Changes in the Mannan Binding Lectin (MBL) Concentration in Human Milk During Lactation

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The mannan binding lectin (MBL) activates the complement system by the lectin pathway after the recognition of some structural motifs (saccharides) present on the surface of microorganisms. MBL has been mostly identified and quantified in human serum by ELISA or microparticle immunonephelometry assays. This article reports the MBL levels as assessed by a microparticle immunonephelometric assay in 76 human milk samples. Immunonephelometry was performed using skim-milk samples diluted 20 times over a calibration range of 0.07–4.82 mg/L. MBL is indeed present in human milk and its concentration

decreases significantly during development from colostrum (0.55 ± 0.09 mg/L) to transitional (0.18 ± 0.02 mg/L) and mature milk (0.17 ± 0.02 mg/L). This innate molecule may be involved in the primary defenses of the mammary gland and the neonate, whose immune system is immature. The high levels observed during the first days of lactation support the hypothesis that this molecule plays a key role in limiting the colonization of the newborn gut by pathogens. *J. Clin. Lab. Anal.* 16:304–307, 2002. © 2002 Wiley-Liss, Inc.

Key words: Mannan binding lectin; MBL; human milk; immunonephelometry; lactation

INTRODUCTION

The mannan binding lectin (MBL), or mannan binding protein (MBP), is a C lectin from the collectin family (1,2). This plasma protein is synthesized by the liver and constitutes an important part of the human innate immune defense system. MBL is a bactericidal factor that activates the complement system by the lectin pathway (3). This activation pathway, independently of the presence of antibodies, takes place at early stages of infection, before specific immune responses are built up (4,5). In association with several serine proteases (including MASP-1 and -2) (6), MBL mediates the activation of the complement system upon binding to specific microbial carbohydrate motifs, such as mannose or N-acetyl glucosamine, that are present on the surface of microorganisms (7). This induces the lysis of microorganisms via formation of the membrane attack complex (5,8). MBL is also a strong opsonin that is recognized by the ubiquitous collectin receptor expressed by phagocytes after its binding to bacterial surfaces (9,10). Furthermore, MBL able to coat viral proteins may neutralize viruses by inhibition of the binding and expansion stages (11). Except for a few investigations of the structure of MBL and its participation in the lectin pathway of complement activation

(12), MBL has been little studied. A few reports have quantified MBL by ELISA in secretions such as saliva and serum, with concentrations varying from 2.5 to 6.4 mg/L (4,13,14). An immunonephelometry assay in serum has also been reported (15). Here we report a study aimed at demonstrating the presence of MBL in human milk, and determining its levels during lactation.

MATERIALS AND METHODS

Milk Samples

Seventy-six samples of human milk were collected from 42 volunteer lactating women at the local maternity ward or at their homes. According to the stage of lactation, these samples consisted of colostrum (from postpartum days 1–5, $n=21$), transitional milk (postpartum days 6–14, $n=27$), and mature milk (postpartum days >14 , $n=28$). All samples were

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collected approximately midway through a single feeding. They were then aliquoted, frozen immediately at -20°C , and stored until they were assayed. All samples were thawed at $+40^{\circ}\text{C}$ in a water bath and vigorously homogenized with a vortex (IKA Works, Inc., Wilmington, NC) before the assay. Preclearing was achieved by adding (v/v) 1,1,2-trichlorotrifluoroethane (Merck, Darmstadt, Germany). After thorough mixing and centrifugation for 20 min at 4,900 g, assays were carried out using the recovered upper phase of each sample. Human milk samples were automatically diluted at 1/20 in a phosphate buffer (0.05 M, pH 7.2) containing NaCl 0.14 M and 3.5% of PEG 6000. For low-concentration samples, a 1/5 predilution was prepared.

Nephelometric Assay

The MBL assay was performed by a microparticle immunonephelometric assay (anti-MBL rabbit IgG coated on polystyrene particles) using an N latex MBP kit (Behring Diagnostics, Marburg, Germany). Rabbit whole immunoglobulins were added to the reaction buffer (phosphate buffer 0.05 M, pH 7.2, containing NaCl 0.14 M and 3.5% of PEG 6000), to eliminate possible interactions between rheumatoid factors and the IgG anti-MBP coated on the microparticles.

A calibration curve was obtained with serial dilutions (from 1/10 to 1/640) of a standard containing 2.41 mg/L of MBL, after reconstitution of the lyophilized product in 0.5 mL distilled water. A total of 44 μL of diluted (1/20) milk sample or diluted standard were incubated with 77 μL of reaction buffer, 44 μL of anti-MBL microparticles, and 10 μL of reaction buffer. After precisely 12 min of incubation, the scattered light was measured automatically by the BNA II laser nephelometer (Behring).

Statistics

Mean and standard error of the mean were calculated for the concentrations of MBL at the three stages of lactation (colostrum, transitional, and mature milk) and comparisons were performed, after verification of normality by the Kolmogorov-Smirnov test, using analysis of variance with a Newman-Keuls post-test. P -values <0.05 were considered statistically significant. Correlations between the concentrations of MBL and levels of other immune molecules (IgA, C3, and C4), as previously determined by classical immunonephelometry studies in the same samples (16–18), was performed by a Pearson test. Version 2.0 of the computer program Prism™ (Graphpad Software Inc, San Diego, CA) was used for all data analyses and statistics.

RESULTS

The standard calibration curve (Fig. 1) allowed the measurement of MBL levels over a large range of concentrations (0.07–4.82 mg/L) for milk samples diluted 1/20. The precision of the method, provided by the manufacturer, was confirmed by coefficients of variation lower than 2% and 4% for within-run and between-run tests, respectively. The reproducibility of the method was demonstrated by the low deviation (1.6%) observed using a known concentration of MBL (2.62 mg/L; Behring) as a control.

The variations in MBL concentration during lactation are shown in Fig. 2. MBL concentrations are highly elevated in the first days of lactation; they then decrease rapidly and strongly before they stabilize at a low level. Comparisons between the MBL concentrations in colostrum, transitional, and mature milk showed that the concentrations were significantly higher ($P < 0.001$) in colostrum ($n = 21$, mean = 0.55 mg/L, SE = 0.09 mg/L) than in transitional ($n = 27$, mean = 0.18 mg/L, SE = 0.02 mg/L) and mature milk ($n = 28$, mean = 0.17 mg/L, SE = 0.02 mg/L). No difference was noted between transitional and mature milk. Positive correlations were observed between the concentration of MBL and the levels of other immune molecules in human milk. These correlations were stronger ($P < 0.0001$) with IgA ($n = 76$, $r = 0.575$) and C3 ($n = 73$, $r = 0.557$) than with C4 ($n = 74$, $r = 0.244$, $P < 0.005$).

Fig. 1. Calibration curve of the microparticle MBL immunoassay. + signs represent the scattered light measured for each serial dilution of the MBL standard.

Fig. 2. Changes of MBL concentration in 76 human milk samples during lactation. Results are plotted as mean $\pm$ SE. a: Concentration significantly different ($P < 0.001$) from that of colostrum.

DISCUSSION

To our knowledge this is the first report dealing with the detection of MBL in human milk, and its evolution during lactation. The immunonephelometric assay developed for human serum was satisfactorily adapted to quantify MBL in human milk. This technique appears to be reproducible and accurate, and allows the measurement of MBL over a wide range of concentrations in milk.

In the present study we observed a rapid decrease of MBL concentration in human milk during the first days postpartum, in accord with previous findings for other proteins, such as IgA (16), IgA1, IgA2 (17), C3, and C4 (18). This decrease may be a result of the diffusion of these molecules from plasma to milk in early colostrum (19). This hypothesis is supported by the fact that MBL plasma concentrations (1.72 ± 1.15 mg/L) are much higher than those observed in human milk, whatever the stage of lactation (20). The higher levels of MBL in colostrum (0.55 ± 0.09 mg/L) as compared to transitional or mature milk, and increases of serum MBL concentrations as pregnancy progresses (21) further support this hypothesis. The transudation of maternal serum MBL to colostrum could be held across the loosened tight junctions of mammary gland acini at the end of pregnancy and beginning of lactation (22). However, the low (but significant) levels of milk MBL observed when lactation is well under way indicate that there may be a local synthesis by cells present in the mammary gland. Macrophages, which are capable of C3 and C4 synthesis, could be also involved in this local MBL production, as could mammary gland epithelial

cells, which were previously reported to secrete C3 and C4 (18,23). Such a local synthesis could provide a direct available source of MBL for the newborn and the mammary gland; however, the role played by this production of MBL is unknown.

MBL plasma levels increase after birth, from 1 mg/L in the newborn to 2.5 mg/L at day 5 postpartum (14). This suggests that the immature immune system of the infant relies heavily on innate immunity (9,24). Because the mucosal immune system of the infant is nearly devoid of any immune constituents, milk-borne MBL could be part of the passive transfer of immunocompetent agents from the mother to her baby (25). MBL has also been described to have a stimulating action on local immune responses, particularly in the neonate gastrointestinal tract (26). MBL could have some functions in the maturation of thymocyte development and selection (27), and therefore may induce the maturation of the immature mucosal immune system of the newborn.

IgAs have been described as noninflammatory, and they cannot bind and activate the complement system (2). However, in a specific or polymeric form, they may cooperate with the complement system for the lysis of Gram-positive bacteria (*Streptococcus pneumoniae*) (28,29). Because MBL presents a strong affinity for the abundant mannose residues of IgA, IgA and MBL are able to activate the lectin complement pathway (30). In this way, MBL and IgA could regulate the gastrointestinal and oropharyngeal bacterial colonization of the neonate (31,32). Indeed, MBL is able to bind and to exclude Gram-negative bacteria, such as *Escherichia coli*, and can decrease the virulence of pathogens (5,33). The high concentrations of IgA in colostrum could stimulate macrophages, via their specific receptor CD89, inducing complement and MBL secretion (34). The correlations observed here in milk samples between the concentrations of MBL and other immune proteins are consistent with their interrelations in the same pathway.

CONCLUSIONS

The measurement of MBL by microparticle immunonephelometry in human milk during different stages of lactation demonstrates that MBL is found in higher concentrations in colostrum. MBL in human milk may provide the newborn with passive protection while the immune system is immature, and may play a role in regulating the colonization of the microflora.

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