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Breast Milk Fatty Acids May Link Innate and Adaptive Immune Regulation: Analysis of Soluble CD14, Prostaglandin E_2, and Fatty Acids

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ABSTRACT: In addition to its role in sensing intraluminal microbial antigens, soluble (s)CD14 may regulate immune responses by its lesser known function as a lipid carrier with possible influences in the production of fatty acid-derived eicosanoids. We investigated the interrelations of fatty acids, prostaglandin E_2 (PGE_2), and sCD14 and their role in infant atopic eczema during the first year of life. Serum and breast milk samples from mothers and serum samples from their infants were collected at infant's age 3 mo and analyzed for sCD14 and PGE_2 concentrations and for fatty acid compositions. The main correlation of sCD14 was with arachidonic acid (20:4n-6) (AA). Dihomo-γ-linolenic acid (20:3n-6) (DHGLA) and the ratio of n-6 to n-3 fatty acids correlated positively and docosahexaenoic acid (22:6n-3) (DHA) and sum of n-3 fatty acid negatively with PGE_2 in mother's serum and linoleic acid (LA) negatively with PGE_2 in breast milk. Soluble CD14 tended to be higher and LA, total polyunsaturated fatty acid (PUFA), and sum of n-6 fatty acids were lower in breast milk received by infants with atopic eczema compared with those without. These results suggest that fatty acids contribute to the regulation of innate and adaptive immune responses and link intraluminal exposures, mother's diet, and microbes. **(*Pediatr Res* 59: 723–727, 2006)**

Characteristic dietary habits in Western societies have constantly been linked to chronic diseases such as diabetes, cardiovascular diseases, and more recently allergic diseases. The accumulating data point to the early fetal period and infancy as the critical phase (1). Specific dietary compounds, including fatty acids and probiotics, have been exploited in the development of prophylactic and therapeutic interventions, the effects ensuing through their immunomodulatory properties (2). For the infant, the effects are drawn together by outstanding breast milk composition, providing not only nourishment but also signals for the maturation of the adaptive immune responses considered imperative with regard to the risk of diseases (3).

Although the major function described for soluble (s)CD14, a pattern recognition receptor, is binding of lipopolysaccharides and thus antimicrobial host defense (4), its binding specificity is not restricted to bacteria. It also binds phospholipids, thus providing a lipid transfer system (5). This innovative function of sCD14 may prove significant, with a potential to modify immune events through phospholipid polyunsaturated fatty acids (PUFAs). Both dietary and cellular fatty acids are evidently important immune regulators via their derivative eicosanoids, particularly PGE_2 and consequent cytokine responses, as suggested in allergic disease (6). The concentration of sCD14 in breast milk has been shown to be up to 20-fold higher than in serum (7), thus providing infants with an external source of sCD14 with potential health benefits as previously observed in allergic disease (8). Experimental studies have linked sCD14 to phospholipid transport in blood (5,9) and a positive correlation between sCD14 and n-3 fatty acids in breast milk has been observed (10), but no studies have examined the relationships, if any, among sCD14, PUFAs, and their derivative eicosanoids.

We sought here to investigate the lesser known function of sCD14 as a lipid carrier and thus the possible immunomodulatory route by evaluating the associations prevailing between serum and breast milk sCD14, fatty acid compositions, and PGE_2. We also explored the associations of these immunomodulatory factors between mother's or infant's serum and breast milk and their role in atopic eczema in the infant.

METHODS

Subjects and study design. A cohort of mothers (n = 38) with atopic disease (asthma, atopic eczema, allergic rhinitis) and their infants were followed from the end of gestation until the infant's age of 1 y (11). Infants were clinically examined at 1, 3, 6, and 12 mo of age. Their weights and heights were measured and hospital records examined for birth data. Atopic eczema was diagnosed as the presence of pruritus, facial and/or extensor involvement, and chronic course of at least 1 mo (12) with positive skin prick test result measured as previously described (11). The study was approved by the Ethical Committee of the Hospital District of South-West Finland, and informed written consent was obtained from the mothers.

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Abbreviations: AA, arachidonic acid, 20:4n-6; **DHA,** docosahexaenoic acid (22:6n-3); **DHGLA,** dihomo-γ-linolenic acid, 20:3n-6; **IQR,** interquartile range; **LA,** linoleic acid, 18:2n-6; **PGE_2,** prostaglandin E_2; **PUFA,** polyunsaturated fatty acid

Analytical methods. Serum samples from mothers and infants and breast milk samples were collected at the infants' age of 3 mo. Samples were stored at −70°C until analyzed. For the analysis of sCD14, the serum or breast milk samples were thawed, mixed, and centrifuged at 14926 × *g* for 10 min at room temperature to separate fat and cells from whey. The concentration of sCD14 (detection limit: 125 pg/mL) in serum or in whey of breast milk were determined using commercial sandwich enzyme-linked immunosorbent assay (ELISA) specific to these molecules (R&D Systems Europe Ltd., Abingdon, UK) as recommended by the manufacturer. For PGE_2 analysis, the samples were first purified by minicolumns (RPN 1900, Amersham Biosciences, Buckinghamshire, UK) and PGE_2 (detection limit: 3.1 pg/mL) was then measured by radioimmunoassay using reagents from Amersham Biosciences (RPA530).

Serum phospholipid fatty acid compositions of mother and infant and the fatty acid composition of breast milk were analyzed. Total lipids in the samples were extracted with chloroform:methanol 2:1 vol/vol (13). Polar lipids were separated from neutral lipids by solid phase extraction with silica columns (14). As samples were assumed not to contain significant amounts of FFA or mono- or diacylglycerols, the polar lipid fraction was taken to consist of phospholipids. Fatty acid methyl esters were prepared from breast milk lipids and the isolated polar lipid fraction with boron trifluoride and analyzed by gas chromatography (PerkinElmer AutoSystem, Norwalk, CT), with DB-23 column (60 m × 0.25 mm i.d., 0.25 μm film thickness; Agilent Technologies, Palo Alto, CA). Diheptadecanoylphosphatidylcholine (Sigma Chemical Co.–Aldrich, St. Louis, MO) was used as an internal standard in plasma samples and triheneicosanoin (Sigma Chemical Co.–Aldrich) in breast milk samples. Breast milk total triacylglycerol concentration was determined in relation to the internal standard.

Statistics. Values are reported as medians with interquartile range (IQR) and as means with ranges for descriptive subject characteristics. Sufficient amounts of breast milk were available from 38 mothers and serum from 35 mothers and 22 infants for all laboratory analyses. Significant correlations were analyzed by Spearman's rank correlation coefficient (ρ) and differences between infants with and without atopic eczema by Mann-Whitney *U* test. All statistical analyses were performed on SPSS for Windows (version 12.0.1; SPSS Inc., Chicago, IL).

RESULTS

All infants were born full-term (mean, 39 wk; range, 37–42 wk of gestation) with a mean birth weight of 3590 g (range, 2730–4890) g and a height of 51 cm (range, 47–55) and were being exclusively or predominantly breast-fed at the age of 3 mo. Atopic eczema with chronic or recurrent eczema from 1 to 12 mo of age and a positive skin prick test result for at least one sensitizing antigen was observed in 29% of the infants (11 of 38 infants).

Mother's and infant's serum and breast milk sCD14 and PGE_2 concentrations and fatty acid compositions are shown in Table 1. The total triacylglycerol content in breast milk was 33 g/L (range, 24 to 54). Associations between mother's serum and breast milk composition were evaluated by correlations. Neither mother's serum sCD14 [$\rho = -0.13$, not significant (NS)] nor PGE_2 ($\rho = 0.21$, NS) correlated with those of breast milk. Of the PUFAs in the mother's serum, α-linolenic acid (18:3n-3; $\rho = 0.44$, $p = 0.009$), eicosapentaenoic acid (20:5n-3; $\rho = 0.94$, $p < 0.001$, DHA (22:6n-3; $\rho = 0.89$, $p < 0.001$), AA ($\rho = 0.56$, $p = 0.001$), sum of n-3 fatty acids ($\rho = 0.46$, $p = 0.006$), and the ratio of n-6 to n-3 fatty acids ($\rho = 0.51$, $p = 0.002$) correlated with those in breast milk.

The relationships between mother and infant were examined by calculating correlations for compositions between breast milk and infant's serum and between mother's and infant's serum. Mother's serum sCD14 ($\rho = 0.48$, $p = 0.003$; Fig. 1) and PGE_2 ($\rho = 0.60$, $p < 0.001$) correlated positively with those in infant's serum, and additionally an inverse correlation was detected between mother's serum PGE_2 and infant's serum DHA ($\rho = -0.44$, $p = 0.03$) and the sum of PUFAs ($\rho = -0.43$, $p = 0.04$). Breast milk sCD14 or PGE_2 did not correlate with infant's serum composition, but breast milk PUFA composition was reflected in the infant's serum, as correlations were observed in breast milk for DHGLA (20:3n-6; $\rho = 0.45$, $p = 0.02$), AA ($\rho = 0.51$, $p = 0.01$), eicosapentaenoic acid ($\rho = 0.84$, $p < 0.001$), DHA ($\rho = 0.83$, $p < 0.001$), sum of PUFAs ($\rho = 0.49$, $p = 0.01$), and ratio of n-6 to n-3 fatty acids ($\rho = 0.45$, $p = 0.02$) with those in infant's serum.

The hypothesis on the relationships between sCD14, PGE_2, and fatty acids was evaluated by calculating their correlations within each medium. In mother's serum, sCD14 correlated positively with AA ($\rho = 0.43$, $p = 0.01$, $n = 35$) and negatively with 20:2n-6 ($\rho = -0.48$, $p = 0.003$) of the fatty acids, whereas no correlation was detected with PGE_2 ($\rho =$

Table 1. *Breast milk and mother's and infant's serum sCD14 (μg/mL) and PGE_2 (pg/mL) concentrations and fatty acid composition (% of total fatty acids)*

	Breast milk ($n = 38$)		Mother's serum ($n = 35$)		Infant's serum ($n = 24$)	
	Median	IQR	Median	IQR	Median	IQR
sCD14	9.0	7.1–11.8	1.3	1.1–1.4	1.0	0.9–1.13
PGE_2	464	312–868	63	36–145	61	35–159
Fatty acid						0.19–0.29
18:3n-3	1.72	1.47–2.32	0.38	0.32–0.49	0.21	9–0.29
20:5n-3	0.11	0.09–0.16	2.00	1.46–2.66	0.98	0.78–1.47
22:6n-3	0.31	0.19–0.42	4.05	2.96–4.79	6.02	4.56–6.77
sum n-3	2.41	1.99–2.80	6.32	5.22–8.06	7.39	5.73–8.43
18:2n-6	9.59	8.89–11.19	21.69	19.72–23.31	18.89	17.94–20.88
18:3n-6	0.09	0.07–0.12	0.08	0.06–0.11	0	0–0.05
20:2n-6	0.21	0.19–0.22	0.43	0.39–0.47	0.41	0.38–0.46
20:3n-6	0.28	0.26–0.33	3.03	2.50–3.51	2.97	2.56–3.19
20:4n-6	0.35	0.32–0.38	8.55	8.02–9.19	10.43	9.12–11.78
sum-6	10.55	9.81–12.13	34.26	32.12–35.06	33.17	32.32–34.31
sum n-6/sum n-3	4.54	3.87–5.16	5.59	4.09–6.56	4.50	3.85–5.98
Total polyunsaturated fatty acids	12.81	11.81–14.96	40.34	39.69–41.44	40.59	40.07–41.05

The results are presented as median and IQR.

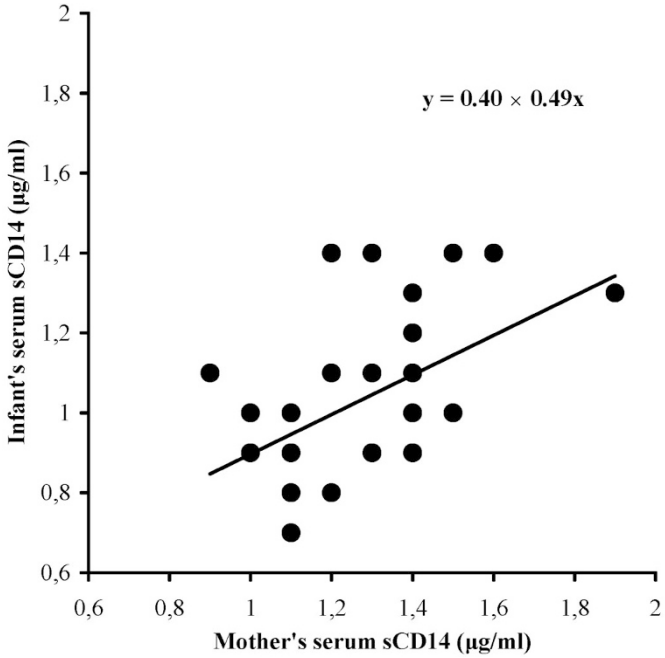

Figure 1. Association between mother's and infant's serum soluble (s)CD14 concentration ($n = 22$). Spearman rank correlation coefficient $\rho = 0.48$, $p = 0.003$. Regression equation by linear regression analysis.

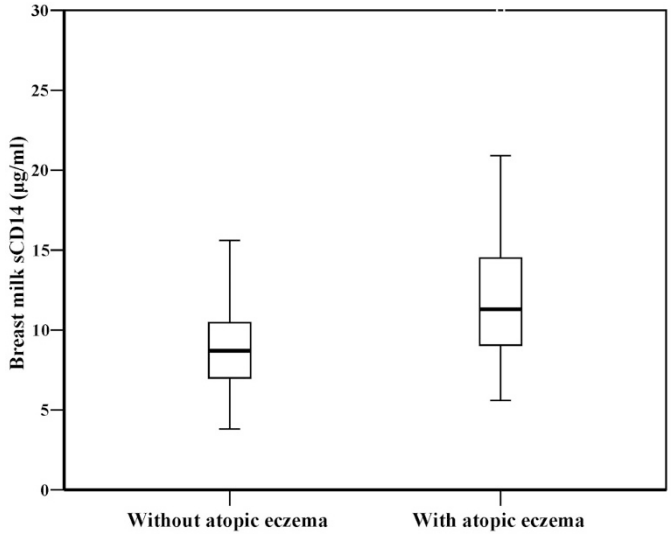

Figure 2. Soluble (s)CD14 concentrations in breast milk received by infants with ($n = 11$) and without ($n = 27$) atopic eczema. Difference between the groups $p = 0.05$, Mann-Whitney U test.

0.03, NS). PGE_2 in turn correlated positively with DHGLA ($\rho = 0.44$, $p = 0.008$) and the ratio of sum of n-6 to the sum of n-3 fatty acids ($\rho = 0.41$, $p = 0.01$), and negatively with DHA ($\rho = -0.44$, $p = 0.009$) and sum of n-3 fatty acid ($\rho = -0.44$, $p = 0.008$) in mother's serum. In infant's serum, no correlations between sCD14 and PGE_2 or fatty acids were detected, but PGE_2 correlated positively with DHGLA ($\rho = 0.42$, $p = 0.04$, $n = 24$). In breast milk, a negative correlation was found between sCD14 and γ-linolenic acid (18:3n-6; $\rho = -0.48$, $p = 0.002$). PGE_2 in breast milk was inversely correlated with LA (linoleic acid) (18:2n-6; $\rho = -0.33$, $p = 0.04$). The total triacylglycerol content in breast milk correlated with both sCD14 ($\rho = 0.360$, $p = 0.026$) and PGE_2 ($\rho = 0.387$, $p = 0.022$).

Differences in the concentrations of sCD14 and PGE_2 and fatty acid composition were evaluated between infants with and without atopic disease. There was a tendency for sCD14 to be higher in breast milk received by infants with atopic eczema [11.3 μg/mL (IQR, 8.3–14.7)] compared with those without [8.7 μg/mL (IQR, 6.8–10.7), $p = 0.05$, Fig. 2]. No differences were observed either for mother's or infant's serum sCD14 or PGE_2 in breast milk or in mother's or in infant's serum according to infant's atopic disease status (data not shown). LA [9.1 (IQR, 8.1–10.2) *versus* 10.3 (IQR, 9.7–11.0), $p = 0.04$], total PUFA [12.4 (IQR, 10.8–14.0) *versus* 13.9 (IQR, 13.0–14.8), $p = 0.04$] and sum of n-6 fatty acids [10.1 (IQR, 9.0–11.2) *versus* 11.3 (IQR, 10.6–12.0), $p = 0.04$] were lower in breast milk received by infants with atopic eczema compared with those without, whereas no differences were observed between the groups in other PUFAs in breast milk or PUFAs in infant's serum (data not shown).

DISCUSSION

The significance of diet in early infancy for the maturing immune system to restrain disease and to ensure normal growth and development has received increasing attention with knowledge accruing. We now investigated the associations among breast milk and serum sCD14, PGE_2, and fatty acids and also differences in them between infants with and without atopic eczema. Our results show that sCD14 and PGE_2 levels are reflected by fatty acid compositions and that these are linked to the manifestation of atopic eczema in the infant.

Our finding of a correlation between mother's and infant's serum sCD14 support the previous conception that the serum sCD14 concentration is regulated by genetic variation (15,16). No associations were observed between mother's serum and breast milk sCD14, which was expected, as a mammary epithelial cell line has been shown to produce sCD14 (7). Nevertheless, the fatty acid composition in mother's serum was associated with that of breast milk and, further, breast milk fatty acid composition was reflected in the infant's serum (17–20). The breast milk composition could thus be amenable to maternal dietary interventions, also suggested previously (10,21).

What would be the basis for suggested relationship between sCD14 and PGE2? In addition to its well-known function in binding of lipopolysaccharides, sCD14 also facilitates the transport of phospholipids and hence the release of fatty acids (5,9), possibly influencing the production of PUFA-derived eicosanoids, particularly AA-derived PGE_2 with properties engaging in immune regulation via immunoglobulin E production (22). Such a view is supported by the result in the present study showing an association between sCD14 with AA and with 20:2n-6 in mother's serum. The association was not, however, seen in infant's serum or in breast milk, but sCD14 in breast milk was positively associated with total breast milk triacylglycerols. Further, although bearing in mind

the small proportion of γ-linolenic acid detected in the samples, an inverse association between breast milk sCD14 and γ-linolenic acid was detected, possibly reflecting the rapid turnover of this precursor fatty acid for AA. Such an interrelation among the fatty acids and sCD14 suggests that sCD14 may regulate the transport of fatty acids, as shown in an experimental study (5) and would thus affect the production of immunomodulatory eicosanoids, giving further insight into the regulation of T helper 3 or T regulatory cells via fatty acids and PGE_2 (22). However, no evidence of an association between sCD14 and PGE_2 was detected, possibly reflecting the excessively indirect mode of assessing the association. Nevertheless, PGE_2 was found to correlate positively with n-6 fatty acids and negatively with n-3 fatty acids, as was expected (23,24). Although no associations between sCD14 and n-3 fatty acids were detected in the present study, a positive correlation has previously been observed between breast milk sCD14 and docosapentaenoic acid (22:5n-3) (10).

The present study showed that the fatty acid composition of breast milk influences the health status of the infant, as LA, the sum of n-6 fatty acids, and PUFAs were lower in breast milk received by infants with atopic eczema compared with those without. Furthermore, sCD14 tended to be higher in breast milk received by infants with atopic eczema. There are previous studies on record both supporting and conflicting with our data (25–28). The immunologic functions of fatty acids in allergy are generally seen to be proinflammatory because n-6 fatty acids may result in increased immunoglobulin E production (6), and against this background, n-3 fatty acid supplementations have been initiated (29,30). According to experimental studies, the availability of sCD14 appears to be rate limiting for the amount of lipid passing out from cell membranes to media (9). Considering our results on higher sCD14 in breast milk received by infants with atopic eczema and also the previous observation of a higher serum sCD14 concentration in atopic mothers compared with those without (31), it may be that the patients with allergic disease have a reduced capacity to exploit sCD14. Alternatively, breast milk sCD14 levels may play a role in mediating responses to beneficial bacteria (7), and the resultant reduced availability of sCD14 via breast milk would increase the risk of infant's eczema, as previously reported (8). Further, sCD14 has been associated with inhibition of immunoglobulin E production (15,32). However, in keeping with our results, no association between serum sCD14 and allergic disease status of the child has been observed (8,31), but higher cord blood sCD14 levels in children with transient atopy during a 5-y follow-up (8) and lower sCD14 levels in atopic children have been reported (33).

The present results on the role of breast milk sCD14 in atopic disease and on the associations of sCD14 with fatty acids appear to confound those of previous studies (8,10). Here, infant's atopic eczema was defined by the presence of chronic eczema with a positive skin prick test result and all mothers had allergic disease, deviating from findings of previous work (8,33), the distinct immunologic characteristics possibly explaining the results. Further, we did not modify mother's diet, in contrast to a previous study (10) in which the breast milk composition may have been influenced by fish oil supplementation. The resultant increase in cellular levels of n-3 fatty acids could give rise to counterregulation of n-6 fatty acids (34). Indeed, fish oil supplementation has been shown to reduce PGE_2 levels (35), and although n-3 fatty acids are generally considered beneficial for health due to their anti-inflammatory effects (36), adverse effects have recently been reported (37,38). In succession, n-6 fatty acids may exhibit a beneficial role in allergic disease via the effects of T helper 3 or T regulatory cells and thus via anti-inflammatory transforming growth factor β_2 and interleukin-10 production (22). In contrast to differences in breast milk composition, neither the infant's serum fatty acid composition nor sCD14 or PGE_2 concentrations differed according to the infant's atopic eczema status. This emphasizes the importance of the first-line defense of the gastrointestinal tract in developing diseases like allergy. Particular importance for the maturation of the innate immune defense system would appear to attach to breast milk composition, providing the infant with an external source of immunomodulatory factors (3).

Our study showed that fatty acids are correlated with the potential immunomodulators sCD14 and PGE_2 and that their composition in breast milk is related to the infant's atopic eczema. Further, intestinal microbes (39) and potential immunomodulators, probiotics, are suggested to exert their effects via similar signaling pathways (40,41). Host-microbe interaction, which is mediated through molecules like CD14, is important for the first-line host defense against allergic, infectious, and inflammatory diseases (42,43). An external source of sCD14 provided via breast milk may be of particular importance providing stimulus for the gut immune system (7). In view of the aberrant barrier functions of the skin epithelium and gut mucosa in children with atopic eczema, the joint effects of nutrients and probiotics promoting the defense mechanisms may confer an important therapeutic advantage (2). This concept calls for more precise analysis of joint effects, particularly when supplementing the diet is becoming an increasingly widespread practice. We advocate the concept that maternal dietary modification offers an intervention target to modulate the infant's innate immune system to benefit health. The results of the study have shown that dietary fatty acids are an important target, the potential immunomodulatory effects of which ensue through sCD14 and PGE_2.

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