Premature Delivery Influences the Immunological Composition of Colostrum and Transitional and Mature Human Milk\textsuperscript{1–3}

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Abstract

Human breast milk is the ideal nutrition for the newborn, and in addition to its nutritional contribution, necessary for infant growth and development, it contains various immune bioactive factors that confer some of the numerous beneficial effects of breastfeeding. The current study analyzed the concentrations of IgA, growth factors such as epidermal growth factor (EGF), TGF\textsubscript{b}1, and TGF\textsubscript{b}2, cytokines IL-6, IL-8, IL-10, IL-13, and TNF\textalpha, and TNF-receptor I (TNF-RI) in colostrum and transitional and mature milk from mothers with mature, premature, and very premature infants. Human milk samples were collected from mothers delivering at term (T), preterm (PT), and very preterm (VPT). Milk from all the mothers was collected at 3 different time points after delivery corresponding to colostrum and transitional and mature milk. After obtaining milk whey, IgA, EGF, TGF\textsubscript{b}1, and TGF\textsubscript{b}2 were determined by ELISA and IL-6, IL-8, IL-10, IL-13, TNF\textalpha and TNF-RI by cytomteric bead array immunoassay. The colostrum of the PT group was extremely rich in most of the factors studied, but higher concentrations than in the T group were only found for IL-6 \((P = 0.051),\) TGF\textsubscript{b}1, and TGF\textsubscript{b}2 \((P < 0.08).\) Conversely, the colostrum of the VPT group had lower concentrations of IgA, IL-8, IL-10, and TNF\textalpha than those in the T group \((P < 0.05).\) Results suggest that maternal lactogenic compensatory mechanisms accelerating the development of immature breast-fed preterm infants may take effect only after wk 30 of gestation. J. Nutr. 141: 1181–1187, 2011.

Introduction

Human breast milk is the ideal nutrition for the newborn; it contains not only nutrients necessary for their growth and development but also numerous bioactive factors that contribute to the beneficial effects of breastfeeding. The list of these factors detected in human milk is growing and includes hormones, growth factors, cytokines, and chemokines. These operate in networks and produce a cascade of effects that contribute to the orchestration, development, and functions of the early immune system through a variety of mechanisms, including direct or indirect antimicrobial activity, modulating immune function, anti-inflammatory effects, and enhancing growth and development of the infant’s tissues. The benefits of human breast milk to the infant are more than the sum of the bioactive factors contained \((1,2),\) and the precise in vivo effects of these agents upon the recipient infant remain to be determined \((3).\)

Among the proteic bioactive factors of milk, Ig, predominantly IgA, act by binding directly to specific microbial antigens, blocking adhesion, enhancing phagocytosis, modulating local immune function, and favoring the normal microbial colonization of the gut \((1,4).\) In addition, IL-6, which is also present in breast milk, is involved in the differentiation of IgA-producing cells \((5,6).\) Among growth factors, the epidermal growth factor \((EGF)\)\textsuperscript{8} and TGF\textsubscript{b}1 and TGF\textsubscript{b}2 promote the functional development of the gastrointestinal mucosa \((1).\) Moreover, these, together with the immunosuppressive cytokine IL-10, promote tolerance of the immune system to dietary and microflora antigens and downregulate inflammation and promote healing of damaged intestinal cells \((7).\) However, variable amounts of proinflammatory cytokines, such as IL-6, IL-8, and TNF\textalpha, have been reported in human milk \((8).\) The inflammatory activity of TNF\textalpha is reduced, however, by TNF-receptor I (TNF-RI), also present in human milk \((9),\) by directly binding it in the intestinal lumen \((10).\)

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\textsuperscript{3} Supplemental Table 1 is available from the “Online Supporting Material” link in the online posting of the article and from the same link in the online table of contents at jn.nutrition.org.
\textsuperscript{4} To whom correspondence should be addressed. E-mail: angelsfranch@ub.edu.

\textsuperscript{8} Abbreviations used: EGF, epidermal growth factor; PT, preterm; T, term; TNF-RI, TNF-receptor I; VPT, very preterm.
The composition of this complex mixture of interacting compounds not only differs among women but also during the lactation period, from colostrum, with the highest concentrations, through transitional to mature milk (7). Moreover, the degree of prematurity may also play a role in milk composition. Although there are several studies about differences between macronutrient concentrations in term and preterm milk, there are currently a limited number of studies concerning immunological and growth factor composition and even fewer studies related to their changes over the course of early lactation.

Thus, the purpose of this study was to ascertain whether, over the lactation period, milk from women delivering at preterm or very preterm contained the same proportion of functional bioactive factors as milk from women delivering at term. Therefore, we analyzed the milk content of IgA, growth factors such as EGF, TGFβ1, and TGFβ2, and cytokines such as IL-6, IL-8, IL-10, IL-13, and TNFα and its receptor, TNF-R1, in milk from mothers with mature, premature, and very premature infants. We also evaluated changes during the lactation period, i.e. in colostrum and transitional and mature milk.

Materials and Methods

Participants. Human milk samples were collected from 3 different groups of mothers delivering at the Clinic-Maternity Hospital of the University of Barcelona. Of these, 22 women delivered at term (T group) between wk 38 and 42 of physiological gestation, 10 women delivered prematurely (PT group) between wk 30 and 37 of gestation, and 10 women delivered very prematurely (VPT group), before wk 30 of gestation. Informed consent was obtained from each mother after she received information about the aim and design of the study. The study protocol was approved by the Hospital Ethical Committee for Human Research. Data of general characteristics (gestational weight increase, baby’s birth weight), diet, therapeutic treatment, and pregnancy history were carefully recorded. The exclusion criteria for mothers were diabetes or gestational diabetes, eclampsia, immunodeficiency, malnutrition, recent local or systemic infectious diseases, psychiatric disorders, and drug addiction.

Colostrum and transitional and mature milk collection and preparation. Milk was collected from mothers corresponding to the T, PT, and VPT groups at 3 different time points postpartum: colostrum (3 ± 1 d), transitional milk (10 ± 2 d), and mature milk (30 ± 2 d). Milk samples from mothers exclusively breastfeeding were collected using an electric breast pump (Ameda, Lactaline) during hospitalization and, after discharge, at home. Milk samples were always collected between 8 and 12 h, at the end of the feeding, in aseptic flasks by completely emptying both breasts for colostrum and by partial suction of both breasts for transitional and mature milk. All samples were maintained at 4°C, aliquoted within 2 h of collection, and stored at −80°C. To avoid interference with the immunosassays with milk fat content, milk whey separation was performed as previously described (11). Briefly, the milk fatty layer and cellular elements were removed by centrifugation at 800 × g for 10 min at 4°C and the intermediate aqueous phase was aliquoted to avoid repeated freeze-thaw cycles and stored at −80°C until analysis.

Quantification of IgA by ELISA. IgA was measured in human milk whey by using an ELISA kit from Bethyl Laboratories following the manufacturer’s instructions. Milk whey samples from colostrum and transitional and mature milk were analyzed at dilutions of 1:30,000, 1:6000, and 1:4000, respectively. Data were expressed as μg/L of human milk whey.

Quantification of EGF, TGFβ1, and TGFβ2 by ELISA. EGF was measured using Human EGF DuoSet (R&D Systems); the assay requires 10 μL of sample and detects from 3.9 ng/L. TGFβ1 and TGFβ2 concentrations in human milk whey were quantified using Human TGFβ1 and TGFβ2 DuoSet (R&D Systems). To quantify both TGFβs, the assays require a previous activation of latent TGF-β and a minimum of 125 μL of milk whey is required for TGFβ1 and TGFβ2 quantification. The limit of detection for both ELISA was 31.25 ng/L. All assays were performed following the manufacturer’s instructions.

Quantification of IL-6, IL-8, IL-10, IL-13, TNFα, and TNF-R1 by Cytometric Bead Array immunoassay. IL-6, IL-10, IL-13, and TNFα cytokines, IL-8 chemokine, and TNF-R1 concentrations were measured using the BD Cytometric Bead Array Human Soluble Protein Flex Set (BD Biosciences). Specific capture beads in suspension with distinct fluorescence intensity was revealed through different detection antibodies conjugated with phycoerythrin and analyzed by a BD FACSAria (BD Biosciences) cytometer and the FCAP Array software (BD Biosciences). All 6 determinations consumed as little as 50 μL of diluted milk whey, and quantitative determinations were performed with the following limits of detection: 1.6 ng/L for IL-6, 1.2 ng/L for IL-8, 0.13 ng/L for IL-10, 0.6 ng/L for IL-13, 0.7 ng/L for TNFα, and 5.2 ng/L for TNF-R1.

Statistics. Because antibody and cytokine concentrations are not normally distributed (12), and considering that data from the same mothers over time are not independent, a nonparametric test was used. Comparisons of the types of milk within the gestational age groups (T, PT, and VPT) were carried out by using the Friedman test for paired samples, and when the type of milk had a significant effect on the concentration of the bioactive molecules, the Wilcoxon’s t test was performed between pairs. For establishing differences among the same type of milk (colostrum or transitional or mature milk) from the 3 study groups, the Kruskal-Wallis unpaired test was performed and the significant effect of gestational age was then analyzed by the Mann-Whitney U test. The presence of a possible interaction between bioactive molecules in human milk whey (e.g. IgA and TGFβ) or between a specific milk bioactive factor (e.g. EGF) and gestational age or birth weight was evaluated by using the Spearman correlation test. Statistical analyses of the mothers’ body weight increase, gestational age, and babies’ weight were performed by conventional 1-way ANOVA followed by the Scheffe post hoc test. The SPSS 16.0 software package was used for the statistical analyses. Differences were considered significant at P < 0.05. All data in the text and figures are expressed as the mean ± SEM.

Results

General data of the studied groups. The clinical mean data of the T, PT, and VPT lactating women included in this study were recorded (Table 1). The socio-economic conditions of the 3 groups were comparable. As expected, the weight increase during pregnancy in the VPT group was lower than in the T and PT groups (P < 0.05), but gestational weight gain did not differ between the T and PT groups. Weight differed between the PT and VPT newborns (P < 0.001), and their body weight was lower than that of the T group (P < 0.001).

Milk whey bioactive factor profile. The quantification of all the bioactive factors in all the different human milk whey samples allowed us to distribute, based on their concentration, the immunological factors into 3 groups. IgA ranged between 2 g/L to hundreds of mg/L and was the “main” immune factor; EGF, TGFβ1, TGFβ2, IL-8, and TNF-R1 in this study were considered as major factors, because they ranged from μg/L to hundreds of ng/L. Finally, IL-6, TNFα, IL-10, and IL-13 constituted the minority compounds, because their concentrations were around ng/L. This profile was generally achieved in colostrum and transitional and mature human milk whey from the T, PT, and VPT groups. However, exceptions were IL-8 in T and PT mature milk and IL-6 in PT colostrum. To show the high variability found in the immunological factor concentrations at each stage of lactation and gestational age groups, mean ± SD and the corresponding range are included (Supplemental Table 1).
Milk whey IgA. In all 3 groups, IgA reached its highest concentration in colostrum; it markedly decreased in transitional milk and also became significantly lower in mature milk than in transitional milk. The IgA concentration in colostrum from the VPT group was significantly lower than that in the T and PT groups (Fig. 1A). Finally, there were good correlations between the concentrations of IgA and TGFβ1, TGFβ2, IL-6, and IL-10 in the colostrum from the T group and the colostrum from the PT and VPT groups, the latter two being considered as a whole (Table 2).

Milk whey major factors. The concentrations of EGF in all the groups (Fig. 1B) were the highest in colostrum and decreased in transitional milk. In mature milk, there was only a significant reduction in the T and VPT groups, in comparison to transitional milk. On the other hand, the EGF concentration in transitional milk from the PT and VPT groups was significantly higher than that of the T group. In the transitional milk of all the groups, there were negative correlations between EGF concentration and birth weight (r = -0.57; P < 0.001) and between EGF concentration and gestational age (ρ = -0.51; P < 0.001).

The TGFβ1 concentration (Fig. 1C) in the T group gradually diminished from colostrum to transitional milk and from transitional to mature milk. The decrease from colostrum to mature milk was significant in the PT and VPT groups. The highest concentration of TGFβ1 was in the PT colostrum, which was significantly greater than that in the T group.

The time course of the TGFβ2 concentration (Fig. 1D) in the T and VPT groups did not significantly change from colostrum to mature milk. However, the TGFβ2 concentration in the PT colostrum was significantly higher than that in the T and VPT groups.

With regards to TGFβ isoforms, the concentration of TGFβ2 was ~600% higher than that of TGFβ1. The TGFβ2:TGFβ1 ratio ranged between 4.93 ± 0.94 in the colostrum from the VPT group and 7.74 ± 1.00 in the transitional milk from the T group. Although there was considerable inter-individual variation in the TGFβ2:TGFβ1 ratio, a positive correlation between the 2 isoforms in human milk whey was always found, regardless of 

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**TABLE 1** Characteristics of T, PT, and VPT lactating women and their newborns

<table>
<thead>
<tr>
<th></th>
<th>T, n = 22</th>
<th>PT, n = 10</th>
<th>VPT, n = 10</th>
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<tbody>
<tr>
<td>Age, y</td>
<td>30.9 ± 1.08 (23–42)</td>
<td>32.8 ± 1.76 (22–39)</td>
<td>33.1 ± 1.18 (29–40)</td>
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<tr>
<td>Parity</td>
<td>1.6 ± 0.19 (1–4)</td>
<td>1.5 ± 0.19 (1–2)</td>
<td>1.4 ± 0.21 (1–3)</td>
</tr>
<tr>
<td>Gestational age, wk</td>
<td>40.6 ± 0.21 (38.6–42.0)</td>
<td>34.0 ± 0.62 (31.4–37.6)</td>
<td>26.7 ± 0.37 (25.4–29.3)</td>
</tr>
<tr>
<td>Weight increase during pregnancy, kg</td>
<td>12.6 ± 1.04 (6.6–25)</td>
<td>14.0 ± 1.31 (8–20)</td>
<td>8.2 ± 1.16 (4–17)</td>
</tr>
<tr>
<td>Type of parturition, % spontaneous/% Caesarean</td>
<td>91/9</td>
<td>40/60</td>
<td>60/40</td>
</tr>
<tr>
<td>Baby’s birth weight, kg</td>
<td>3.5 ± 0.10 (2.8–4.5)</td>
<td>2.2 ± 0.18 (1.4–2.9)</td>
<td>0.89 ± 0.05 (0.7–1.2)</td>
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</table>

1 Data are mean ± SEM (range). Means in a row without a common letter differ, P < 0.05.
gestation period or type of milk considered. Taking together all the samples throughout the lactation period, a high positive correlation was obtained ($r = 0.78; P < 0.001$).

IL-8 concentration (Fig. 1) in colostrum from the T and PT groups was higher than in transitional milk, which in turn was higher than in mature milk. It is remarkable that the IL-8 concentration in the colostrum from the VPT group was significantly lower than that in the T and PT groups. Surprisingly, all changes in IL-8 evolution for all groups were almost identical to those described for IgA but at a 0.0001% lower concentration.

Considering all the groups, a negative correlation between IL-8 concentration in mature milk and birth weight was obtained ($r = -0.33; P < 0.05$).

In all 3 groups there was a significant reduction in the TNF-RI concentration (Fig. 1) from colostrum to transitional milk. TNF-RI values in transitional milk remained in the same range in mature milk.

**Milk whey minority factors.** The time course of the IL-6 concentration (Fig. 2A) in the T group gradually and significantly decreased from colostrum to mature milk. The highest concentration of IL-6 was in PT colostrum, which was markedly higher than that in the colostrum from the T ([$P = 0.051$]) and VPT ([$P < 0.05$]) groups. The IL-6 concentration correlated positively with that of IgA, TGF/1, and TGF/2 in the colostrum from the T group and in the colostrum from the PT and VPT groups considered together (Table 2). Moreover, there was a positive correlation between the IL-6 and IgA concentration in the mature milk of the PT and VPT groups ($r = 0.74; P < 0.01$).

TNFα, IL-10, and IL-13 were found in very low concentrations in human milk and were undetectable in some milk whey samples (Fig. 2). Those samples that had concentrations below the cutoff received a value corresponding to one-half the cutoff value, as described (13).

In all 3 groups, TNFα reached the highest concentration in colostrum and decreased markedly in transitional milk (Fig. 2B). Moreover, TNFα concentration was significantly reduced in mature milk compared with transitional milk. The TNFα concentration in the colostrum from the VPT group was significantly lower than in the T and PT groups. A positive correlation between TNFα and IL-6 was always found independently of the gestation period and type of milk considered ([$r = 0.66; P < 0.001$], taking together all samples throughout lactation).

There was a gradual decrease of the IL-10 concentration during lactation in the T group (Fig. 2C). The time course of the IL-10 content in the PT group was very similar. However, the colostrum IL-10 content in the VPT group was lower than that in the T and PT groups and did not significantly vary in transitional and mature milk. The colostrum concentration of IL-10 correlated with those of IgA, TGF/1, TGF/2, and IL-6 (Table 2).

The colostrum IL-13 contents in the T and PT groups were similar (data not shown). In both groups, these concentrations diminished up to 50% in transitional milk (detectable in 7/22 samples from the T group and 5/10 from the PT group) and became practically nonexistent in mature milk (detectable only in 4/22 and 1/10 in the T and PT groups, respectively). In the VPT group, the IL-13 concentration was extremely low, being undetectable in 87% of the milk samples.

![FIGURE 2 Time course of IL-6 (A), TNFα (B), and IL-10 (C) quantified in milk whey of T (n = 22), PT (n = 10), and VPT (n = 10) groups. Bars are the mean ± SEM. If there were samples in which they were undetectable, the number of positive samples vs. total samples is indicated at the top of the bar. Labeled means without a common letter within same gestational age group (a, b, c) or within same type of milk (x, y, z) differ, P < 0.05.](image)

<p>| TABLE 2 | Correlations between IgA and some cytokines in colostrum from T group (in bold) and PT and VPT groups considered together1 |
|---------|-----------------|-----------------|-----------------|-----------------|-----------------|</p>
<table>
<thead>
<tr>
<th>IgA</th>
<th>TGFβ1</th>
<th>TGFβ2</th>
<th>IL-6</th>
<th>IL-10</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgA</td>
<td>—</td>
<td>0.60**</td>
<td>0.43*</td>
<td>0.64**</td>
</tr>
<tr>
<td>TGFβ1</td>
<td>0.59**</td>
<td>—</td>
<td>0.80***</td>
<td>0.60**</td>
</tr>
<tr>
<td>TGFβ2</td>
<td>0.73**</td>
<td>0.87***</td>
<td>—</td>
<td>0.52*</td>
</tr>
<tr>
<td>IL-6</td>
<td>0.80***</td>
<td>0.67**</td>
<td>0.73***</td>
<td>—</td>
</tr>
<tr>
<td>IL-10</td>
<td>0.72**</td>
<td>0.51*</td>
<td>0.76***</td>
<td>0.48*</td>
</tr>
</tbody>
</table>

1 Asterisks indicate: *P < 0.05, **P < 0.01, ***P < 0.001.
2 NS, nonsignificant, $P \geq 0.05.$
Discussion

The composition of human milk is subject to changes during lactation; however, little is known about the changes in its immunological constituents. Moreover, there are very few studies regarding the content of immunoactive factors in milk from mothers who deliver at preterm or very preterm. In the present study, we showed the time course in the content of 10 immunoactive factors during the lactation period and we compared the results in the milk from mothers delivering at T, PT, and VPT. In general, there was a high inter-individual variability in the milk concentration of the studied factors at the same collection time. This variability may contribute to the poor correlation with other studies and may also reflect the different milk sample collection methods used, the inclusion of a wide range of gestational ages, and methodological approaches for biological factor detection that influence these kinds of results (14). On the other hand, the highest variability has been detected in the colostrum of the PT group. This could be due to the nonuniform, compromised initiation of lactation in PT women that has been described by Gurgan et al. (15).

The IgA content in milk, the main mediator of the local first-line immune system, in mothers delivering at T and during lactation, completely agrees with that of other studies (16–18). In colostrum and mature milk, IgA was present at a similar concentration to that previously reported (19–21). Conversely, other results referring to milk IgA described extremely high or low values (22,23). The IgA concentration in the PT group was in line with previous data (16,18,21,23), which also show a higher colostrum IgA concentration in mothers delivering at PT than at T. TGFβ1, IL-6, and IL-10 are of particular interest for the development and differentiation of IgA-producing cells (24). In this sense, the positive correlation in the concentration of these cytokines and between them and the IgA in the colostrum and mature milk (data not shown) from the T, PT, and VPT groups, found here and partially by Böttcher et al. (22), supports their involvement in IgA synthesis in the mammary gland. Therefore, our results suggest the robustness of IgA production assurance by the organism irrespective of the gestation period. Our results showed that the ratio of TGFβ1:TGFβ2, which also have a crucial role in the induction of oral tolerance and maintenance of the mucosal barrier function (25,26), in milk in the T group is in line with some previously reported data (26–28) and in contrast with others (29). Moreover, both isofoms are the least variable factors throughout the lactation period in the T group, which could be linked to the neonate’s high requirement of these factors during the first 12 wk of lactation (28). The decreasing pattern of IL-6 content throughout the lactation period in the T group is partially in agreement with previously reported data (5,8,28,29). The IL-10 concentration in colostrum in our study is in line with that of other studies (22,30,31), although they had a lower percentage of samples. The similar decreasing IL-10 concentration profile in the T and PT groups agrees with Meki et al. (8), who also found similar IL-10 values in the mature milk of the same groups.

EGF is abundant in human milk (32) and has a key role in the development and protection of the gastrointestinal tract (33). The present study showed that, regardless of gestation length, concentration of EGF decreased during lactation, which partially agrees with the results of Dvorak et al. (34). It must be pointed out that EGF is the only factor studied whose concentration in PT and VPT transitional milk was higher than that of the T group, in accordance with Read et al. (35). Moreover, the negative correlation of EGF and birth weight described in this study highlights the importance of this factor in early life or low-weight babies (36). The fact that extremely premature infants may require higher local growth factors such as EGF supports the hypothesis that the EGF content in milk may be influenced by intrauterine growth retardation, although the mechanisms of this phenomenon are unknown. Moreover, the intestinal role of EGF is further shown by the demonstrated presence of EGF receptors in both the fetal and neonatal gut (37). Similar to EGF, the key role of IL-8 in baby growth was reflected by its negative correlation between milk concentration and birth weight. IL-8, a chemokine with a local maturation role in the developing human intestine (38), had intercohort colostral differences between our results and other studies (8,13,39,40). However, the large reduction in IL-8 content from colostrum to mature milk in the T and PT groups is in agreement with other studies (8,30,39,40). In contrast to previous studies (41,42), we found a lower concentration of colostrum IL-8 in the VPT group than in the T and PT groups. The high content of EGF and IL-8 in premature infants and their inverse correlations to birth weight suggest that their concentration in milk is modulated by the neonate’s requirements.

TNFα, whose production is defective in the neonate, and its receptors in their soluble form, TNF-R1 and TNF-RII, are present in milk (9). In the present study, TNF-R1 had a decreasing concentration pattern in the T, PT, and VPT groups, and this bioactive factor is the only one that reaches a plateau in accordance with others (8). Thus, the high TNF-R1 concentrations can act by modulating the biological effect of TNFα and contribute to the antiinflammatory effects of human milk. Also, TNF-R1 may be useful in ensuring the blocking of TNFα from luminal endogenous production by the lactating infant. A similar concentration of free TNFα was found in the T and PT groups, but colostrum from the VPT group had lower concentrations, in accordance with results reported by Ustundag et al. (30).

Overall and regarding the effect of gestational length on immune factors concentration, the colostrum of the PT group was richer than that of the T group in most of the studied factors. The mechanisms responsible for differences in the composition of milk between gestational ages and stages of lactation have yet to be established. It has been described, to a lesser extent for TNFα but markedly for IL-6, IL-8, and IL-10, that neonatal cells have a lower expression than adult counterpart cells (43). We think that the premature infant (with a delay in the development of his immune system) has to compensate this imbalanced situation and ensure a good host defense through the arrival of an increase in the proportion of these components contained in human milk. We suggest that the breast milk content is adapted to better collaborate in the immune defense and development of premature babies. Thus, breast milk from mothers delivering at PT have to perform a rapid immunological adaptation to PT newborn requirements as suggested (16,23).

Conversely, the colostrum of VPT had lower contents in most of the bioactive factors than in the T group. Thus, because colostrum from mothers delivering a VPT infant contained lower concentrations of these immune components than that of mothers delivering at T or PT, it is plausible to think that when delivery occurs before wk 30 of gestation, the lactation adaptation is not as efficient as in PT deliveries. Thus, at VPT delivery (before wk 30), when in normal physiological conditions neonates are not expected to be born, breast milk cannot compensate for that situation. However, regardless of the high content of colostrum immune factors in the PT group or the low content in the VPT group, their concentration was similar in all
the groups later in mature milk, when the neonate has already completed the first stages of life. This implies that the supply of these essential factors is assured irrespective of the number of weeks of gestation.

Although this study provided novel insight into immunological factors in breast milk from mothers of T, PT, and VPT babies and their changes over the course of early lactation, there were some limitations of the study. The main limitation was the small sample size, especially due to the high inter-individual variability. Also, the collection of only hindmilk and the partial breast emptying may have resulted in an under- or overestimation of the immunologically factor content in milk samples. Further studies are needed to ensure that immunological factor concentrations are representative of the whole breast emptying period and also of a larger population.

In conclusion, breast milk immune factor composition varies, not only depending on the length of lactation, but also on gestational length. From the results presented here, it can be inferred that maternal lactogenic compensatory mechanisms only accelerate the development of immature breast-fed PT infants after wk 30 of gestation. In shorter gestational ages, it seems difficult to trigger compensatory maternal response and to promote extremely premature babies’ maturity. Further studies in VPT babies are needed to confirm these results and increase the very small amount of literature available in this regard.

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