, in plasma the sCD14 can be found in response to LPS stimulation, where two major isoforms coexist [4, 5]. Thus, both membrane-bound CD14 and sCD14 are competing for LPS and are able to bind it [6, 7].

In breast milk only one isoform of sCD14 exists; however, much higher levels than in serum occur [8]. Levels of sCD14 in breast milk have been suggested as important immune modulators and may play an important role in the defence of the neonatal intestine [8, 9]. sCD14 may monitor the bacterial load of the gut and initiate an appropriate immune activity to commensals and pathogens. An evaluation of several small-scale studies suggested that low sCD14 levels in breast milk are associated with development of atopy and eczema [10]. However, results of large population-based studies are lacking so far.

We analysed data of a large prospective birth cohort study to examine determinants of sCD14 in breast milk and investigated whether breastfeeding practice and sCD14 level in breast milk are determinants of AD and asthma in children within the first 2 years of life after controlling for confounding factors.

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Methods

Study design and study population

All women who came to the Department of Gynecology and Obstetrics at the University of Ulm between November 2000 and November 2001 for the delivery of their baby, their partners and their offspring were recruited for the study. In Germany, on average, women stay in the hospital for about 5 days after delivery, and recruitment was carried out during this time window. During the time of recruitment, the Department of Gynecology and Obstetrics at the University of Ulm was the only major department of obstetrics in the study area and served the vast majority of childbearing women in the city of Ulm and the nearby communities.

We excluded women with less than 32 gestational weeks, with a baby of less than 2500 g birth weight, or transfer of their infants to in-patient paediatric care immediately after delivery. Furthermore, we excluded women with no understanding of the German, Turkish or Russian language, as well as all women who left the hospital immediately after birth. Overall, 1066 families could be included in this study (67% of all 1593 eligible families who fulfilled the inclusion criteria).

Active follow-up of all children included in the baseline examination was performed at 12 and 24 months of age. A participation rate of 82% for all families, and of 76% for the primary health care paediatricians of the children included in the baseline examination was achieved for the 2-year follow-up.

Participation was voluntary, and informed consent was obtained in each case. The study was approved by the Ethics Boards of the University of Ulm and of the Physicians' Boards of the states of Baden-Wuerttemberg and Bavaria.

Data collection

At baseline examination all mothers underwent standardized interviews conducted by trained interviewers during hospitalization after delivery. Interviews included detailed questions about family demographics, socioeconomic status, housing and living conditions, and family history. Maternal and paternal atopy was recorded from the parents by means of a questionnaire at baseline. The question was (for both the father and the mother separately): 'Has a physician ever diagnosed one of the following atopic diseases?' Then, 'AD', 'asthma', 'atopic rhinitis', and 'urticaria' were listed as possible answers. A history of atopic diseases for the mother or, respectively, for the father was assumed if at least one disease was ever diagnosed by a physician. The interview was also conducted in Turkish and Russian as they were the mothers tongue of a large proportion of mothers.

Evaluation of atopic diseases in children

At both follow-up examinations, the parents again filled out a detailed mailed questionnaire and medical information was also provided from the child's primary health care paediatricians at both time-points, who gave a detailed medical history during the first and second year of life (in Germany, children usually undergo several screening health examinations including doctor visits after the first and second year of life). In particular, the physicians answered the following questions at the 1- and 2-year follow-up: 'Has a physician

diagnosed one of the following atopic diseases?' Then, 'AD' and 'asthma bronchiale or obstructive bronchitis' were listed as possible answers (yes, no categories).

Evaluation of breastfeeding and collection of breast milk

All women included at baseline were contacted by phone 6 weeks and 6 months post-partum and were asked whether they were breastfeeding at that time (and if not how long they did). If the mother continued to breastfeed longer than 6 months, the information was supplemented from the mothers' questionnaire response of the 1-year follow-up and, if breastfed longer than 1 year, from mothers' questionnaire response of the 2-year follow-up. Exclusive breastfeeding was defined as no nutrition other than breast milk and eventually tea.

A trained nurse visited all women who were still breastfeeding 6 weeks post-partum and collected 10 mL of breast milk, which was immediately aliquoted and frozen at -80°C until analysis. It was possible to obtain milk samples from 98% of the mothers who were breastfeeding at that time ($n = 636/647$).

Measurement of soluble CD14 in breast milk

Levels of sCD14 in breast milk were determined by a commercial sandwich-type ELISA (IBL, Hamburg, Germany), using an oligoclonal capture antibody, followed by an enzyme-tagged monoclonal detection antibody. This ELISA measures only free sCD14 and not sCD14 bound in any complex with LPS. The protocol was slightly modified: to remove fat, we centrifuged breast milk for 5 min at 12350 g in a Heraeus Biofuge (Kendro Laboratory Products GmbH, Langenselbold, Germany), and used the fat-free phase for determination of sCD14. As sCD14 levels in human breast milk are about three times higher than in human serum, we used a predilution of 1:401 or 1:801 instead of 1:101.

Statistical analyses

The main characteristics of the study population and the sCD14 concentrations in breast milk were summarized in a descriptive way. Then, the relationship between various possible determinants and the distribution (in quartiles) of sCD14 in breast milk was quantified by means of a χ^2 test. The cumulative incidence of AD and asthma (both as reported from the caring physicians) was then enumerated. The association of duration of breastfeeding, respectively, duration of exclusive breastfeeding, sCD14 concentration in breast milk, and the cumulative incidence rate of AD and of asthma was assessed by a χ^2 test, after stratification for maternal history of atopy, and, in summary, after adjustment for the latter factor by calculating a Mantel-Haenszel χ^2 statistic; if expected cell numbers were <5 , Fisher's exact test (two-sided) was used. In addition, among children who were breastfed, a test for trend was performed. We then used unconditional logistic regression to estimate prevalence odds ratios (OR) and their 95% confidence interval (CI) to describe the independent association of duration of breastfeeding, and exclusive breastfeeding (independent variables) with cumulative incidence of AD or asthma in children (dependent variables). The following covariates were adjusted for in the models: atopy of father (yes, no), ethnic origin of mother (German, other), and duration of school education (≤ 9 , >9

years). Finally, we compared the association of sCD14 concentration in breast milk (lower two quartiles vs. upper two quartiles) with AD and asthma in categories of breastfeeding (≥ 6 months, respectively, ≥ 3 months in exclusive breastfeeding). All analyses were carried out with the SAS statistical software package (SAS Institute, Inc. SAS Language: Reference. Version 8, 1st ed. Cary, NC, USA).

Results

Overall, 803 mothers and their children were included in this analysis. Table 1 shows some major characteristics of the study population. Most of the families were of German ethnicity. The mean age of the mothers was 31.5 years. Thirty-nine percent of the mothers breastfed the children 9 months or longer, whereas 38.5% reported exclusive breastfeeding for a duration of 6 months or longer, with no nutrition other than breast milk and eventually tea. The median concentration of sCD14 in breast milk collected 6

weeks post-partum was 25.0 $\mu\text{g/mL}$ with an interquartile range from 19.6 to 30.6 $\mu\text{g/mL}$.

Table 2 shows the association of levels of potential determinants with the sCD14 levels in breast milk in the upper quartile of the overall distribution. Mothers of nationality other than German, mothers aged younger than 25 years, and mothers with a school education of 9 years or less had the highest proportions of high sCD14 levels. A maternal history of atopic disease, parity, body mass index, smoking, and alcohol consumption (both during pregnancy) were not related to sCD14 concentrations in breast milk.

Overall, for 165 (20.6%) of the 2-year-old children an AD was reported and asthma was reported for 157 (19.6%) of the children by the caring physicians. There was no statistically significant difference in incidence rates with respect to ethnic origin of the mother ($P = 0.5$ and 0.7 , respectively).

Table 3 shows the cumulative incidence of AD in categories of breastfeeding and sCD14 concentration of breast milk (as measured in samples collected 6 weeks post-partum). In

Table 1. Some major characteristics of the study population

	N (total = 803)	%
Sex of the children		
Male	420	52.4
Female	382	47.6
Nationality of children		
German	723	90.0
Turkish	19	2.4
Other*	61	7.6
Number of siblings		
None	386	48.1
One	304	37.9
Two	85	10.6
Three or more	27	3.4
School education of mother (years)		
≤ 9	146	18.2
10–11	322	40.1
≥ 11	324	40.4
Unknown	11	1.4
Age in years (<i>n</i> , mean (SD)) of		
Mother	802	31.5 (4.7)
Father	799	33.9 (5.4)
Duration of breastfeeding (months)		
Never	76	9.5
<3	91	11.3
3 to <6	131	16.3
6 to <9	190	23.7
≥ 9	315	39.2
Duration of exclusive breastfeeding (months)		
Never	94	11.7
<3	134	16.7
3 to <6	266	33.1
≥ 6	309	38.5
sCD14 concentrations in breast milk† ($\mu\text{g/mL}$) (<i>n</i> , median) (25th/75th percentile)	636	25.0 (19.6/30.6)

*Other included Italy ($n = 6$), former Yugoslavia ($n = 6$), Poland ($n = 5$), Austria ($n = 5$), Hungary ($n = 4$), and others. †Collected 6 weeks post-partum.

sCD14, soluble CD14.

Table 2. sCD14 in breast milk of breastfeeding mothers collected 6 weeks post-partum according to potential determinants

		Number and proportion in upper quartile of sCD14 distribution		
	<i>N</i> (total = 636)	<i>n</i>	%	<i>P</i> -value
Nationality of mother				
German	570	133	23.3	0.01
Other	66	23	34.9	
Maternal history of atopy				
No	455	106	23.3	0.07
Yes	181	50	27.6	
Parity				
First	304	89	26.3	0.7
Second	236	54	22.9	
Third	73	14	19.2	
Fourth	23	8	34.8	
Age of mother (years)				
16–25	59	22	37.3	0.03
>25–35	443	102	23.0	
>35	134	32	23.9	
Body mass index (kg/m ²)				
<20	85	23	27.1	0.8
20<25	381	91	23.9	
25<30	133	34	25.6	
30+	35	8	22.9	
School education of mother				
≤9 years	103	33	32.0	0.006
10–11 years	250	59	23.6	
≥11 years	281	64	22.8	
Alcohol consumption during pregnancy				
No	510	128	25.1	0.2
Sometimes	124	28	22.6	
Smoking during pregnancy				
No	227	50	22.0	0.8
Yes	46	9	19.6	

sCD14, soluble CD14.

Table 3. Cumulative incidence of atopic dermatitis (as reported from the caring physician) in 2-year-old children and association* with breastfeeding practice and sCD14 levels in breast milk

	Cumulative incidence of atopic dermatitis in 2-year-old children					
	Maternal history of atopic disease				All†	
	No		Yes			
	<i>n</i>	%	<i>n</i>	%		
Duration of breastfeeding (months)						
Never	6/55	10.9	4/21	19.1	10/76	13.2
<3	16/63	25.4	9/28	32.1	25/91	27.5
3 to <6	21/104	20.2	8/27	29.6	29/131	22.1
6 to <9	16/129	12.4	18/61	29.5	34/190	17.9
≥9	44/217	20.3	23/98	23.6	67/315	21.3
	<i>P</i> trend = 0.49		<i>P</i> trend = 0.30		<i>P</i> trend = 0.24	
	<i>P</i> = 0.09		<i>P</i> = 0.75		<i>P</i> = 0.16	
sCD14 concentration in breast milk in mothers with ≥6 months breastfeeding						
1st and 2nd quartile	31/171	18.1	21/73	28.8	52/244	21.3
3rd and top quartile	28/163	17.2	19/77	24.7	47/240	19.6
	<i>P</i> = 0.82		<i>P</i> = 0.57		<i>P</i> = 0.60	

**P*-value (*P*) for overall difference among all categories and *P* for trend (*P* trend) among children who were breastfed. †*P*-values after adjustment for maternal history of atopic diseases.

sCD14, soluble CD14.

general, the cumulative incidence of AD was higher in children with a maternal history of atopic disease compared with other children. It was lowest in the small group of children who have never been breastfed ($n = 76$) and, if breastfed, decreased with increasing duration of breastfeeding. These patterns, although not statistically significant, were tentatively similar whether duration of exclusive breastfeeding was considered (data not shown). In the groups of children who were breastfed more than 6 months, the cumulative incidence of AD was similar irrespective of sCD14 concentration in breast milk. If exclusive breastfeeding was considered, the rates were tentatively lower if the group with sCD14 breast milk levels in the upper two quartiles was compared with the group with levels in the first two quartiles, but again this difference was not statistically significant (data not shown).

Table 4 shows the cumulative incidence of asthma in categories of duration of breastfeeding and sCD14 concentration of breast milk. Here, a protective effect of duration of breastfeeding with cumulative incidence of asthma was evident, which reached statistical significance in the group of children with no history of maternal atopic disease and, overall, after adjustment for maternal history of atopic diseases. In the group of children who were breastfed more than 6 months, the cumulative incidence was tentatively lower in children whose mothers had a sCD14 concentration in breast milk in the upper two quartiles compared with children whose mothers had a sCD14 concentration in the lower two quartiles. Considering the duration of exclusive breastfeeding, the patterns were tentatively similar and if breastfed, the duration of exclusive breastfeeding was associated with decreasing risk of asthma after adjustment for maternal history of atopic disease (*P* trend = 0.02).

Table 5 shows the results of the multivariate analysis for AD. The few children who had never been breastfed had a tendency towards lower risk of AD but the CI included the

null effect value in both strata. Compared with children who were breastfed for less than 3 months, children of mothers with no history of atopic disease who were breastfed between 6 and <9 months had the lowest OR = 0.42 (95% CI 0.19–0.91). In children of mothers with a history of atopic diseases, the risk decreased with increasing duration of breastfeeding; however, it did not reach statistical significance. When sCD14 levels were also considered, no major differences could be observed when concentrations in the lower two quartiles were compared with concentrations in the upper two quartiles in children who were breastfed ≥6 months. Likewise, no significant association with duration of breastfeeding of exclusive breastfeeding or with sCD14 levels among those who were exclusively breastfed ≥3 months was seen (data not shown).

Table 6 shows the results of the multivariate analysis for asthma. Again, the few children who had never been breastfed had a tendency towards a lower risk for asthma; however, the CI included the null effect value in both strata. In children of mothers without a history of atopic diseases, the risk decreased with increasing durations of breastfeeding (*P* for trend = 0.02). In children of mothers with a history of an atopic disease, the risk also decreased with a longer duration of time; however, the differences were not statistically significant. When exclusive breastfeeding was considered, children of mothers with no history of atopic diseases had a statistically significantly lower risk if exclusively breastfed ≥3 months when compared with children exclusively breastfed <3 months.

In children who were breastfed ≥6 months, the OR for asthma was lowest in the category with the highest sCD14 levels in mothers' breast milk, and it reached statistical significance in children of mothers with no history of atopic diseases (*P* for trend = 0.0005). This pattern was similar when the duration of exclusive breastfeeding and sCD14 levels were considered (*P* for trend = 0.005).

Table 4. Cumulative incidence of asthma (as reported by the caring physician) in 2-year-old children and association* with breastfeeding practice and sCD14 levels in breast milk

	Cumulative incidence of asthma in 2-year-old children					
	Maternal history of atopic disease					
	No		Yes		All†	
	<i>n</i>	%	<i>N</i>	%	<i>n</i>	%
Duration of breastfeeding (months)						
Never	11/55	20.0	5/21	23.8	16/76	21.1
<3	23/63	36.5	6/28	21.4	29/91	31.9
3 to <6	23/104	22.1	4/27	14.8	27/131	20.6
6 to <9	25/129	19.4	9/61	14.8	34/190	17.9
≥9	37/217	17.1	14/98	14.3	51/315	16.2
	<i>P</i> trend = 0.003		<i>P</i> trend = 0.44		<i>P</i> trend = 0.003	
	<i>P</i> = 0.02		<i>P</i> = 0.76		<i>P</i> = 0.01	
sCD14 concentration in breast milk in mothers with ≥6 months of breastfeeding						
1st and 2nd quartile	37/171	21.6	11/73	15.1	48/244	19.7
3rd and top quartile	23/163	14.1	10/77	13.0	33/240	13.8
	<i>P</i> = 0.07		<i>P</i> = 0.71		<i>P</i> = 0.09	
Duration of exclusive breastfeeding (months)						
Never	14/71	19.7	5/23	21.7	19/94	20.2
<3	28/96	29.2	7/38	18.4	35/134	26.1
3 to <6	38/198	19.2	15/68	22.1	53/266	19.9
≥6	39/203	19.2	11/106	10.4	50/309	16.2
	<i>P</i> trend = 0.09		<i>P</i> trend = 0.10		<i>P</i> trend = 0.02	
	<i>P</i> = 0.19		<i>P</i> = 0.17		<i>P</i> = 0.09	
sCD14 concentration in breast milk in mothers with ≥3 months of exclusive breastfeeding						
1st and 2nd quartile	22/98	22.5	5/50	10.0	27/148	18.2
3rd and top quartile	15/97	15.5	5/49	10.2	20/146	13.7
	<i>P</i> = 0.21		<i>P</i> = 0.97		<i>P</i> = 0.28	

**P*-value (*P*) for overall difference among all categories and *P* for trend (*P* trend) among children who were breastfed. †*P*-values after adjustment for maternal history of atopic diseases.
sCD14, soluble CD14.

Table 5. Adjusted odds ratio (OR) with 95% confidence interval (CI) for atopic dermatitis in 2-year-old children according to breastfeeding practice and soluble CD14 (sCD14) levels in breast milk and history of atopic disease in mothers

	Odds ratio (95% CI) for atopic dermatitis in children after multivariate adjustment*	
	No history of atopic disease in mother	History of atopic disease in mother
Duration of breastfeeding (months)		
Never	0.36 (0.13–1.01)	0.49 (0.12–1.95)
<3	1 (Reference)	1 (Reference)
3 to <6	0.74 (0.35–1.56)	0.91 (0.28–2.97)
6 to <9	0.42 (0.19–0.91)	0.88 (0.32–2.41)
≥9	0.76 (0.39–1.47) <i>P</i> = 0.51†	0.59 (0.22–1.59) <i>P</i> = 0.16†
Duration of breastfeeding and sCD14 in breast milk (months)		
Never	0.43 (0.16–1.16)	0.62 (0.17–2.37)
<3	1 (Reference)	1 (Reference)
3 to <6	0.86 (0.42–1.79)	1.18 (0.39–3.62)
≥6 and		
sCD14 1st and 2nd quartile	0.77 (0.39–1.52)	1.09 (0.43–2.74)
sCD14 3rd and top quartile	0.71 (0.36–1.42) <i>P</i> = 0.18†	0.83 (0.33–2.10) <i>P</i> = 0.27†

*After adjustment for atopy of father, ethnic origin of mother, duration of school education of mother. †All *P*-value represents tests for trend among breastfed children.

Discussion

In this prospective birth cohort study including 803 children up to the age of 2 years, we found the lowest risk for a

physician-reported AD in children of mothers without a history of atopic diseases if breastfed for 6 to less than 9 months. Furthermore, we found an inverse association between duration of breastfeeding and risk of asthma, which

Table 6. Adjusted odds ratio (OR) with 95% confidence interval (CI) for asthma in 2-year-old children according to breastfeeding practice and soluble CD14 (sCD14) levels in breast milk and history of atopic disease in mothers

	Odds ratio (95% CI) for asthma in children after multivariate adjustment*	
	No history of atopic disease in mother	History of atopic disease in mother
Duration of breastfeeding (months)		
Never	0.44 (0.19–1.01)	1.17 (0.29–4.69)
<3	1 (Reference)	1 (Reference)
3 to <6	0.50 (0.25–1.00)	0.64 (0.15–2.67)
6 to <9	0.42 (0.21–0.82)	0.70 (0.21–2.32)
≥9	0.35 (0.18–0.66) <i>P</i> = 0.02†	0.70 (0.22–2.21) <i>P</i> = 0.69†
Duration of breastfeeding and sCD14 in breast milk (months)		
Never breastfed	0.50 (0.22–1.14)	1.16 (0.32–4.25)
<3	1 (Reference)	1 (Reference)
3 to <6	0.57 (0.29–1.13)	0.63 (0.17–2.41)
≥6 and		
sCD14 1st or 2nd quartile	0.56 (0.30–1.04)	0.72 (0.25–2.06)
sCD14 3rd or top quartile	0.31 (0.16–0.60) <i>P</i> = 0.0005†	0.62 (0.21–1.80) <i>P</i> = 0.55†
Duration of exclusive breastfeeding (months)		
Never	0.58 (0.28–1.21)	1.37 (0.36–5.22)
<3	1 (Reference)	1 (Reference)
3–6	0.55 (0.31–0.99)	1.40 (0.50–3.94)
≥6	0.56 (0.32–0.99) <i>P</i> = 0.08†	0.59 (0.21–1.71) <i>P</i> = 0.17†
sCD14 concentration in breast milk in mothers with ≥3 months of exclusive breastfeeding (months)		
Never	0.63 (0.30–1.30)	1.43 (0.40–5.11)
<3	1 (Reference)	1 (Reference)
≥3 and		
sCD14 1st and 2nd quartile	0.76 (0.44–1.31)	1.01 (0.38–2.68)
sCD14 3rd and top quartile	0.47 (0.26–0.85) <i>P</i> = 0.005†	0.90 (0.34–2.34) <i>P</i> = 0.78†

*After adjustment for atopy of father, ethnic origin of mother, duration of school education of mother. †All *P*-values represent tests for trend among breastfed children.

was especially evident in children of mothers without a history of atopic disease. This protective effect seemed to be synergistic with sCD14 concentrations in breast milk, supporting the idea that increased amounts of the sCD14 in breast milk might have an additional beneficial role in immunomodulation, which could decrease the risk of atopic diseases during early childhood.

Breastfeeding and risk of atopic diseases

To date many studies have been published on the association between breastfeeding and atopic diseases [11–13]. The Results are inconsistent (for details, Ref. [13]). Some studies described a beneficial effect of breastfeeding, no difference, or a positive association (increased risk if breastfed). However, many of the positive studies only reported crude associations, and did not or only incompletely control for potentially confounding factors. One other reason for a positive association may be that propaganda for breastfeeding for prophylaxis against atopic diseases may lead to a higher rate of breastfeeding in atopic families, and therefore the reported associations are not causal because of breastfeeding; instead, they may have a reversed causal relationship as mothers with a history of atopic diseases in their families may decide to breastfeed more often.

With respect to AD, the evidence so far is also quite controversial. The results of a meta-analysis [14] and more recent published studies support [15–18], however, a bene-

ficial effect of breastfeeding on AD, especially suggested in children of parents with a history of atopic disease and in combination with avoidance of solid food within the first months of life [18]. We also found the cumulative incidence of AD to decrease with increasing duration of breastfeeding. This pattern seemed even stronger in children without a history of maternal atopic disease, and it reached statistical significance in the 6 to <9 months category.

With respect to asthma, breastfeeding has been reported to reduce its risk [19, 20]. About 70% of wheezing episodes within the first year of life are associated with viral respiratory infections [21], and the latter are reduced by breastfeeding. The results of a prospective population-based study including 4089 children showed a clear protective effect of breastfeeding on the risk of asthma within the first 4 years of life, which was especially pronounced in children with parents without a history of atopic disease [22]. This is in line with our findings where a clear inverse association was evident, especially in children of mothers with no history of atopic diseases.

Soluble CD14 in breast milk

So far, very few data from large-scale epidemiological studies exist to describe the effects of sCD14 in breast milk on the immunocompetence of the newborn. We found higher levels of sCD14 in breast milk 6 weeks post-partum to be associated with ethnicity other than German, younger age, and a low school education. sCD14 in breast milk may have an

important role in cell-derived immunity. An *in vitro* study showed an important role of breast milk sCD14 in B cell growth and differentiation [9]. Although the function is not known yet, it may play a role in colonization of the gastrointestinal tract, and as a result in the generation of an atopic phenotype [23]. Jones et al. [10] in a small study, showed, lower levels of sCD14 in breast milk to be associated with eczema at 6 months of age. In our study, a protective synergistic effect of sCD14 levels and breastfeeding on the risk of asthma was suggested, especially in children of mothers without a history of atopic diseases.

Breastfeeding has many beneficial effects on a variety of infant illnesses as it contains a large number of bioactive compounds that confer specific and unspecific protection against infectious agents [24]. It may well confer a better protection against infectious agents that are suggested as risk factors for atopy in various studies [25], thus explaining the described patterns. That the few children (7.5%) who were not breastfed at all had a lower risk when compared with children who were breastfed for less than 3 months may be because of the fact that they comprise a special group of selected children. The mothers who did not breast feed at all had a lower school education, had a lower prevalence of atopic diseases, and also, less often they had a spouse with a history of atopic diseases. Finally, when adjusted for a variety of factors, the risk estimates of this relatively small category were not statistically significant and included the null effect value.

When considering our results, the following limitations must be considered: when assessing the outcomes, we only relied on the reports of the caring paediatricians. We could include follow-up information of the physicians of 75.3% of the subjects. Subjects lost to follow-up were less likely to be of German ethnicity. We cannot exclude that subjects who did not participate were either more or less likely to have children with atopic diseases. However, our incidence rates are in agreement with the results of another population-based birth cohort study from Germany, which reported identical cumulative rates for AD at age two as described in our study [26]. The results for asthma were also in line with other population-based studies [1].

In addition, we cannot rule out some misclassification of maternal and paternal history of atopic diseases and of duration of breastfeeding and exclusive breastfeeding, all of which relied on self-reports by the parents, even when we specifically asked for a history of physician-diagnosed atopic diseases among the parents. It is unlikely, however, that such a misclassification could explain the positive results of this study.

Furthermore, sCD14 levels in breast milk were only measured in 6-week milk samples. Although concentrations are known to vary with the course of the lactation period [10], inter-individual differences might be relatively stable over time. Unfortunately, in this study, we were unable to take sCD14 levels in the serum of the children into account. CD14 levels in serum of the children may additionally determine the risk for atopy. As shown recently, blood cells of farmers' children (who are at a lower risk for atopic diseases) showed higher amounts of CD14 [27]. Furthermore, several polymorphisms on the child's side influence CD14 expression, thus aiding in determining T helper response and subsequently risk

of atopy after LPS contact [28]. Unfortunately, in this study, we could not take these issues into consideration as we had not collected blood from the children during follow-up.

Despite these limitations, the results of this prospective birth cohort study led us to the conclusion that breastfeeding does not increase the risk for atopic diseases in childhood. We even found a beneficial association, especially in children of mothers without a history of atopic disease. The beneficial effects of breastfeeding, especially on the incidence of asthma, might be further supported by high levels of sCD14 in breast milk.

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