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Inflammation markers and cytokines in breast milk of atopic and nonatopic women

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Background: Breast milk is thought to contain its own complex immune system. Whether or not this is altered in allergic individuals is not yet known.

Methods: By ELISA techniques, inflammatory markers (MIP-1 α , sICAM-1) and T_H1 (interferon-gamma [IFN- γ]), as well as T_H2 cytokines (interleukin [IL]-4, IL-10), were investigated in serum and milk samples from nonallergic ($n=23$) lactating women and those with respiratory allergies ($n=19$) during the first week postpartum.

Results: IFN- γ was not detected in either serum or milk. IL-10 was more often found to be above the detection limit in both milk and serum samples from allergic mothers. IL-4 was detected in almost all serum samples with a wide variation. In milk, IL-4 was found in about 20% of the samples. MIP-1 α was not detected in the serum but was detected in the milk of 23% of the nonatopic and 53% of the allergic mothers. Soluble ICAM-1 was present in all samples. Surprisingly, serum levels of sICAM-1 in allergic mothers (271 ± 97 ng/ml) were significantly lower ($P < 0.001$) than in nonatopic subjects (375 ± 86 ng/ml). Concentrations of sICAM-1 in milk were similar in both groups.

Conclusions: The concentrations of proinflammatory markers and cytokines in breast milk did not differ significantly between allergic and nonatopic mothers. In some individuals, high levels of MIP-1 α , IL-10, and sICAM-1 could be found. However, the significance of these components for the breast-fed infant is still unclear.

Human milk contains its own immune system consisting of a variety of components, such as macrophages, immunoglobulins, or antimicrobial proteins, which are thought to protect against infection and inflammation in the gastro-

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Table 1. Detection of cytokines and inflammation markers in serum and milk of nonallergic and allergic mothers (given as percentage of number of samples analyzed)

Parameter	Detection limit (pg/ml)	Serum		Milk	
		Nonallergic	Allergic	Nonallergic	Allergic
IFN- γ	2	None	None	None	None
IL-4	45	95%	94%	18%	24%
IL-10	5	9%	21%	17%	37%
MIP-1 α	7	None	None	23%	53%
sICAM-1	3×10^3	100%	100%	100%	100%

intestinal and respiratory tract of the human milk-fed infant. In addition, the presence of other potentially immunomodulating factors (e.g., complex oligosaccharides, growth factors, enzymes, hormones, or cytokines) has been acknowledged (1–3). These beneficial properties together with the high availability of nutrients and the low antigen content of human milk are the physiologic basis of the current recommendation by pediatric nutritional committees that breast milk is the best food for infants, especially those with a family history of allergies (4–6).

It has previously been reported that components that modulate allergic reactions, such as cytokines, chemokines, and adhesion molecules, are also secreted in milk at various stages of lactation (7–11). Although the role of proinflammatory and immune regulatory cytokines in the pathogenesis of allergies is becoming increasingly evident, these molecules have not been studied in the breast milk of allergic mothers so far.

In the pathogenesis of allergic reactions, the balance of T-helper cell types (T_{H1} and T_{H2}) seems to be changed, and the increase in T_{H2} cells is thought to play a critical role (12, 13). Therefore, we selected interferon-gamma (IFN- γ) as a characteristic T_{H1}-type cytokine and interleukin-4 (IL-4) and interleukin-10 (IL-10), produced by T_{H2} cells, for analysis in milk as well as in serum samples. Serum levels of IL-4 and IFN- γ are thought to be indicators for the deviation of T-cell function (14). In addition, two inflammatory markers, the chemokine macrophage inflammatory protein-1 alpha (MIP-1 α) and the soluble intercellular adhesion molecule-1 (sICAM-1), were included in this study, because both have been implicated in the pathogenesis of allergy; the early-response mediator MIP-1 α , as well as IL-4, promotes the mononuclear cell and eosinophil recruitment to the airways. On the other hand, IL-10 deactivates macrophage functions and thus exerts an immunosuppressive effect (15–18). Adhesion molecules such as ICAM-1 play a key role in the early stage of the

Table 2. Characteristics of patient population

	Nonallergic mothers (n=23)	Allergic mothers (n=19)
Age (years [range])	28 $\pm$ 6 (20–38)	29 $\pm$ 5 (20–39)
Delivery (% cesarean section)	39	42
Smokers (%)	26	10
Total IgE (kU/l) (mean $\pm$ SD [range])	42 $\pm$ 68 (2–310)	121 $\pm$ 226 (8–982)
IgE >100 kU/l (n)	3	4
Antigen-specific IgE (RAST-positive subjects)	4	19
Clinical symptoms (n)	1*	19

* Asthma attack caused by aspirin 2 years before this study.

signal cascade leading to cellular extravasation and development of the inflammatory response (19).

In lactating atopic mothers, it has not yet been shown whether or not the systemic effect of allergic reactions affects the immune system of breast milk. Cytokines, chemokines, or soluble adhesion molecules could be either beneficial or disadvantageous to the breast-fed infant. Thus, milk as well as serum was analyzed for cytokines and inflammatory markers to compare the milk composition in women with and without respiratory allergies, and to determine whether or not a correlation exists between serum and milk concentrations of these immune factors.

Material and methods

Subjects and sample collection

Lactating mothers of full-term neonates (n=42) were clinically categorized by family history, case history, and clinical status. Furthermore, a blood sample and a milk sample were collected at the same time 3–9 days/postpartum. The milk sample was obtained by manual expression at least 1 h after the last feeding.

Allergy testing

Blood samples were analyzed by RAST (Pharmacia & Upjohn GmbH, Freiburg, Germany) for total IgE and for specific IgE antibodies against common inhalant antigens (pollen of timothy grass, rye, birch, and mugwort as well as allergens of *Cladosporium herbarum*, house-dust mites, and

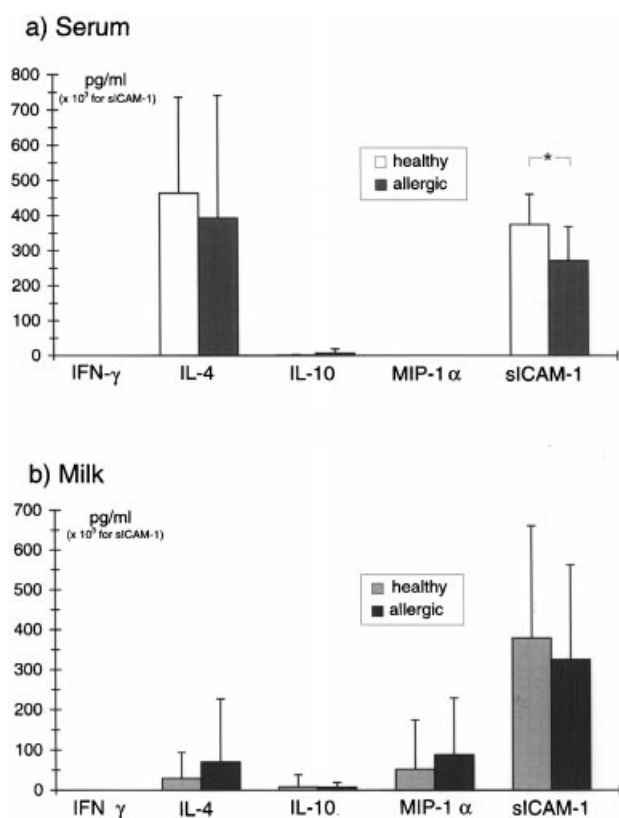


Figure 1. Concentrations of inflammation markers in serum and milk from nonatopic and allergic mothers (*significantly different at $P < 0.001$).

cat and dog dander}. Subjects were defined as “allergic” when both antigen-specific antibodies were detected (positive RAST; i.e., specific IgE > 0.7 kU/l) and clinical symptoms of respiratory allergies were reported.

Sample preparation

Serum was prepared from peripheral venous blood samples. Milk was centrifuged in two consecutive steps to remove

cells (2000 rpm, 10 min, room temperature) and fat (6000 rpm, 10 min, 4°C). Cell- and fat-free milk samples were then frozen at -20°C until further analysis.

Cytokine (IFN- γ , IL-4, and IL-10) and chemokine (MIP-1 α) assays

Cytokines and chemokines in serum and milk samples were detected with ELISA according to the manufacturer’s instructions. Detection of IL-4 and IL-10 was performed by two-step procedures (Genzyme, Cambridge, MA, USA). IFN- γ and MIP-1 α were measured in a similar fashion with a dual antibody sandwich ELISA (R&D Systems, Wiesbaden, Germany). Tetramethylbenzidine and hydrogen peroxide were used as substrates in a peroxidase reaction. Plates for chemokines and cytokines were read at 450 nm. The assay sensitivities as given by the manufacturers are shown in Table 1.

Assay for sICAM-1

Soluble ICAM-1 in serum and milk samples (defatted and cell-free) was determined with a sandwich ELISA in a one-step immunoreaction (Boehringer, Mannheim, Germany). The assay is based on the reaction of two monoclonal antibodies against different epitopes of sICAM-1. In one incubation step, sICAM-1 in standards and samples is simultaneously bound by the biotin-labeled antibody linked to the streptavidin-coated surface of the microtiter plate and by the peroxidase-conjugated anti-ICAM-1 antibody. Subsequent to the washing step, the peroxidase bound in the antibody/antigen-complex was developed with tetramethylbenzidine as a substrate and determined photometrically at 450 nm.

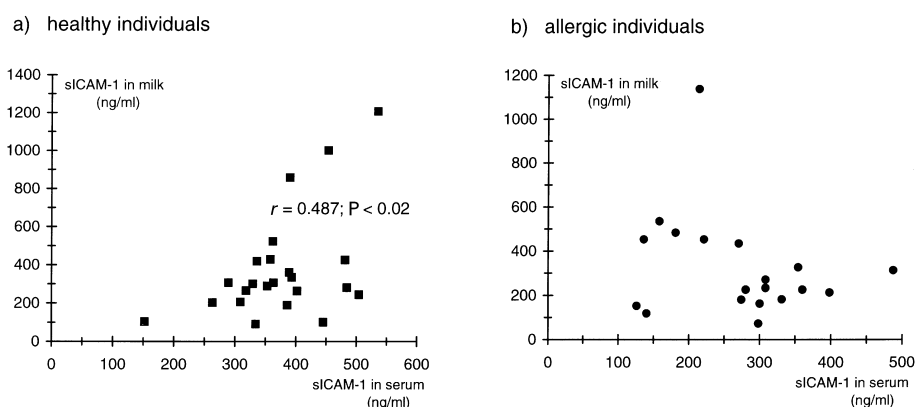


Figure 2. Correlation of sICAM-1 in serum and milk from nonatopic (■) and allergic (●) mothers.

Statistics

Statistical analysis was done by the software package SAS. The Wilcoxon test was performed for group comparison accepting a statistical difference at $P < 0.05$. For correlation analysis, the Pearson correlation coefficient is given when a significant correlation between serum and milk parameters was determined.

Results

As the criterion for a respiratory allergy, we used the presence of antigen-specific IgE in serum (positive RAST) in combination with a history of clinical symptoms (rhinoconjunctivitis and/or bronchial asthma) and categorized 19 mothers as “allergic”. The duration of allergic symptoms was at least 2 years; most of these mothers had had respiratory allergies for more than 5 years before pregnancy. However, 17 allergic mothers were reported to have been free of clinical symptoms since their pregnancy, although they were studied during an allergy season. No difference was found in age and method of birth between the groups of nonallergic and allergic mothers; however, more women in the nonallergic group were smokers. Total IgE, which is often used as an additional serologic parameter for the diagnosis of atopy, varied widely in our study and was not significantly different in the two groups. Neither the threshold value for IgE of 100 kU/l for this test nor the IgE level itself correlated well with the presence of clinical signs of respiratory allergies (Table 2).

Among the cytokines analyzed, IFN- γ was detected neither in the serum nor in the milk of either group. Analysis of IL-10 revealed low concentrations with maximum values of 7 and 20 pg/ml in serum and 143 and 48 pg/ml in milk of nonallergic and allergic subjects, respectively. Although IL-10 was more often found to be above the detection limit in milk and serum samples from mothers with respiratory allergies (Table 1), the mean differences were not significant due to the large variations (Fig. 1a, b). However, IL-4 was detected in almost all serum samples but in only about 20% of the milk samples of both nonallergic and allergic individuals. The concentrations varied widely and were found to be 464 ± 272 and 392 ± 348 pg/ml (mean $\pm$ SD) in the serum of nonallergic and allergic mothers, respectively (Fig. 1a). Lower concentrations of IL-4 were found in milk than in serum (29 ± 65 vs 70 ± 157 pg/ml, nonallergic vs allergic subjects, respectively), although

the differences between both groups were not statistically significant (Fig. 1).

The chemokine MIP-1 α was not detected in the serum but in the milk of 23 % of nonallergic and 53 % allergic mothers (Fig. 1a, b) with concentrations up to 504 and 569 pg/ml, respectively. Differences between the group means did not reach statistical significance.

Soluble ICAM-1 was the only inflammatory parameter that was present in all serum and milk samples we analyzed. Serum levels of sICAM-1 in allergic mothers (271 ± 97 ng/ml) were significantly lower ($P < 0.001$) than in nonallergic subjects (375 ± 86 ng/ml) (Fig. 1a). However, concentrations of sICAM-1 in milk were similar in both groups with very large variations (325 ± 238 vs 379 ± 282 ng/ml in allergic and nonallergic subjects, respectively) (Fig. 1b).

Correlations of serum and milk concentrations were investigated, and a significant positive trend was found only for sICAM-1 in healthy subjects ($r = 0.487$; $P < 0.02$) (Fig. 2a); no correlation, however, could be seen in mothers with respiratory allergies (Fig. 2b).

Discussion

Breast-feeding is currently recommended as the first choice in feeding infants at risk of developing allergies (4–6). However, it has not yet been determined whether the immune system in breast milk is altered in atopic women, an effect which might affect the recipient child. In the present study, we investigated whether or not inflammatory markers and cytokines regularly secreted with breast milk in healthy mothers (7–11) show a different profile in women with respiratory allergies. Lactating women were defined as allergic when both a positive case history was reported and a positive RAST was present. Serum samples were analyzed for T_H1-type cytokines (IFN- γ), T_H2-type cytokines (IL-4 and IL-10), and the inflammatory markers MIP-1 α and sICAM-1 to determine whether the immune system present in breast milk is affected by the profile of immune factors in serum.

The T_H1-type cytokine IFN- γ was not found in serum or milk from our subjects (Fig. 1a), although Sanchez-Guerrero et al. (20) reported increased serum levels of IFN- γ in allergic vs nonallergic asthmatic patients. However, IL-4, a T_H2-type cytokine known to function as an early response mediator in allergic airway inflammation (16), was detected in almost all serum samples. The concentrations varied widely (Fig. 1a), and no significant difference was found between allergic and nonallergic mothers. In contrast, the serum concentra-

tions of IL-10, a potent immunosuppressive component, tended to be higher in the allergic than the nonallergic group ($P < 0.08$).

Serum sICAM-1, which has been proposed as an indicator for acute-phase allergic reactions, was lower in mothers with respiratory allergies than in nonallergic subjects ($P < 0.001$). Since 17 out of the 19 allergic mothers in our study were reported to have been symptom-free throughout their pregnancy and during the investigation period, it is possible that sICAM-1 concentrations increase only after allergen provocation, as has been reported by Ciprandi et al. (21). These authors found that symptomless pollinosis patients and healthy subjects did not express ICAM-1 on epithelial cells. Shiota et al. (22) also observed that only active bronchial asthma is associated with the presence of increased levels of sICAM-1 in serum. Our serum data might be interpreted as an additional indication of the stable, symptomless condition which was reported by most of our allergic subjects for the time of their pregnancy. This is in agreement with observations by Schatz & Zeiger (23) that allergic rhinitis and asthma appear to improve in a number of women during pregnancy and the early postnatal period. According to Matthiesen et al. (24), a state of systemic suppression of the maternal immune system seems to be present during this period; i.e., the number of leuko- and lymphocytes responsible for the production of inflammation markers was found to be decreased.

However, the immune system of breast milk is mainly derived from the cellular activity of the mammary gland itself, where components such as secretory IgA, anti-microbial proteins, and complex carbohydrates are synthesized. In addition, immune cells secreted in milk, such as macrophages or lymphocytes, are thought to contribute to the effective host defense in early lactation. The origin of other, potentially immunomodulating compounds, such as cytokines or soluble adhesion molecules, is not yet known.

Concentrations of cytokines and soluble adhesion molecules in milk were compared with the corresponding serum levels in order to get an indication of the origin of milk components; however, there did not seem to be a clear correlation. Thus, it appears that the inflammation markers in milk are not directly derived from the blood. Possible sources of these compounds in milk are cellular components such as macrophages or epithelial cells found especially in early milk. Indeed, maternal cells in breast milk have been found to have mRNA for various cytokines, revealing a unique cytokine profile of breast milk (10). Cytokines such as IL-10 may be produced *in vivo* by mammary gland cells

(7). However, with regard to sICAM-1 we obtained similar results when we compared sICAM-1 in cell-containing milk and cell-free milk (data not shown). In this case, shedding of ICAM-1 from the cell surface of different cells within the mammary gland via shear force during milk expression might be a source of sICAM-1 in milk.

From the immunologic parameters we determined, IFN- γ and MIP-1 α were not detected in serum, but MIP-1 α was found in milk, more often from allergic than from nonallergic women. Srivastava et al. (10), who analyzed preterm and term milk for various other cytokines, did not detect MIP-1 α or IL-4, possibly because of the low number of samples from an early lactation period in their study. However, we found IL-4 to be present in about 20% of the milk samples from both groups.

Our data on IL-10 in milk were in the range reported by Srivastava et al. (10) but were much lower than those published by Garofalo et al. (7). An explanation for this discrepancy could be that these high IL-10 values were determined in samples collected during the first 24 h (7), whereas our samples were taken 3–9 days postpartum.

In general, the concentrations of these immune factors in milk varied widely, in agreement with findings from other research groups (7, 8, 10). Whether milk cytokines are biologically significant for the breast-fed infant remains to be elucidated.

In some individuals from both study groups, milk concentrations of sICAM-1 were found to be very high. Therefore, it is tempting to speculate that sICAM-1 in milk might be beneficial for the recipient infant by preventing overshooting immune reactions in the gastrointestinal tract and diminishing the risk of disorders such as chronic inflammation (25).

In conclusion, the milk profile of cytokines and inflammatory factors was not reflected by the concentrations in serum. Although the acute-phase marker MIP-1 α was more often found in the milk of mothers with respiratory allergies, the mean difference between the groups was not statistically significant. However, since most women in the allergy group were reported to have been free of clinical symptoms during pregnancy and the early postpartum period, the immune status could change later in lactation. The question of whether cytokines and soluble adhesion molecules in milk have immunomodulating, anti-inflammatory effects on the alimentary tract of the recipient infant, as has been suggested by Garofalo et al. (7), needs further investigation, especially since the concentrations of these components in milk are very high in some individuals.

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