The Immunological Components of Human Milk and Their Effect on Immune Development in Infants[1,2]

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ABSTRACT There have been considerable advances in our understanding of the diverse mixture of bioactive components in human milk that influence the immune status of infants by not only providing protection but also facilitating development, tolerance, and an appropriate inflammatory response. It could be suggested that milk is the communication vehicle between the maternal immune system and the infant, a system actively directing and educating the immune, metabolic, and microflora systems within the infant, while conferring multiple means of protection from pathogens. The physiological and protective functions of many of the immune components in human milk have been deduced not from studies in infants but from what is known in other species and in vitro models. This update briefly reviews immune development in infants and focuses on current knowledge of how both the "classical" immune and the nonimmune ingredients found in mature human milk promote immune development, facilitate the development of tolerance, and regulate the inflammatory response of infants. J. Nutr. 135: 1–4, 2005.

KEY WORDS: • infants • human milk • inflammation • lymphocytes • cytokines

Neonates are exposed to a large number of microorganisms, foreign proteins, and chemicals and resistance to infection relies both on the protective factors in milk and on the infant developing his/her own innate and adaptive (antigen-driven) immunity. The neonatal immune system functions differently from that of an adult (1). Whether one can define the infant's immune system as classically "immunosuppressed" is somewhat debatable. Because little antigen exposure occurs in utero, from an immunological standpoint the infant's cells require considerable "education" in the early postnatal period. T cell immaturity is contributed to by deficiencies in antigen presentation (1). The germ-free status of the intrauterine environment favors T-helper 2 (Th2)[4] type cytokine response over a T-helper 1 (Th1) response (2). Together, the naïveté and altered cytokine response by the infant's immune system will contribute to lower immune competence in the infant. As a result, inflammatory cells of the innate immune system, predominantly macrophages and neutrophils, become responsible for the clearance of foreign antigen.

At birth cells of the innate immune system (macrophages, neutrophils, dendritic cells) and IgM- and IgG-producing cells are present in the intestine, but mucosal IgA cells are either absent or extremely rare (3). The infant's intestinal immune system develops rapidly in the early postnatal period as it contacts dietary and microbial antigens (3). In addition to eliminating infectious agents and minimizing the damage they cause, their immune system must develop the ability to discriminate between antigens that are harmless (tolerance) and those that are potentially dangerous. Induction of tolerance is believed to occur primarily in the gut and is facilitated by the specialized B and T cells, the production of sIgA, and the skewed Th2 response (4). Failure to regulate tolerance and active immune responses is hypothesized to contribute to food-related allergy, autoimmunity, and inflammatory bowel disorders.

Epidemiological data support the benefits of breast-feeding in preventing gastrointestinal and, to a lesser extent, respiratory infections in both developing and developed countries (5). More recently, it has been recognized that protection, provided through breast milk, against some infections may extend well beyond weaning (6). Considerable controversy exists as to the potential benefits of breast milk on the infant's response to vaccines, which may be influenced by neutralizing antibodies identified in milk [reviewed by (7)]. Recently, a systematic review concluded that breast-feeding appears to protect infants from the development of atopic diseases (eczema and food and respiratory allergies), particularly if there is a family history (8). Other epidemiological studies have associated breast-feeding with reduced incidence of immune-mediated diseases, including celiac disease, inflammatory bowel disease, type 1 diabetes, rheumatoid arthritis, asthma, eczema, necrotizing enterocholitis, and multiple sclerosis [reviewed in (3,6)].

Antimicrobial Properties of Human Milk. Breast milk contains a variety of antimicrobial substances (relatively resistant against intestinal proteolysis) that function to both safeguard the lactating mammary gland and provide protection to the suckling infant at a time when its immune system is still immature (**Table 1**). Excellent recent reviews are available on the antimicrobial properties of human milk (3,6,9) and will not be addressed in this review.

Constituents in Milk That Promote Immune Development. Human milk contains its own immune system and a wide range of soluble and cellular factors (Table 1), which likely facilitate immune development and maturation in infants.

Components of the maternal immune system. Depending on the phase and stage of lactation, a variety of leukocytes are present in colostrum ($\sim 4 \times 10^9$/L) and mature milk ($\sim 10^8$–

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[4] Abbreviations used: LCP, long-chain PUFA; LF, lactoferrin; MHC, major histocompatibility complex; NK, natural killer cell; TGF, transforming growth factor; Th1, T-helper 1; Th2, T-helper 2.

TABLE 1

Compounds with immunological properties in human milk

Anti-microbial compounds	Immune development compounds
Immunoglobulins: sIgA, SIgG, SIgM	Macrophages
Lactoferrin, lactoferricin B and H	Neutrophils
Lysozyme	Lymphocytes
Lactoperoxidase	Cytokines
Nucleotide-hydrolyzing antibodies	Growth factors
κ-Casein and α-lactalbumin	Hormones
Haptocorrin	Milk peptides
Mucins	Long-chain polyunsaturated fatty acids
Lactadherin	Nucleotides
Free secretatory component	Adhesion molecules
Oligosaccharides and prebiotics	Anti-inflammatory compounds
Fatty acids	Cytokines: IL-10 and TGFβ
Maternal leukocytes and cytokines	IL-1 receptor antagonist
sCD14	TNFα and IL-6 receptors
Complement and complement receptors	sCD14
β-Defensin-1	Adhesion molecules
Toll-like receptors	Long-chain polyunsaturated fatty acids
Bifidus factor	Hormones and growth factors
	Osteoprotegerin
	Lactoferrin
Tolerance/priming compounds	Long-chain polyunsaturated
Cytokines: IL-10 and TGFβ	Hormones and growth factors
Anti-idiotypic antibodies	fatty acids

10^9/L) (10) that one could assume play a role in promoting the development of the neonatal immune response. Macrophages (55–60%) and neutrophils (30–40%) dominate over lymphocytes (5–10%) (10). Viable leukocytes from milk have been isolated in feces from infants fed human milk (11), and it is also possible that key surface molecules on these cells could remain antigenically intact in the gut.

Macrophages. Breast milk–derived macrophages (CD14+) likely affect infant T and B cell function because they are reported to express activation markers (i.e., CD11c), demonstrate phagocytic activity, and secrete immunoregulatory factors (12). Additionally, milk macrophages have been reported to contain engulfed sIgA, which they could release on contact with bacteria in the gut (3).

Neutrophils. Human milk neutrophils demonstrate decreased adherence, polarity, and motility (13) and express high levels of CD11b and low levels of L-selectin (14), all indicative of prior activation. Little is known about the impact of milk neutrophils on immune development in infants but most researchers suggest that their main role is maternal protection, because they have limited functional capacity once they are secreted into milk.

Lymphocytes. The majority of lymphocytes in milk are T cells [>80% (1)]. The higher proportion of CD8+ (expressing L-selectin, α4β7 integrin, mucosal addressin cell adhesion molecule-1) and γδ+ lymphocytes, compared to blood, suggests that these CD8 cells have selectively homed from the maternal mucosal immune system to the mammary gland. Breast milk CD4+ cells are also present in an activated state (expressing activation markers CD40L, sCD30, IL-2 receptor, human mucosa lymphocyte antigen-1, or late activation protein-1) and express CD45RO+, a surface protein associated with immunological memory (15). It has been hypothesized that activated T cells from maternal origin both compensate for the immature function of neonatal T cells and promote their maturation. Additionally, activated antigen mature lymphocytes might help compensate for the low antigen presenting capacity of macrophages. In animal models, milk-derived lymphocytes can traverse the neonatal intestine, suggesting that their influence extends beyond the intestine [reviewed in (6)]. Some recent studies have shown that immunophenotypic differences in systemic lymphocyte populations occur following exposure to maternal milk. These differences include a decrease in CD4+:CD8+ cells and an increase in natural killer (NK) cells (16). The functional consequences of a report that breast-fed infants have a thymus twice the size of that of non-breast-fed infants (17) have yet to be explained but support the role of human milk on T cell development.

Cytokines. Human milk contains an array of cytokines and chemokines. This list includes IL-1 β (18,19), IL-4 (20), IL-5 (20), IL-6 (19,20), IL-8 (18,21,22), IL-10 (20,23), IL-12 (24), IL-13 (20,24), TNFα (19), TGF (transforming growth factor)β (20,25), INFγ (20), granulocyte-colony stimulating factor (26), monocytes chemotactic protein 1 (15), and RANTES (21,22). The primary source of these cytokines is the mammary gland. However, leukocytes recovered from expressed human milk have been shown to be capable of producing a number of cytokines (19). The extent to which cytokines survive passage through the infant stomach is largely unknown, but recent work has suggested that some cytokines/chemokines may be sequestered and protected until they reach the intestine (26). Although particular cytokines can be in high concentrations in some women's breast milk, in general the concentrations of cytokines vary widely, making it difficult to assess their roles (individually or together) in the development of the infant's immune system. However, the intake of cytokines through human milk clearly has the potential to influence the maturation and development of immune cells in infants. For example, maternal cytokines (TGFβ, IL-6, and IL-10) in milk could contribute to the development and differentiation of IgA-producing cells (20) and maturation of the naïve intestinal immune system (27). Unfortunately, most of the research on milk cytokine activities has been conducted in vitro and there are many factors in breast milk that could either facilitate or inhibit cytokine activities (i.e., adhesion molecules and soluble receptors and antagonist receptors for cytokines) that are not accounted for in these studies.

Microbial colonization and immune development. Unlike pathogens, which strongly activate immune defense mechanisms, bacterial antigens from the microflora have the potential to promote maturation of the infant's immune system by a yet to be defined mechanism. An optimum flora in early life that promotes Th2 response is critical for infants to promote the cytotoxic response required to clear intestinal pathogens (3).

Growth factors and other proteins. Many hormones, growth factors, and partially digested milk peptides have been detected in human milk, including cortisol, estrogen, pregnanediol, progesterone, thyroid hormones, erythropoietin, gonadotropin, human-chorionic gonadotropin, insulin, leptin, prolactin and procalcitonin, epidermal growth factor, insulin-like growth factors and binding proteins, nucleotides, α-lactalbumin, β-lactoglobulin, and lactoferrin (LF) [reviewed in (9,28)]. These compounds have clearly been demonstrated in other situations to modulate the immune system and therefore most likely affect immune development in infants.

Long-chain PUFA (LCPs). Dietary (n-6) and (n-3) LCPs modulate Th1 and Th2 cell generation in adults (29). Docosahexanoic and arachidonic acid constitute a relatively small fraction of the total fatty acids in human breast milk, but have recently been suggested to participate in immune development

(30). We reported that infants fed LCP-supplemented formula had lymphocyte populations and produced cytokines more similar to human milk–fed infants than infants who received unsupplemented formula (30). Conjugated linoleic acid, found in variable amounts in breast milk, has also been suggested to contribute to immune development (31).

Nucleotides. Dietary nucleotides are reported to benefit the systemic immune system by promoting lymphocyte proliferation, NK activity, and macrophage activation and producing a variety of other immunomodulatory factors [reviewed in (28)]. Additionally, animal models have demonstrated beneficial effects of feeding nucleotides on mucosal immunity (28). Feeding nucleotide-supplemented formula to full-term and preterm infants improved responses to immunizations, promoted T cell maturation, and reduced the risk of diarrheal disease [reviewed in (28)]. Although the mechanisms remain somewhat unclear, animal studies suggest that dietary nucleotides promote a Th1 response and modulate maturation and differentiation of B cells (28).

Constituents in Milk That Promote Tolerance/Priming of the Immune System. During infancy there is a fine balance between antigen responses that results in tolerance (suppression) to responses, which results in sensitization (priming). Food intolerance in infancy is common and hypothesized to be the result of the failure to adequately develop tolerance. It is hypothesized that the successful development of tolerance contributes to lower incidences of food-related allergies in breast-fed infants (8). Tolerance is an active process and in vitro studies suggest that the dietary antigens present in breast milk coupled with the immunosuppressive cytokines (i.e., IL-10 and TGFβ) aid in promoting tolerance to dietary and microflora antigens (3). There are also clinical data to support breast-feeding in tolerizing infants to maternal major histocompatibility complex (MHC) antigens. For example, kidney transplants from a maternal donor were shown to survive better if the recipient had been breast-fed by the mother (32). Tolerance to maternal MHC would be necessary for milk-derived immune cells to come in contact with the infant's immune system. More recently, there is support for the LCP found in milk promoting tolerance (33).

In rat studies, perinatal antigen exposure primes the immune system of suckling animals (4). There is growing interest in harvesting this specialized priming capacity in milk to transfer maternal vaccinations. It has been suggested that different vaccine priming responses reported, when beast milk is used as the vehicle, might be influenced by exposure to anti-idiotypic antibodies in milk (6). Anti-idiotypic antibodies are naturally occurring antibodies with specificity against other autologous antibodies. In the case of breast milk, anti-idiotypic antibodies are proposed to have the capability of priming the infant's antibody response against the antigen the idiotype is directed against. Animal studies have demonstrated that a relatively small amount of anti-idiotypic antibody given in the neonatal period influences the immune system in a manner so profound that the effects can still be detected two generations later (6).

Immunomodulatory/Anti-Inflammatory Components in Human Milk. Although inflammation is a beneficial defense to the infant, an exaggerated inflammatory response will result in reduced intake, illness, and gut damage. It is not entirely clear whether the exaggerated or unchecked inflammatory response to an infectious challenge occurs only in the gut or whether this extends to the infant's systemic immune system. The overall the balance of factors in breast milk appears to dampen the inflammatory response.

Anti-inflammatory cytokines. IL-10, a potent immunosuppressive cytokine, is found in breast milk (20,23), produced by mammary cells (23), but also is present in lymphocytes and macrophages in milk (1). IL-10 dampens the Th1 response, thereby inhibiting pro-inflammatory cytokine release. TGFβ (both TGFβ1 and TGFβ2), which has been reported in human milk (25) and in animals, survives passage through the infant gut (34). TGFβ is rapidly taken up by the neonatal intestine (34), suggesting that this protein may influence immunity beyond the gut. TGFβ is implicated in immune regulation and can downregulate inflammation and promote healing of intestinal cells damaged by cytokines or infection [reviewed in (27)].

Proinflammatory cytokines. The pro-inflammatory cytokines IL-1β (18,19), IL-6 (19,20), IL-8 (18,21,22), and TNFα (19) are reportedly present in variable amounts in milk. However, the IL-1 receptor antagonist and both soluble TNFα and IL-6 receptors are also present, which could bind these cytokines in the lumen and reduce their activity [reviewed in (35)]. The much higher concentration of IL-10 in milk would be predicted to downregulate the production of any potential effects of IL-8.

Other immune modulatory compounds. A number of soluble molecules that could modulate the inflammatory response have been identified in human milk. This list includes sCD14 (36) and a number of soluble adhesion molecules [i.e., intracellular adhesion molecule-1 and vascular adhesion molecule-1 (37)]. LCPs have the potential to alter lymphocyte production of cytokines and eicosanoids and the expression of cell surface markers such as adhesion molecules (29). Our work has demonstrated that feeding LCPs to infants alters the ability of the infant's peripheral mononuclear cells to produce cytokines (38). In addition to their effect on cell growth, enzyme activities, and metabolism, many of the hormones and growth factors in milk could modulate inflammatory reactions. For example, osteoprotegerin, a member of the TNF superfamily, found in high concentrations in breast milk, is suggested to prevent TNF-induced inhibition of T cell proliferation, thereby enabling T cells to dampen an inflammatory response (39).

LF. LF has been demonstrated to inhibit the production of proinflammatory cytokines (IL-1β, IL-6, TNFα, and IL-8) and mediators (nitric oxide, granulocyte-macrophage colony stimulating factor), most likely through its effect on nuclear factor κB expression (7,9).

Human milk is a complex mixture of interacting compounds, of which the composition differs not only between women but also within the lactation period. Our understanding of the importance of this complex nutritional supplement on immune development, tolerance, and regulation of inflammation is still in its infancy. This will be a fruitful area of research for nutritionists for many years.

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