The Immunologic Significance of Breast Milk

Susan Orlando, RNC, MS

The importance of breast milk in protecting the newborn from infection is recognized worldwide. Infant morbidity and mortality have been directly affected by a decline in breastfeeding. Health care providers are working toward meeting the national goal of increased initiation and duration of breastfeeding. This article focuses on the protective factors transferred to the infant through breast milk. A discussion of maximizing the immunologic benefits of breast milk for the high-risk infant is presented.

There is no substitute for breast milk. Commercial formulas may meet the nutritional needs of premature and term infants; however, the protective properties of breast milk are unique and cannot be duplicated in the laboratory. The components identified in breast milk are multifunctional and interactive. The composition of breast milk complements the developing host defense system in the newborn infant.

Host Defense Mechanisms

The host defense mechanisms of the newborn can be categorized into nonspecific (innate) and specific (acquired) responses. Nonspecific mechanisms function effectively with no prior exposure to a microorganism or its antigens. Intact skin, mucous membranes, gastric acid, and digestive enzymes serve as barriers to microorganisms. Phagocytic cells ingest and kill bacteria and other microorganisms. Serum proteins of the complement system mediate opsonization and lysis of bacteria. The alternative pathway of complement may be activated by immunoglobulin A (IgA) and acts directly on the third component (C3). This pathway is activated in the absence of specific antibodies. In contrast to these nonspecific mechanisms, specific host defense mechanisms function most effectively after exposure to the infecting agent or its antigens. Antibody-mediated immunity involves B lymphocytes, plasma cells, immunoglobulins, and antibodies. Cell-mediated immunity refers to the reaction of sensitized T lymphocytes and the interaction with macrophages.

Transfer of Immunity

Passive immunity results from active placental transfer of specific antibodies from mother to fetus. Most IgG transfer occurs during the 3rd trimester and increases with increasing gestational age. IgG antibodies to viruses and bacterial toxins provide transient protection to the newborn. Antibody protection against the encapsulated pyogenic organisms such as Streptococcus, Hemophilus influenzae, Staphylococcus, and Pneumococcus is present for the first several months of life. There is limited or no transfer of antibodies to agents that produce IgA or IgM antibody response. This places the newborn at high risk for infection caused by gram-negative organisms such as Escherichia coli, Salmonella, and Shigella. In addition, the infant cannot be protected against organisms to which the mother has little or no immunity.

Immunologic Properties of Breast Milk

Host resistance factors are abundant in colostrum and fresh breast milk (Table 1). Specific and nonspecific factors are transferred to the newborn through breast milk. The most important role for breast milk in host defense against infection appears to be the supply of local protective factors to the infant's gastrointestinal tract (Cates, Rowe, & Ballow, 1983). Immunoglobulins, components of the complement system, carrier proteins, enzymes, and hormone-like substances are present in breast milk. Nonspecific factors, such as bifidus factor and epithelial growth factor, have local effects on the gastrointestinal tract. The cells identified in breast milk include T and B lymphocytes, macrophages, monocytes, epithelial cells, and polymorphonuclear leukocytes (PMNs). Soluble factors active against viruses, streptococci, and staphylo-

Table 1. *Major Host Defense Factors in Colostrum and Fresh Human Milk*

Immunoglobulins
 Secretory IgA
 IgM
 IgG
Lactoferrin
Lysozyme
Complement proteins
Bifidus factor
Cellular components
 Macrophages
 T and B lymphocytes
 Polymorphornuclear leukocytes (PMN)

cocci also are present in breast milk. The immune factors in breast milk have shared features:

1. they are common to mucosal sites;
2. they are capable of surviving in the gastrointestinal tract because they are resistant to digestive enzymes;
3. they kill certain bacterial pathogens synergistically;
4. their protection is achieved without triggering inflammatory reactions; and
5. the secretion of many soluble immune factors by the mammary gland is inversely related to the ability of the recipient to produce them at mucosal sites (Goldman, 1993).

Secretory IgA

The most abundant immunoglobulin present in breast milk is secretory IgA. The secretory component enables the IgA molecule to resist digestion by pepsin and trypsin and hydrolysis by gastric acid. The concentration of IgA is highest in colostrum and peaks during the first 3–4 postpartum days. As the volume of milk increases, the concentration of IgA declines and reaches a plateau that remains higher than maternal serum levels during lactation (Ogra & Ogra, 1978).

Secretory IgA found in breast milk protects the infant from a variety of enteric and respiratory pathogens. Antibodies to bacteria, viruses, parasites, and fungi have been identified in breast milk (Table 2). Most of the antibodies present in breast milk are directed against organisms that colonize or are pathogenic to the gastrointestinal and respiratory tracts. The unique antibody composition of breast milk compensates for the lack of protection against enteric antigens in placentally transferred IgG. Transfer of antibodies via breast milk is specific for pathogens present in the mother's environment. Sensitized lymphocytes are transported from the maternal gastrointestinal and bronchotracheal-associated lymphatic tissues to the mammary gland, where they differentiate into plasma

cells and secrete antibodies. Maternal exposure to the infant's microorganisms results in the production of milk antibodies, which in turn protect the breastfed infant.

Breastfeeding protects against upper respiratory infection, specifically against respiratory syncytial virus (Laegreid, Kolstøotnæss, Ørstavik, & Carlsen, 1986). Secretory IgA binds to mucosal epithelium and inhibits bacterial or viral adherence to mucosal cells. Protection of the respiratory tract may be linked to regurgitation of milk into upper airways (Hayward, 1983). Secretory IgA antibody protects by neutralizing enterotoxins (Stoliar, Pelley, Kaniecki-Green, Klaus, & Carpenter, 1976). This effect may explain why infants with diarrhea caused by certain strains of *E. coli* show improvement when fed breast milk.

IgM and IgG

The level of IgM is highest in colostrum and decreases during the first week of lactation. The IgG concentration is constant during the first 6 months of lactation (Ogra & Ogra, 1978). IgM and IgG bind complement. Bacteria coated with these antibodies can be opsonized for phagocytosis.

Lactoferrin

Lactoferrin is an iron-binding protein that functions in a bacteriostatic manner by depriving organisms of iron. Lactoferrin also acts on microbes by blocking carbohydrate metabolism, attacking the cell wall, and binding calcium and magnesium (Sanchez, Calvo, & Brock, 1992). Organisms with high iron requirements, such as

Table 2. *Organsisms Affected by Anti-infective Factors in Human Milk*

Bacteria	Viruses
Escherichia coli	Rotavirus
Salmonella	Rubella
Shigella species	Poliovirus 1, 2, 3
Vibrio cholerae	Echovirus
Bacteriodes fragillis	Coxsackie viruses A and B
Bordetella pertussis	Respiratory syncytial virus
Clostridium tetani	Cytomegalovirus
Clostridium difficile	Influenza A virus
Corynebacterium diptheriae	Arboviruses
Streptococcus mutans	Herpes simplex type 1
Streptococcus pneumoniae	Other
Haemophilus pertussis	Candida albicans
Diplococcus pneumonae	Giardia lamblia
Staphylococcus aureus	Entamoeba histolytica
Haemophilus influenza type B	
Mycobacterium tuberculosis	
Chlamydia trachomatis	

coliforms and yeast, are inhibited by lactoferrin. Secretory IgA and lactoferrin act synergistically to produce a greater antibacterial effect against *E. coli.* The anti-infective activity of lactoferrin is reduced when cow milk-based infant formula is added to the diet of the breastfed infant. However, this effect is not seen with the addition of human milk fortifier (Kerner, Yang, & Stevenson, 1988). The concentration of lactoferrin is high in colostrum and progressively drops during the following 12 weeks of lactation. The level remains stable after the first 4 months.

Lysozyme

The antimicrobial factor lysozyme is an enzyme that is synthesized by the milk macrophage. The concentration increases over time during lactation. Preterm milk contains higher levels of lysozyme. Lysozyme digests a bond in the cell wall of bacteria. It also has anti-inflammatory properties. The synergistic effect of secretory IgA and lysozyme kills resistant *E. coli.* The beneficial effects of lysozyme are reduced when cow milk-based formula is added to breast milk. A similar decrease in lysozyme activity was seen when soy-based formulas were combined with breast milk. No detrimental effect on the anti-infective properties was demonstrated with the addition of powdered human milk fortifier (Kerner et al., 1988). Lysozyme is stable in the gastrointestinal tract. The stools of breastfed infants contain large concentrations of lysozyme, which acts with lactoferrin to control the growth of pathogens.

Complement Proteins

The level of complement proteins in breast milk is lower than that in maternal serum. The interaction of complement proteins and immunoglobulins amplifies antibody activity. IgG and IgM activate complement. When activated, the C3 component of complement has opsonic, anaphylactic, and chemotactic properties. It plays a role in the lysis of bacteria bound to a specific antibody.

Bifidus Factor

The predominant organism in the stool of breastfed infants is Bifidobacterium. Breast milk contains a specific factor that promotes the growth of *Bifidobacterium bifidum.* This organism rapidly increases in number with the initiation of breastfeeding. By day 5 of life, Bifidobacterium organisms significantly outnumber enterobacteria (Yoshioka, Ken-ichi, & Fujita, 1983). The predominance of gram-positive lactobacilli prevents the proliferation of coliform and other potentially pathogenic organisms known to cause neonatal disease. The acidic environment resulting from lactose fermentation inhibits the growth of organisms such as pathogenic *E. coli,* Salmonella, and Shigella.

Cellular Components

Breast milk contains macrophages, T and B lymphocytes, neutrophils, and epithelial cells. The cell numbers are highest in colostrum and early milk. Most of the leuko-

Breast milk and colostrum provide the infant with secretory IgA, which acts as a barrier to antigen in the immature gut.

cytes in breast milk are macrophages. These cells synthesize lysozyme, lactoferrin, and the complement proteins C3 and C4. Other functions of the milk macrophage include phagocytosis, bacterial killing, and interaction with lymphocytes present in breast milk (Pitt, 1979).

Lymphocytes in breast milk produce secretory IgA and interferon (Welsh & May, 1979). Interferon provides protection against viral infections. The response of milk T cells to common antigens differs from that of peripheral blood T cells. Milk T cells are highly reactive to organisms invading the gastrointestinal tract. Antigen introduced in the maternal gastrointestinal tract stimulates the development of antibody in the breast milk. Sensitized lymphocytes originating in the maternal gut lymphoid tissue migrate to the mammary gland and synthesize IgA antibody. A similar immune response is seen after the introduction of antigens in the maternal bronchopulmonary tract. IgA antibody to specific respiratory tract viruses is present in breast milk (Fishaut, Murphy, Neifert, McIntosh, & Ogra, 1981).

Polymorphonuclear leukocytes present in breast milk protect the mammary gland. The functions of these cells include chemotaxis and phagocytosis. The highest concentration of cells occurs during the first few days of lactation. There is a significant increase in the number of milk PMNs during episodes of mastitis.

Other Protective Factors in Breast Milk.

An antistaphylococcal factor present in the lipid fraction of breast milk provides protection from infection caused by *Staphylococcus aureus.* Glycolipids, glycoproteins, and free oligosaccharides inhibit bacterial adhesion to epithelia. Bile salt-stimulated lipase is the major factor in breast milk that inactivates protozoans. Lipid-enveloped bacteria and viruses also are inactivated by lipase. Macromolecules are thought to inhibit attachment and penetration of herpes simplex, Coxsackie B, rotavirus, and cytomegalovirus (CMV) (Lawrence, 1994).

Antiallergenic Properties of Breast Milk

The neonate's small intestine is immature and has increased permeability to foreign macromolecules. The production of secretory IgA by the intestinal tract is delayed until 6 weeks of age or later (Lawrence, 1994). Allergic responses can develop after the ingestion of cow's milk protein. Lack of secretory IgA in the formula-fed newborn may permit more antigen to reach the immune system (Hayward, 1983). Breast milk and colostrum provide the infant with secretory IgA, which acts as a barrier

Table 3. Definitions of Breast Milk Preparations

Fresh raw milk: Milk stored continuously at 4°C for use within 72 hours after expression.

Fresh frozen milk: Fresh raw milk that has been frozen and held at −20°C for less than 12 months.

Heat-processed milk: Fresh raw or fresh-frozen milk that has been heated to a minimum of 56°C for 30 minutes.

Holder pasteurization: Rapidly heating fresh raw or fresh-frozen milk to 62.5°C and holding it at that temperature for 30 minutes.

Pooled milk: Milk from more than one donor

to antigen in the immature gut. A significant decrease in the incidence of eczema, wheezing, and otitis media can result when infants with a family history of allergic disorders are breastfed (Ogra & Fishaut, 1990).

Breast Milk and Necrotizing Enterocolitis

Necrotizing enterocolitis remains a major cause of morbidity and mortality in high-risk infants. Early studies have examined the use of breast milk as protection against necrotizing enterocolitis. However, there are case reports of infants with the disease despite exclusive feedings of fresh or frozen breast milk.

The value of breast milk in protecting the high-risk infant from necrotizing enterocolitis has been re-explored in a prospective multicenter study. In exclusively formula-fed infants, necrotizing enterocolitis was 6–10 times more common than in those fed breast milk alone and three times more common in those who received a combination of formula and breast milk. Infants who received pasteurized donor milk seemed to have the same protective effect as those who received raw maternal milk. The greatest difference in the incidence of disease was seen in infants older than 30 weeks of gestation. Necrotizing enterocolitis was 20 times more common in the infants who received only formula. When formula feeding was delayed, the incidence of necrotizing enterocolitis was lower. However, early introduction of enteral feedings of breast milk did not increase the incidence of disease in the exclusively breastfed group (Lucas & Cole, 1990).

Breast milk provides the high-risk infant with secretory IgA, which exerts a local protective effect on the gut lumen. The percentage of IgA in colostrum of mothers who deliver preterm is significantly higher than that of those who deliver at term. Exclusive use of colostrum as an early enteral feeding for preterm infants supplies the infant with specific antibacterial and antiviral antibodies during the period when function of the mucosal immune system is delayed. Variables such as the time of feeding initiation, the choice of diet, and the rate of increase in volume of feeding may affect the overall rate of necrotizing enterocolitis in some neonatal intensive care units.

Maximizing the Protective Factors in Breast Milk for the High-Risk Infant

The immunologic benefits of providing breast milk to the newborn infant are accepted worldwide. High-risk infants who are unable to feed directly at the breast may be given milk expressed from their mothers. Table 3 defines the various preparations of breast milk available for high-risk infants. When properly collected and stored, fresh or fresh-frozen milk from an infant's mother provides the highest quality of anti-infective properties. The Centers for Disease Control and the Food and Drug Administration recommend heat treatment of donor milk to destroy harmful bacteria and viruses. Holder pasteurization of breast milk requires heat treatment at 2.5°C for 30 minutes. Milk leukocytes and complement are destroyed in this process. Secretory IgA may be reduced by as much as 30%. Lactoferrin is reduced by two-thirds. Many antiviral factors in human milk remain stable during pasteurization (Welsh & May, 1979). Guidelines for the establishment and operation of breast milk banks have been published to ensure the safety, quality, and standard of banked milk (Arnold & Tully, 1991).

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The benefits of providing breast milk must be weighed against the risk of transmitting pathogens to the compromised infant. Contamination of breast milk occurs during pumping, collection, storage, and final preparation for feeding. Milk contaminated with *S. aureus*, group B hemolytic Streptococcus, and Salmonella species has been associated with neonatal infection (Losonsky & Ogra, 1992). Nosocomial infection caused by *Staphylococcus epidermidis* is common in intensive care nurseries. The gastrointestinal tract is a possible portal of entry for pathogenic organisms in the preterm infant. The consequences of feeding breast milk containing potential pathogens to the vulnerable infant may be devastating. Thus, guidelines for pumping and storing maternal milk for the high-risk infant in the hospital are more stringent than are recommendations for storing milk for the healthy infant during maternal absence. Expressed breast milk should be free of all bacteria other than normal skin flora. Skin flora should be present in minimal concentrations, usually 10^2–10^4 colony forming units per milliliter (Meier & Wilks, 1987). Protocols for bacteriologic surveillance of expressed maternal milk aid in identifying potential sources of contamination.

Viral contaminants of breast milk include rubella,

herpes simplex, hepatitis B and CMV. The greatest risk for severe CMV infection is in the premature infant of a nonimmune mother who has no serum antibody to CMV. Donor screening for CMV or pasteurization of donor milk should be done to avoid transmission of the virus to the nonimmune infant (Goldfarb, 1993).

Human immunodeficiency virus (HIV) has been isolated from the breast milk of infected women. The Centers for Disease Control and the Public Health Service recommend that women in the United States who test positive for HIV should not breastfeed. This will avoid postnatal transmission to the infant, who may not be infected (Lawrence, 1994). Death rates in developing countries among infants not breastfed far exceed the risks of HIV transmission by this route. The World Health Organization has recommended that unless safe infant formulas are readily available, breastfeeding should continue to be promoted, even in areas where the HIV epidemic is most severe (Goldfarb, 1993).

Methods of collection and storage of expressed breast milk may alter the available protective factors. Ideally, fresh breast milk should be expressed before each feeding. This method maximizes the anti-infective properties of breast milk received by the compromised infant. The need for refrigeration and freezing is eliminated. However, most mothers are unable to remain near their infants in the hospital for extended periods of time. Many premature and ill infants are unable to consume a large volume of milk. Refrigeration and freezing of expressed milk are needed for these infants.

Bacteria found in breast milk when collected under strict conditions usually reflect normal skin flora. Concentrations of normal flora in refrigerated milk samples decrease with time. The bacterial inhibitory factors in breast milk remain active during refrigeration and freezing (Hernandez, Lemons, Lemons, & Todd, 1979; Sosa & Barness, 1987). It appears that factors involved in suppressing bacterial growth are heat labile because pasteurization destroys bacterial inhibition properties in breast milk.

Anti-infective properties of breast milk are affected by the type of container used for storage. Glass, polyethylene bags, and rigid polyethylene containers are commonly used to store breast milk. Nylon-laminated bags also have been introduced. Early research in breast milk banking reported decreased leukocyte counts in milk stored in glass containers (Paxson & Cress, 1979). Later studies reported cell count to be affected more by storage length than by type of container (Goldblum, Garza, Johnson, Nichols, & Goldman, 1981). Because live cells are destroyed by freezing, the effect of container type on the remaining immune properties becomes critical. Storing breast milk in polyethylene bags dramatically reduces the amount of secretory IgA antibodies. Difficulty in filling and handling the bags increases the risk of contamination. Rigid polypropylene plastic containers maintain the stability of cells and immunoglobulins (Goldblum, Goldman, Garza, Johnson, & Nichols, 1982). Glass containers are the best choice for storing breast milk because

there is no absorption of secretory IgA antibodies and other proteins of proven benefit to the infant (Hopkinson, Garza, & Asquith, 1990).

Refrigeration of breast milk protects cell viability and function. A significant loss of cellular viability is noted when breast milk is stored at 4°C for more than 48 hours. Guidelines on the recommended storage time for refrigerated breast milk vary. Refrigeration times that exceed 48 hours result in approximately 50% reduction of viable and functional cells (Pittard & Bill, 1981). Based on research findings, a more stringent protocol for high-risk infants in the hospital is required to provide maximal protective properties. The Committee on Nutrition of the American Academy of Pediatrics and the Canadian Pediatric Society recommend that milk be stored at 3-4°C if it can be used within 48 hours and at −20°C or lower for longer storage (Goldfarb, 1993).

Freezing breast milk significantly alters its cellular stability, but there is only minimal effect on its antibody content.

Freezing breast milk significantly alters its cellular stability, but there is only minimal effect on its antibody content. No viable cells remain in breast milk after freezing. Samples that were subjected to deep freezing at −20°C for 3 months had no significant decrease in lactoferrin, lysozyme, IgA, IgG, and C3 (Evans, Ryley, Neale, Dodge & Lewarne, 1978). Bacterial growth-inhibiting activity remains present in milk samples frozen for as long as 3 weeks (Hernandez et al., 1979).

Methods of thawing frozen breast milk can adversely affect the anti-infective factors. Use of microwave ovens to thaw frozen breast milk or to warm freshly expressed, refrigerated milk can be detrimental. The greatest effect is seen at high temperatures. A marked decrease in activity of anti-infective factors was noted when samples were subjected to microwave temperatures of 72–98°C. Warming in the microwave at low temperatures (20–53°C) resulted in significant decreases in lysozyme and specific IgA to E. coli serotype 06. When temperatures were controlled at 20–25°C, the growth of E. coli was five times greater than the control sample (Quan et al., 1992). For optimal benefit, frozen milk should be thawed in the refrigerator and used for feeding within 24 hours. Contamination can occur during warming when milk containers are submerged in warm tap water. Slow warming at a steady temperature can be accomplished in the hospital using a standard laboratory incubator (McCoy, Kadowaki, Wilks, Engstrom, & Meier, 1988).

The use of breast milk as prophylaxis against infection is optimized when the infant is allowed to feed directly at the breast. Specific protocols aimed at maximiz-

ing the immunologic benefits of breast milk should be used for the high-risk infant. Mothers should receive detailed instruction in proper techniques of pumping and storing milk. The importance of fresh colostrum for early feeding should be emphasized. Routine bacteriologic surveillance of expressed breast milk assures no pathogens are transmitted to the high-risk infant via breast milk. Protocols for continuous feeding of breast milk should address frequent change of syringe and tubing to prevent bacterial overgrowth. Intermittent gavage feeding of fresh breast milk should be used when possible. Neonatal intensive care unit protocols that provide a comprehensive approach to breastfeeding support for the preterm infant are essential. Early transition to breastfeeding with the availability of experienced nurses to provide ongoing support and consultation are key in preventing breastfeeding failure (Meier et al., 1992).

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Address for correspondence: Susan Orlando, RNC, MS, 23 West Woodlawn Drive, Destrehan, LA 70047.

Susan Orlando is the unit director of the neonatal intensive care unit at Ochsner Foundation Hospital in New Orleans, LA.