

Antiinfective Properties of Human Milk^{1,2}

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Abstract

The unfavorable effects of neonatal immunodeficiency are limited by some naturally occurring compensatory mechanisms, such as the introduction of protective and immunological components of human milk in the infant. Breast-feeding maintains the maternal-fetal immunological link after birth, may favor the transmission of immunocompetence from the mother to her infant, and is considered an important contributory factor to the neonatal immune defense system during a delicate and crucial period for immune development. Several studies have reported that breast-feeding, because of the antimicrobial activity against several viruses, bacteria, and protozoa, may reduce the incidence of infection in infants. The protection from infections may be ensured either passively by factors with antiinfective, hormonal, enzymatic, trophic, and bioactive activity present in breast milk, or through a modulator effect on the neonatal immune system exerted by cells, cytokines, and other immune agents in human milk. J. Nutr. 138: 1801S–1806S, 2008.

Introduction

The increased neonatal susceptibility to infection is a direct consequence of the challenging immunological adaptation during the transitional period from intra- to extrauterine life. Indeed, the ontogeny of the immune system starts early in the embryo and continues during fetal life but is completed only several years after birth (1,2). In addition, the fetus, who develops in a highly protective germ-free environment, lacks antigenic experience. These factors are responsible for the “physiological” immaturity of the immune function in newborn infants.

The reduced cytotoxic response during fetal life, the poor T-lymphocyte response to mitogens, the immaturity of T and B lymphocytes, the inadequate cytokine synthesis, the marked deficiency of antibody production, and the reduced neutrophil, complement, and natural killer cell activity are important contributory factors to the complex deficiency of immunological function in the neonate and may represent the biological basis for the increased susceptibility to various infections and the reduced clearance of intracellular pathogens. In preterm neonates, the immunodeficiency is more severe and prolonged and is associated with higher incidence of infection and sepsis and increased risk of morbidity, mortality, or neurological sequelae (3).

The unfavorable effects of neonatal immunodeficiency are limited by some naturally occurring compensatory mechanisms such as the transplacental passage of IgG antibodies from mother to fetus during pregnancy and the introduction of protective and immunological components of human milk in the infant. Breast-feeding maintains the maternal-fetal immunological link after birth, may favor the transmission of immunocompetence from the mother to her infant, and is considered an important contributory factor to the neonatal immune defense system during a delicate and crucial period for immune development.

Antiinfective factors in human milk

There is convincing evidence that breast-feeding, because of the antimicrobial activity against several viruses, bacteria, and protozoa, may reduce the incidence of gastrointestinal (4) and nonenteric infections in infants (5). For example, the risk of nonviral diarrhea is higher for non-breast-fed infants in the first 4–6 mo of life, with odds ratios of diarrhea in the range of 3.0–6.0 (6). In 1 study, the protective effect of breast-feeding against enterovirus infections was primarily mediated by maternal antibodies in breast milk (7). In addition, a recent meta-analysis suggested that infants who were breast-fed for >4 mo showed a three times reduced incidence of severe respiratory tract infection requiring hospitalization, as compared with infants who were not breast-fed (8). In other studies, protection against otitis media and urinary tract infections was provided by breast-feeding (9,10).

Several studies have been carried out to evaluate the ability of human milk to prevent infection in preterm infants; although most of the published reports suggested a beneficial effect, there were serious methodological flaws in all of the cohort studies (such as poor study design, inadequate sample sizes, or the neglect to account for some confounders), suggesting that benefits of human milk feeding in preventing infection in preterm, very

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TABLE 1 Antiinfective and immunological components in human milk

Adaptive immunity compounds:	Immunoglobulins sIgA (11S), 7S IgA, IgG, IgM, IgE, IgD, free secretory component, antiidiotypes
Innate immunity agents:	Complement, chemotactic factors, properdin factors, interferon, α -fetoprotein, antistaphylococci factors, mannose binding lectin, β -defensin-1, antiadherence substances (oligosaccharides, mucins, lactadherin, glycolipids and glycosaminoglycans, κ -casein), milk fat globule, hormones and growth factors (prolactin, cortisol, insulin, thyroxine, prostaglandins, vascular-endothelial growth factor, nerve growth factor, TGF, erythropoietin), antiviral factors (fatty acids and monoglycerides), migration inhibition factor, α -lactalbumin
Cytokines, chemokines, and receptors:	IL-1 β , IL-2, IL-4, IL-5, IL-6, IL-8, IL-12, IL-13, IL-16, IL-18, IFN γ , TNF α , G-CSF, M-CSF, GM-CSF, GRO α , monocyte chemotactic protein-1, TGF β 1 and -2, sCD14, Toll-like receptor, sFas, sFasL
Antiinflammatory factors:	IL-10, TGF β 2, glucocorticoids, antioxidants (α -tocopherol, β -carotene, lutein, vitamin E, catalase, glutathione peroxidase,), lactoferrin, IL-1Ra, soluble TNF α receptors I and II, CD59
Prebiotics:	Bifidus factor, oligosaccharides
Histocompatibility antigens	
Carrier proteins:	Lactoferrin, transferrin, vitamin B-12 binding protein, steroid binding protein
Enzymes:	Lysozyme, lipoprotein lipase, leukocyte enzymes, antiproteases, platelet-activating factor-acetyl-hydrolase
Others:	Nucleotides, long-chain polyunsaturated fatty acids
Cellular	
Total counts:	Colostrum, $1-3 \times 10^9/L$; mature milk, $\sim 1 \times 10^9/L$
Cell types:	Macrophages, $\sim 60\%$; neutrophils, $\sim 25\%$; lymphocytes, $\sim 10\%$; epithelial cells

low-birth-weight (VLBW)³ infants are not conclusively proven by the currently available evidence (11). However, a recent report has confirmed a favorable effect of mother's milk on reduction of late-onset septicemia in a cohort of extremely premature infants (12).

Use of donor milk. As suggested by a recent systematic review (13), donor human milk may offer some protection against the development of necrotizing enterocolitis in VLBW infants, although fresh mother's milk is much more effective than pasteurized donor human milk (14).

Infectious agents in human milk. On the other hand, breast milk may increase the risk of vertical transmission from mother to infant of certain viral infections, such as HIV or cytomegalovirus (CMV) in VLBW infants (15). Maternal HIV infection is considered a contraindication to breast-feeding, at least in developed countries. Yet in settings where replacement feeding is not considered acceptable, feasible, affordable, sustainable, or safe, exclusive breast-feeding is recommended for HIV-infected women for the first 6 mo of life (16). The risk of CMV infection in VLBW neonates must be counterbalanced carefully against several distinct advantages of using fresh mother's milk. Indeed, the consequences of neonatal CMV infection seem limited, as suggested by the reassuring findings observed at follow-up after a 8.5-y study period in VLBW infants who developed transient neonatal symptoms related to postnatal CMV infection transmitted by breast milk (17). For other infections, breast milk is not an important mode of transmission, and continuation of breast-feeding is usually warranted. Appropriate immune prophylaxis [varicella-zoster virus and varicella-zoster immunoglobulin, hepatitis A virus and immunoglobulin, hepatitis B virus (HBV), and HBIG plus HBV vaccine] or antimicrobial therapy (e.g., *T. pallidum*, *M. tuberculosis*, *H. influenzae*) to protect the infant against transmission should be provided when indicated.

In a few situations, temporary cessation of breast-feeding or the avoidance of breast milk may be required for a limited time (24 h for *N. gonorrhoeae*, *H. influenzae*, group B streptococci, and staphylococci, and longer for others including *T. pallidum* and *M. tuberculosis*) (15).

Bioactive factors in human milk. A host of factors with immunological, hormonal, enzymatic, trophic, and bioactive activity present in breast milk can offer passive protection (18) (Table 1). In addition, breast milk is rich in maternal cells (19) that may produce cytokines and exert a modulatory effect on the neonatal immune system. Macrophages and leukocytes, which are largely concentrated at the beginning of the lactation, are mainly included among the cellular components (Table 1). Breast-milk feeding, therefore, may represent an important means of transmission of immunocompetence from the mother to her infant.

Most of the protective components of human milk may interact synergistically with each other or with factors related to the mucosal or systemic immune response (20). Other factors may develop or may be activated only after they reach the intestinal tract or may behave differently depending on the context (21).

The nondigestible fucosylated and sialylated oligosaccharides, either free or conjugated with proteins or lipids, may directly inhibit the adhesion of diarrheal pathogens (22) and protect the infant against infectious diarrhea (23) or indirectly produce a protective and immunomodulatory result through a prebiotic effect on the infant intestinal microflora (24). In addition, a mixture of synthetic oligosaccharides (fructooligosaccharides and galactooligosaccharides) was recently shown to reduce the incidence of atopic dermatitis during the first 6 mo of life (25) and to stimulate the response to influenza vaccination in an experimental model (26).

Particularly well known is the protective role of secretory IgA, which is lacking in newborn infants but is present at very high concentration in the colostrum (~ 10 g/L) and in mature milk (~ 1 g/L). The percentage of IgA₂, more resistant to the peptic acidity in the stomach and to digestion by enteric enzymes and bacterial proteases, is much higher in mother's milk than plasma; indeed, the IgA₁/IgA₂ ratio is $\sim 6:4$ in milk, as opposed to 9:1 in plasma. Neonatal IgA intake with milk is $\sim 0.5-1$ g/d.

³ Abbreviations used: BAL, tracheobronchial tree mucosa; CMV, cytomegalovirus; GALT, gut-associated lymphoid tissue; G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte-macrophage colony-stimulating factor; HBV, hepatitis B virus; IFN γ , interferon- γ ; M-CSF, macrophage colony-stimulating factor; TGF β , transforming growth factor- β ; VLBW, very low-birth-weight.

IgA concentration may be higher in milk from mothers delivering prematurely (27). IgG and IgM are also present at lower concentrations and provide the infant with ~10–100 mg/d (5). The distribution of specific antibodies within IgG subclasses in human milk may compensate for the reduced transplacental transfer of some antibodies, as those against pneumococci (28).

The entero-broncho-mammary link of IgA⁺ B lymphocytes and mucosal immune system is considered a means of transfer of highly specific protection from a mother to her infant. When the nursing mother is exposed to antigenic material from environmental pathogens, M cells of Peyer's patches in the gut-associated lymphoid tissue (GALT) or tracheobronchial tree mucosa (BALT) acquire and present the antigen to B cells that become active to secrete IgA and migrate to local and regional lymph. Then they reach salivary and lacrimal glands, intestine, upper airways, and the urogenital tract. During pregnancy and lactation, because of hormonal stimuli, IgA⁺ B lymphocytes colonize mammary glands and produce specific secretory IgA that may bind to pathogen and prevent infection (29). The antimicrobial effects of IgA antibodies are related both to immune exclusion, by inhibition of epithelial adherence and penetration or microbial agglutination and neutralization, and immune elimination, by phagocytosis and cytotoxicity via FcαRI (30). However, HIV-specific IgA in human milk from HIV-infected mothers do not show a protective role; on the contrary, specific IgA antibodies may be associated with an enhanced transmission of the infection (31,32).

Among the carrier proteins, lactoferrin, a proteolysis-resistant glycoprotein, is the dominant whey protein. Its protective effect may be linked to competition with siderophilic bacteria and fungi for ferric iron and to the epithelial growth-promoting activity (1,5).

Human milk and the infant immune response

Several reports seem to confirm that the immunological components of human milk can influence the infant immune response (33–47).

Spontaneous integrin expression on CD4⁺, CD8⁺, and CD19⁺ lymphocytes at 6 mo were reported to be significantly lower in breast-fed than formula-fed infants. In addition, breast-fed 12-mo-old children showed increased production of IFNγ and increased percentages of CD56⁺ and CD8⁺ cells after measles-mumps-rubella vaccination, as compared with formula-fed infants (48).

Cell-mediated immune response to bacillus Calmette-Guérin vaccine given at birth, measured in terms of lymphocyte blastogenesis stimulated by purified protein derivative of *Mycobacterium tuberculosis*, was significantly enhanced in breast-fed infants (49).

Ultrasound assessment of the thymic index suggested decreased thymus size in formula-fed infants compared with breast-fed infants at 4 mo of age (50). Because CD8⁺ cells may be correlated to the thymic index, a higher CD8 percentage was reported in breast-fed infants than formula-fed infants at 8 mo (51).

Ribonucleotide in human milk may support increased T-cell maturation, affect immunoregulatory natural killer cell subsets (52), and stimulate significantly higher poliovirus type 1 (53) or tetanus toxoid antibody responses (54).

Breast-fed infants show a better-developed response to *Haemophilus influenzae* type b polysaccharide, oral poliovirus, tetanus, and diphtheria toxoid vaccines (1,5,55). Specific secretory IgA synthesis results particularly improved (56). Antibody response to HBV vaccine was slightly reduced in infants born to HBsAg-positive mothers (57), whereas no significant differences were observed when mothers were HBsAg negative (58).

Response to rhesus rotavirus reassortant vaccines was similar in breast-fed and non-breast-fed children (59).

Human milk may offer a long-term protection against the development of allergy (60), insulin-dependent diabetes, Crohn disease, ulcerative colitis, and tumors in infancy (61). The latter effect may also be favored by the human α-lactalbumin made lethal to tumor cells, a novel type of protein produced after the modification of α-lactalbumin, under the conditions that exist in the stomach of a nursing infant, that induces apoptosis of tumor cells (62,63).

Of the several factors with immunological, hormonal, enzymatic, and trophic activity, cytokines are believed to play a significant role in the immune-modulation and immune-protection of breast milk. Most of the cytokines that are known to be deficient in the neonate, particularly in preterm infants, have been found in significant amounts in breast milk: IL-1β, IL-2, IL-6, IL-8, IL-10, IL-12, IL-18, IFNγ, TNFα, transforming growth factor-β (TGFβ), granulocyte colony-stimulating factor (G-CSF), macrophage colony-stimulating factor (M-CSF), and granulocyte-macrophage colony-stimulating factor (GM-CSF) (64–73). Breast milk cytokines may reach the neonatal intestine intact because of protection from digestion by protease inhibitors, mainly α₁-antichymotrypsin and α₁-antitrypsin, that are present in maternal milk. In addition, the gastric digestion of proteins is reduced during the first 3 mo of life because of the limited secretion of pepsin and H⁺ ions and the general immaturity of the newborn's digestive abilities. These factors favor the survival and the intestinal absorption of undamaged polypeptides so that they can exert their biological activities (74).

Breast milk is rich in cytokine-producing cells, mainly macrophages and activated T lymphocytes; most milk T cells display the CD45RO marker of activation (75), at variance with the neonatal T cells, which predominantly express the "naïve" CD45RA phenotype (Fig. 1).

Cord blood T lymphocytes are less able to perform Th1- and Th2-like responses. Because of the more important IFNγ deficiency, it is suggested that the Th1-like response is more compromised in neonates (76) and that a progressive maturation toward the Th1-like response occurs with age (77). We recently evaluated cytokine production in breast milk by CD4 and CD8 lymphocytes, expressing either the CD45RA or the CD45RO antigen. Intracellular cytokine synthesis of fluorescent-stained cells was measured by 3-color flow cytometry. Our data showed a substantially higher percentage of cytokines producing cells in breast milk than in cord blood. IFNγ was the most relevant

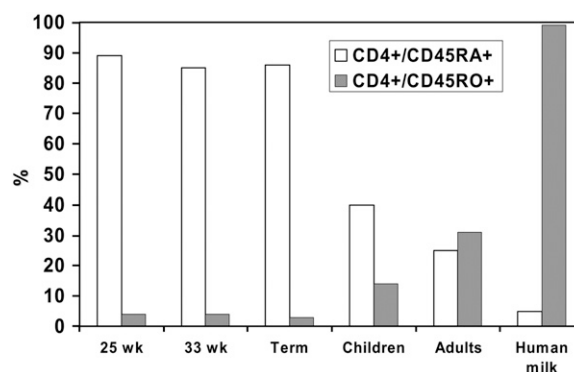


FIGURE 1 Distribution of memory (CD45RO⁺) and naïve (CD45RA⁺) T cells in human milk and in neonates at various gestational ages, children, and adults. Adapted from Gasparoni et al. (2,81).

cytokine found in breast milk, as opposed to the very low levels found in cord blood (78). The high percentages of Th1-type cytokines (IL-2 and, particularly, IFN γ) may play an important role in the differentiation of neonatal T cells toward a preferential Th1, rather than Th2, pathway. Such a hypothesis seems to be in accordance with a report on the differential immune response to measles vaccination, showing a preferential Th1 activation in breast-fed infants (79).

Immunological characteristics of maternal milk may vary according to the race. We recently measured the levels of breast milk IL-4, IL-8, IL-10, TNF α , and IFN γ to evaluate their race-related variations. These cytokines were chosen because of their possible role in modulation of neonatal immune development (80). IL-8 levels were significantly higher in milk samples from Asian mothers as compared with African ones; in addition, IL-10 concentration was significantly higher in Italian than in African women (81).

These data suggest that the immunological factors of breast milk may contribute to one of the most important and peculiar characteristics of human milk, that is, the dynamic variability of its nutritive, bioactive, and functional components.

Other articles in this supplement include references (82–91).

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