JOGNN

RESEARCH

Changes in the Immune Components of Preterm Human Milk and Associations With Maternal and Infant Characteristics

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Keywords
human milk
immunity
preterm infants

ABSTRACT

Objective: To describe difference in cytokines, chemokines, and growth factors (CCGFs) and secretory immunoglobulin A (sIgA) in the breast milk of mothers who gave birth preterm and maternal or infant characteristics related to these immune components.

Design: A prospective, repeated-measures, one-group design.

Setting: Data were collected at an 82-bed NICU in West Central Florida.

Participants: Seventy-six very-low-birth-weight infants weighing less than 1,500 g and their mothers.

Methods: Daily aliquots of breast milk from mothers of preterm infants were collected from the daily infants' feedings and pooled at the end of each week, and CCGFs and sIgA were measured weekly with MagPix multiplexing (Luminex, Austin, TX) and enzyme-linked immunosorbent assay. Q1 Q2

Results: The CCGFs showed high individual variability, but the levels of most CCGFs and sIgA fell over time. Immune variables were generally greater in milk from mothers of infants smaller than 1,000 g. The breast milk of mothers of male preterm infants had significantly greater sIgA than the breast milk of mothers of female preterm infants. We found relationships between age, body mass index, parity, sIgA, and some of the CCGFs in the breast milk of women who gave birth preterm.

Conclusion: Immune molecules declined in concentration over time in the breast milk of mothers who give birth preterm during the NICU stay, and maternal and infant factors appeared to play some role in the levels of these immune molecules. Further exploration of this relationship is warranted.

JOGNN, ■, ■–■; 2016. http://dx.doi.org/10.1016/j.jogn.2016.04.009

Accepted April 2016

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The authors report no conflict of interest or relevant financial relationships.

All infants are born immunologically naive; human milk bridges this developmental gap, provides essential components that protect against pathogens, and promotes maturation of the infant gastrointestinal and immune systems (Newburg & Walker, 2007). Immune components of breast milk include antibodies, primarily secretory immunoglobulin A (sIgA); pro- and anti-inflammatory cytokines; many chemokines; growth factors; various antiseptic molecules, such as lactoferrin and lysozyme; pre- and probiotics, and leukocytes. All of these components interact with each other and with the infant gut according to the stage of lactation and postmenstrual age.

Immunologic immaturity is most marked in preterm infants because of decreased placental immunoglobulin transfer, decreased ability to launch proinflammatory cytokine responses (A. A. Sharma, Jen, Butler, & Lavoie, 2012), and immature adaptive immunity with bias toward T helper–2 lymphocyte activation (humoral immunity; Adkins, Leclerc, & Marshall-Clarke, 2004). Nearly one third of the morbidity in the neonatal population is due to infection (A. A. Sharma et al., 2012), and preterm infants are most at risk.

The immunology of breast milk of mothers who give birth at term (hereafter referred to as *term milk* [TM]) has been better characterized than the breast milk of mothers who give birth before 37 weeks gestation (hereafter referred to as *preterm milk* [PTM]). The unique properties of PTM were not initially recognized, and the American Academy of Pediatrics did not recommend the routine use of mother's milk for preterm infants until 1996 (Work Group on Breastfeeding, 1997).

Very-low-birth-weight infants are physiologically immature, and mothers' own milk bridges the gap between early birth, development of immunity, and gastrointestinal maturation.

Although the evidence of benefits of feeding mother's milk to preterm infants has increased during the past two decades, many preterm infants still receive little of their mothers' own milk (MOM; Snijders et al., 2007). Donor milk is primarily pooled TM that contains lower levels of cytokines and sIgA than PTM, particularly the PTM produced in the first month of life (Groer et al., 2014). Infants may transition from banked donor to cow's milk–based infant formula by 34 weeks corrected gestational age (cGA) because that is the time point at which the risk for necrotizing enterocolitis and infection becomes greatly reduced (R. Sharma & Hudak, 2013). Preterm infants receive variable amounts of MOM, donor milk, and bovine-based formula as the sources of their feeds during their NICU stay.

Although the benefits of human milk are well known for term infants, there is a gap in knowledge about how long preterm infants should be fed human milk to reap all the potential benefits. Many infants leave the NICU continuing to receive MOM, but many factors can thwart the mother's intention for long-term breastfeeding, including return to work, lack of support, stress, and infant condition (Purdy et al., 2012). Short-term benefits of human milk include reduced incidences of sepsis, necrotizing enterocolitis, and urinary tract infections (Ahrabi & Schanler, 2013). Longer-term benefits include more positive mental and emotional regulation development at age 30 months in 773 infants (Vohr et al., 2007) and positive effects on verbal intelligence quotient and white matter volume when measured at age 7.5 to 8.0 years (Isaacs et al., 2010).

There is some evidence that PTM contains generally higher levels of immune variables, is more anti-inflammatory, and contains higher sIgA concentration than TM (Gregory & Walker, 2013). There is controversy about PTM from mothers of differing gestational ages. Milk from mothers of extremely-low-birth-weight infants (<1,000 g) showed lower concentrations of immune components compared with TM or milk from mothers of very-low-birth-weight (VLBW) infants (<1,500 grams; Moles et al., 2015). Epithelial growth factor (EGF) is very high in PTM and TM. It functions to promote integrity and growth of the intestinal mucosa (Wagner, Taylor, & Johnson, 2008). Cytokines are small molecules that allow communication between cells, and chemokines are a class of cytokines that attract cells. Milk cytokines are grouped as pro- and anti-inflammatory, and milk is generally considered an anti-inflammatory secretion; some of the proinflammatory cytokines in milk act differently than in other body fluids. In a review of studies of human milk cytokines, researchers pointed out that studies of human milk cytokines, chemokines, and growth factors (CCGFs) have used a variety of methods, timings, populations, and types of milk (fore vs. hind), and there is a great deal of variation in the literature about the levels of even the most common CCGFs in PTM and TM (Agarwal, Karmaus, Davis, & Gangur, 2011).

The variability of PTM immune components has been rarely addressed in studies. Large variations in protein concentrations of five different proteins, including sIgA, were reported in 30 term, preterm, and very preterm mothers' milk (Broadhurst et al., 2014). Variations in sIgA levels in PTM compared with TM across lactation have also been reported (Ballabio et al., 2007). Variation may be very important in determining benefits to infants, and very little is known about influences on milk components variation. Jang et al. (2011) found that composition at 3 months postpartum in preterm and term mothers' milk showed differences in milk fatty acids, although this was not corrected for gestational age. In another study of PTM, Bauer and Gerss (2011) reported that protein levels were higher than in TM over 8 weeks of lactation. A steady drop in sIgA and cytokines occurred over the time of lactation in PTM, which coincides with maturation of the neonatal gut and immune system (Castellote et al., 2011; Hawkes, Bryan, James, & Gibson, 1999; Kverka et al., 2007; Ustundag et al., 2005). An important question is whether infant or maternal characteristics influence the variability of immune components of PTM. We previously reported that the mood states of anger and vigor were positively correlated with higher PTM sIgA (Groer, Humenick, & Hill, 1994). In another study, we found that stress and mood variables negatively influenced milk sIgA levels in term mothers (Groer, Davis, & Steele, 2004). These questions and gaps in the literature led to the following research questions:

- How variable are the concentrations of selected immune components of PTM provided by mothers of VLBW infants,

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