HUMAN MILK*

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Mother's milk delivered naturally through breast-feeding has been the sole source of infant nutrition in mammalian species for millions of years. Since human beings learned to domesticate cattle about 10,000 years ago, nonhuman mammalian milk also has been used to supplement or replace maternal milk in the human infant. The development and widespread use of commercially prepared infant formula products have been phenomena of the 20th century and notably of the past 6 decades. Additional information acquired during the last several years has reinforced existing concepts on the role of breast-feeding in protecting the infant against infections, in providing an ideal source of infant nutrition, in modulating infectious immune responses, and in suppressing the evolution of neoplasms and autoimmune disease later in life.[1]

Over the past few decades, the immune responses on intestinal and respiratory mucosal surfaces to local infections have been intensely studied. These investigations have led to the development of concepts of immunity on mucosal surfaces of gastrointestinal, respiratory, and genitourinary tracts and identification of mucosa-associated lymphoid tissue (MALT) and local mechanisms of defense that are distinct from the internal (systemic) immune system.

This chapter reviews existing information on major aspects of the physiologic, nutritional, immunologic, and anti-infective components of the products of lactation. Also discussed is the most recent evidence on the contribution of human milk to the development of immunologic integrity in the infant and its influence on the outcome of infections and other host-antigen interactions in the neonate.

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*This chapter is dedicated to Lars A. Hansen, MD, PhD, the discoverer of sIgA in human milk, the father of modern "mother's milk feeding practices," and a remarkable human being.

PHYSIOLOGY OF LACTATION

Developmental Anatomy of the Mammary Gland

The rudimentary mammary tissue undergoes several developmental changes during morphogenesis and lactogenesis: In the 4-mm human embryo, the breast tissue appears as a tiny mammary band on the chest wall[2,3]; by the 7-mm embryonic stage, the mammary band develops into the mammary line, along which eventually develops the true mammary anlage; by the 12-mm stage, a primitive epithelial nodule develops; by the 30-mm stage, the primitive mammary bud appears. These initial phases of development take place in both genders (Table 5-1). Further development in the male, however, appears to be limited by androgenic or other male-associated substances.[4,5] Castration in male rat embryos early in gestation leads to female breast development, whereas ovariectomy in the female does not alter the course of development of the mammary anlage. Toward the end of pregnancy, initial phases of fetal mammary differentiation seem to occur under the influence of placental and transplacentally acquired maternal hormones, with transient development of the excretory and lactiferous ductular systems. Such growth, differentiation, and secretory activities are transient and regress soon after birth.[5,6]

At thelarche, and later on at menarche, true mammary growth and development begin in association with rapidly increasing levels of estrogens, progesterone, growth hormone, insulin, adrenocorticosteroids, and prolactin.[6,7] Estrogens appear to be important for the growth and development of the ductular system, and progestins, for lobuloalveolar development (see Table 5-1). Final differentiation of the breast associated with growth and proliferation of the acinar lobes and alveoli continues to be influenced by the levels of estrogen and progesterone. Other peptide hormones, such as prolactin, insulin, and placental chorionic somatomammotropin, appear to be far more important for the subsequent induction and maintenance of lactation (see Table 5-1).

It appears that prolactin secretion from the pituitary gland is under neural control and that the increasing innervation of the breast observed throughout pregnancy is regulated by estrogens.[7] Intense neural input in virgin and parturient but not in currently pregnant mammals has been shown to result in lactation. For example, lactation in goats can be induced by milking maneuvers. Adoptive breast-feeding also is well documented in primitive human societies. Sudden and permanent cessation of suckling can result in the termination of milk secretion and involution of the breast to the prepregnant state as the concentrations of prolactin decline. Estrogen and progesterone also may amplify the direct effects of prolactin or may induce additional receptors for this peptide hormone on appropriate target tissues in the breast.

Table 5–1   **Possible Endocrine Factors in Growth of Human Female Mammary Glands**

Clinical State	Growth Characteristics	Maturational Hormones
Prenatal	Rudimentary	None
Infancy	Rudimentary	None
Puberty	Growth and budding of milk ducts	Growth hormone, prolactin-estrogen, adrenocortical steroids, prolactin (high doses)
Pregnancy	Growth of acinar lobules and alveoli	Estrogen, progesterone, prolactin, growth hormone, adrenocortical steroids
Parturition	Alveolar growth	Prolactin, adrenocortical steroids
Lactational growth of tissue	None	None
Secretory products	Casein, α-lactalbumin	Prolactin, insulin, adrenocortical steroids

Endocrine Control of Mammary Gland Function

Breast tissue is responsive to hormones, even as a rudimentary structure, as illustrated by the secretion of "witch's milk" by both male and female newborns in response to exposure to maternal secretion of placental lactogen, estrogens, and progesterone.[3] The secretion of this early milk ceases after exposure to maternal hormones has waned. Sexual differentiation, marked by puberty, is the next major stage in mammary development. As pointed out earlier, androgens inhibit the development of mammary tissue in the male, whereas the development of mammary tissue in the female is dependent on estrogen, progesterone, and pituitary hormones.[8] The postpubertal mammary gland undergoes cyclical changes in response to the release of hormones that takes place during the menstrual cycle. The last stage of development occurs during menopause, when the decline in estrogen secretion results in some atrophy of mammary tissue.

During the menstrual cycle, the mammary gland responds to the sequential release of estrogen and progesterone with a hyperplasia of the ductal system that continues through the secretory phase and declines with the onset of menstruation. The concentration of prolactin modestly increases during the follicular stage of the menstrual cycle but remains constant during the secretory phase.[9] Prolactin secretion appears to be held in readiness for the induction and maintenance of lactation.

Initiation and Maintenance of Lactation. Pregnancy is marked by profound hormonal changes reflecting major secretory contributions from the placenta, the hypothalamus, and the pituitary gland, with contributions from a number of other endocrine glands (e.g., the pancreas, thyroid, and parathyroid). Increased estrogen and progesterone levels during pregnancy stimulate secretion of prolactin from the pituitary, whereas placental lactogen appears to inhibit the release of a prolactin-inhibiting factor from the hypothalamus. Prolactin, lactogen, estrogen, and progesterone all aid in preparing the mammary gland for lactation. Initially in gestation, an increased growth of ductule and alveolobular tissue occurs in response to estrogen and progesterone. In the beginning of the second trimester, secretory material begins to appear in the luminal cells. By the middle of the second trimester, mammary development has proceeded sufficiently to permit lactation to occur should parturition take place.

Once the infant is delivered, a major regulatory factor, the placenta, is lost, and new regulatory factors including the maternal-infant interaction and neuroendocrine regulation are gained for control of lactation. Loss of placental hormone secretion results in an endocrine hypothalamic stimulation of prolactin release from the anterior pituitary gland, as well as neural stimulation of oxytocin from the posterior pituitary. The stimulation of the nipple by suckling activates a neural pathway that results in release of both prolactin and oxytocin. Prolactin is responsible for stimulating milk production, whereas oxytocin stimulates milk ejection (the combination is known as the *let-down reflex*). Oxytocin also stimulates uterine contractions, which the mother may feel while she is breast-feeding; this response helps to restore the uterus to prepregnancy tone.

Milk production and ejection are thus dependent on the complex interaction of stimulation by the infant's suckling, neural reflex of the hypothalamus to such stimulation, release of hormones from the anterior and posterior pituitary, and response of the mammary gland to these hormones to complete the cycle.

Milk Secretion. Milk is produced as the result of synthetic mechanisms within the mammary gland, as well as the transport of components from blood. Milk-specific proteins are synthesized in the mammary secretory cells, packaged in secretory vesicles, and exocytosed into the alveolar lumen. Lactose is secreted into the milk in a similar manner, whereas many monovalent ions, such as sodium, potassium, and chloride, are dependent on active transport systems based on sodium-potassium adenosine triphosphatases (Na^+, K^+-ATPases). In some situations, the mammary epithelium, which may behave as a "mammary barrier" between interstitial fluid derived from blood and the milk because of the lack of space between these cells, may "leak," permitting direct diffusion of components into the milk. This barrier results in the formation of different pools or compartments of milk components within the mammary gland and is responsible for maintaining gradients of these components from the blood to the milk.

Lipid droplets can be observed within the secretory cells of the mammary gland and are surrounded by a milk fat globule membrane. These fat droplets appear to fuse with the apical membrane of the secretory cells and then to be either exocytosed or "pinched off" into the milk.[8] Some whole cells also are found in milk, including leukocytes,

macrophages, lymphocytes, and broken or shed mammary epithelial cells. The mechanisms by which these cells enter the milk are complex and include, among others, specific cellular receptor-mediated homing of antigen sensitized lymphocytes.

As the structure of the mammary gland is compartmentalized, so is that of the milk. The gross composition of milk consists of cytoplasm encased by cellular membranes in milk fat globule membranes (fat compartments made up of fat droplets), a soluble compartment containing water-soluble constituents, a casein-micelle compartment containing acid-precipitable proteins with calcium and lactose, and a cellular compartment. The relative amounts of these components change during the course of lactation, generally with less fat and more protein in early lactation than in late lactation. Thus, the infant consumes a dynamic complex solution that has physical properties permitting unique separation of different functional constituents from one another, presumably in forms that best support growth and development.

Lactation Performance. Successful lactation performance depends on continued effective contributions from the neural, endocrine, and maternal-infant interactions that were initiated at the time of delivery. The part of this complex behavior most liable to inhibition is the mother-child interaction. An early attachment of the infant to the breast is mandatory to begin stimulation of the neural pathways essential to maintaining prolactin and oxytocin release.

A healthy newborn infant placed between the mother's breasts will locate a nipple and begin to suck spontaneously within the first hour of birth.[10] This rapid attachment to the mother may reflect olfactory stimuli from the breast received by the infant at birth.[11] Frequent feedings are necessary for the mother to maintain an appropriate level of milk production for the infant's proper growth and development. Programs to support lactation performance must emphasize proper maternal-infant bonding, relaxation of the mother, support for the mother, technical assistance to initiate breast-feeding properly and to cope with problems, and reduction of environmental hindrances. Such hindrances may include lack of rooming-in in the hospital, use of extra formula feeds, and lack of convenient day care for working mothers.

Lactation ceases when suckling stops; therefore, any behavior that reduces the amount of suckling by the infant initiates weaning or the end of lactation. Introduction of water in bottles or of 1 or 2 bottles of formula a day may begin the weaning process regardless of the time after parturition but can be most damaging to the process when the mother-infant dyad is first establishing lactation.

Secretory Products of Lactation: Nutritional Components of Human Colostrum and Milk

Colostrum and milk contain a rich diversity of nutrients, including electrolytes, vitamins, minerals, and trace metals; nitrogenous products; enzymes; and immunologically specific cellular and soluble products. The distribution and relative content of various nutritional substances found in human milk are presented in Table 5-2. The chemical composition often exhibits considerable variation among lactating women and in the same woman at different times

Table 5–2   Distribution of Secretory Products in Human Colostrum and Milk[a]

Water 86%-87.5%; Total Solids 11.5 g

Nutritional Components
Lactose 6.9-7.2 g
Fat 3.0-4.4 g
Protein 0.9-1.03 g
α-Lactalbumin 150-170 mg
β-Lactoglobulin trace
Serum albumin 50 mg

Electrolytes, Minerals, Trace Metals
Sodium 15-17.5 mg
Potassium 51-55 mg
Calcium 32-43 mg
Phosphorus 14-15 mg
Chloride 38-40 mg
Magnesium 3 mg
Iron 0.03 mg
Zinc 0.17 mg
Copper 15-105 µg
Iodine 4.5 µg
Manganese 1.5-2.4 µg
Fluoride 5-25 µg
Selenium 1.8-3.2 µg
Boron 8-10 µg

Nitrogen Products Total 0.15-2 g
Whey protein nitrogen 75-78 mg
Casein protein nitrogen 38-41 mg
Nonprotein nitrogen 25% of total nitrogen
Urea 0.027 g
Creatinine 0.021 g
Glucosamine 0.112 g

Vitamins
C 4.5-5.5 mg
Thiamine (B_1) 12-15 µg
Niacin 183.7 µg
B_6 11-14 µg
B_{12} <0.05 µg
Biotin 0.6-0.9 µg
Folic acid 4.1-5.2 µg
Choline 8-9 mg
Inositol 40-46 mg
Pantothenic acid 200-240 µg
A (retinol) 54-56 µg
D <0.42 IU
E 0.56 µg
K 1.5 µg

[a]Estimates based on amount per deciliter.

of lactation,[12] as well as between samples obtained from mothers of low-birth-weight infants and from mothers of full-term infants.[13,14] Mature milk contains the following average amounts of major chemical constituents per deciliter: total solids, 11.3 g; fat, 3.0 g; protein, 0.9 g; whey protein nitrogen, 760 mg; casein nitrogen, 410 mg; α-lactalbumin, 150 mg; serum albumin, 50 mg; lactose, 7.2 g; lactoferrin, 150 mg; and lysozyme, 50 mg. Human milk contains relatively low amounts of vitamins D and E (see Table 5-2) and little or no β-lactoglobulin (the major whey protein in bovine milk). The fat globule membrane appears to have a high content of oleic acid, linoleic acid, phosphatidylpeptides, and inositol.[15] In addition, a binding ligand that promotes absorption of zinc has been identified in human milk.[16,17] Temporal studies have indicated that concentrations of many

chemical components, especially nitrogen, calcium, and sodium, decrease significantly as the duration of lactation increases.[18,19] Several components, however, have been found to change in concentration as a function of water content, because their total daily output appears to be remarkably constant, at least during the first 8 weeks of lactation.[20,21]

Milk production progresses through three distinct phases, characterized by the secretion of colostrum, transitional (early) milk, and mature milk. Colostrum comprises lactational products detected just before and for the first 3 to 4 days of lactation. It consists of yellowish, thick fluid, with a mean energy value of greater than 66 kilocalories (kcal)/dL, and contains high concentrations of immunoglobulin, protein, fat, fat-soluble vitamins, and ash. Transitional milk usually is observed between days 5 and 14 of lactation, and mature milk is found thereafter. The concentrations of many nutritional components decline as milk production progresses to synthesis of mature milk. The content of fat-soluble vitamins and proteins decreases as the water content of milk increases. Conversely, levels of lactose, fat, and water-soluble vitamins and total caloric content have been shown to increase as lactation matures.[22,23]

As the result of several manufacturing errors, the nutrient composition of infant formulas has been legislated,[24] resulting in the paradoxical situation that human milk may not always meet the recommended standards for some nutrients, whereas infant formulas may exceed the recommendation. Human milk nutrient composition varies with time of lactation (colostrum versus early milk versus mature milk) and, to some extent, maternal nutritional status. The appropriate amounts of each nutrient must be considered within these constraints.

Minerals. The mineral content of human milk is low relative to that of infant formulas and very low compared with that of cow's milk, from which most formulas are prepared, so that although human milk is sufficient to support growth and development, it also represents a fairly low solute load to the developing kidney. The levels of major minerals tend to decline during lactation, with the exception of that of magnesium, but with considerable variability among women tested.[25] Sodium, potassium, chloride, calcium, zinc, and phosphorus all appear to be more bioavailable in human milk than in infant formulas, reflecting their lower concentrations in human milk. Iron is readily bioavailable to the *infant from human milk* but may have to be supplemented later in lactation.[26,27] Preterm infants fed human milk may need supplements of calcium and sodium.[28]

Vitamins. Human milk contains sufficient vitamins to maintain infant growth and development, with the caveat that water-soluble vitamins are particularly dependent on maternal intake of these nutrients.[29] The preterm infant may require supplements of vitamins D, E, and K when fed human milk.[30,31] The low content of vitamin D in human milk has been related to the development of rickets in a few breast-fed infants, as discussed later.[32] Vitamin D deficiency may be a particular problem in breast-fed infants who are not exposed to at least 30 minutes of sunshine per week.[33]

Carbohydrates and Energy. Lactose is the primary sugar found in human milk and usually is the carbohydrate chosen for the preparation of commercial formulas. Lactose supplies approximately half the energy (of a total 67 kcal/dL) taken in by the infant from human milk. Lactose (a disaccharide of glucose and galactose) also may be important to the neonate as a carrier of galactose, which may be more readily incorporated into gangliosides in the central nervous system than galactose derived from glucose in the neonate.[34] Also, glycogen may be synthesized more efficiently from galactose than from glucose in the neonate because of the relatively low activity of glucokinase in early development.[35] Human milk also contains other sugars, including glucose and galactose and more than 100 different oligosaccharides.[36] These oligosaccharides may have protective functions for the infant, especially with respect to their ability to bind to gastrointestinal pathogens.[37]

Lipids. Fats provide almost half of the calories in human milk, primarily in the form of triacylglycerols (triglycerides).[38] These lipids are supplied in the form of fat globules enclosed in plasma membranes derived from the mammary epithelial cells.[39] The essential fatty acid, linoleic acid, supplies about 10% of the calories derived from the lipid fraction. The triacylglycerols serve as precursors for prostaglandins, steroids, and phospholipids and as carriers for fat-soluble vitamins. The lipid profiles of human milk differ dramatically from those of commercial formulas, and despite considerable adaptation of such formulas, human milk lipids are absorbed more efficiently by the infant.

Cholesterol, an important lipid constituent of human milk (12 mg/dL), usually is found in only trace amounts in commercial formulas. It has been suggested that cholesterol may be an essential nutrient for the neonate.[40] A lack of cholesterol in early development may result in turning on of cholesterol-synthetic mechanisms that are difficult to turn off later in life, influencing induction of hypercholesterolemia.[41] Some studies suggest that breast-feeding of the neonate is associated with lowered adult serum cholesterol levels and reduced deaths from ischemic heart disease.[42]

Recently, interest has increased in the role that long-chain polyunsaturated fatty acids (LC-PUFAs) may play in human milk, especially docosahexaenoic acid (DHA) and arachidonic acid (AA). These LC-PUFAs are not found in unsupplemented infant formulas but are present in human milk. They are structural components of brain and retinal membranes and thus may be important for both cognitive and visual development. In addition, they may have a role in preventing atopy.[43] Numerous studies have found that infants fed formula without DHA or AA have reduced red blood cell amounts of these fatty acids[44,45]; however, findings in visual and cognitive functional studies in term infants have been inconsistent.[46,47] These studies have been complicated by the finding of slower growth in some preterm infants fed LC-PUFA–supplemented formulas.[48] The inconsistent findings in supplemented formula–fed babies may reflect the difficulty in determining the optimal amounts of DHA to AA, and their precursors linoleic and linolenic acids, in such supplemented formulas. Thus, these lipids appear to be best delivered from human milk.

Protein and Nonprotein Nitrogen. The exact protein content of mature human milk is variable but falls close to 1.0 g/dL, in contrast with that of infant formulas, which usually contain 1.5 g/dL; the milk from mothers who deliver preterm infants may have slightly more protein.[49] The

nutritionally available protein may be even less than 1.0 g/dL—as low as 0.8 g/dL—as a result of the proportion of proteins that is utilized for non-nutritional purposes. In addition, human milk contains a considerably greater percentage of nonprotein nitrogen (25% of the total nitrogen) when compared with formulas (5% of the total nitrogen).[50]

Human milk protein is primarily whey predominant (acid-soluble protein), whereas formulas prepared from bovine milk classically reflect the 18% whey–82% casein protein composition in that species. The whey-to-casein protein ratio in humans may change during lactation, with the whey component ranging from 90% (early milk) to 60% (mature milk) to 50% (late milk).[51] Formulas for preterm infants have been reconstituted from bovine milk to provide 60% whey and 40% casein proteins; all of the major formulas for term infants in the United States are now bovine whey protein–predominant preparations, in an attempt to make them closer to human milk in composition. These differences in protein quality are reflected by differences in the plasma and urine amino acid responses of infants fed human milk or formulas that are casein protein predominant or whey protein predominant.[52-54] In general, however, term infants do not respond with the dramatic differences seen in preterm infants when fed formulas with different protein quality.[55-58]

The nonprotein nitrogen component of human milk contains a variety of compounds that may be of importance to the development of the neonate: polyamines, nucleotides, creatinine, urea, free amino acids, carnitine, and taurine.[59] The significance of the presence of these components is not always clear, but when they are not fed, as in the case of infant formulas that contain little taurine[52] or of soy formulas that contain little carnitine,[60] apparent deficiencies that may influence the development of the infant occur. Taurine is important for bile salt conjugation, as well as for support of appropriate development of the brain and retina,[40] whereas carnitine appears to be important for appropriate fatty acid metabolism.[61]

Nucleotides, in particular, appear to bridge the gap between the nutritional and the immunologic roles of human milk components. Human milk contains a majority of these compounds in the form of polymeric nucleotides or nucleic acids,[62,63] whereas formulas contain nucleotides (when they are supplemented) only in the monomeric forms (Table 5-3). Nucleotides appear to enhance intestinal development, promote iron absorption, and modify lipid metabolism in their nutritional role.[64] On the other hand, these compounds perform an immunologic function by promoting killer cell cytotoxicity and interleukin-2 (IL-2) production by stimulated

mononuclear cells from infants either breast-fed or fed nucleotide-supplemented formulas.[65] Nucleotide supplementation also has been reported to reduce the number of episodes of infant diarrhea in a group of lower-socioeconomic-status infants in Chile, in a manner analogous to that for protection afforded by human milk.[66] In 1998 it was reported that nucleotide-supplemented formulas promote the immune response of infants to Haemophilus influenzae type b polysaccharide immunization at 7 months of age, and a similar response was observed for diphtheria immunization.[67] Infants fed human milk for more than 6 months demonstrated a similar response and also exhibited an enhanced titer response to oral polio vaccine; this latter response was not observed in the nucleotide-supplemented formula–fed group.[67] Thus, nucleotides are emerging as both nutritional and immunologic components of human milk.

Nutritional Proteins. As noted, the nutritional proteins in human milk are classified as either whey (acid-soluble) or casein (acid-precipitable). Within these two classes of proteins, several specific proteins are responsible for supporting the nutritional needs of the infant.

Human casein is made up primarily of β- and κ-casein, although the actual distribution of these two proteins is not clear.[68] By contrast, bovine milk contains α_{s1}- and α_{s2}-casein (neither of which is found in human milk), in addition to β- and κ-casein.[69] These two human milk casein proteins appear to account for approximately 30% of the protein found in human milk, in contrast with the earlier calculation of 40% (the amount commonly used to prepare reconstituted, so-called humanized formulas from bovine milk, which normally contains 82% casein proteins).

The whey protein fraction contains all of the proposed functional proteins in human milk (immunoglobulins, lysozyme, lactoferrin, enzymes, cytokines, peptide hormones), in addition to the major nutritional protein, α-lactalbumin. The whey proteins make up approximately 70% of human milk proteins, in contrast with 18% in bovine milk. Whereas α-lactalbumin is the major whey protein in human milk, β-lactoglobulin is the major whey protein in bovine milk (and is not found in human milk).[50] A consistent fraction of human milk whey protein is made up of serum albumin. Its source remains unclear; some evidence indicates that it may be synthesized in the mammary gland.[70] Most of the serum albumin, however, probably is synthesized outside the mammary gland.

Thus, milk proteins are characterized by their site of synthesis, as well as being species specific. Therefore, proteins such as α-lactalbumin and β-lactoglobulin are species and organ specific, whereas proteins such as serum albumin are species specific but not organ specific.[71] The net result of these differences in proteins utilized for nutrition by the neonate is that different amounts of amino acids are ingested by the neonate, depending on the source of milk; even reconstitution of the whey and casein classes of proteins from one species in a ratio similar to that of another species does not result in an identical amino acid intake. These differences are reflected in plasma amino acid profiles of infants fed commercial milks versus human milk, regardless of the ratios of reconstitution.[54]

Bioactive Proteins and Peptides. Whereas a major proportion of human milk protein is composed of the nutritional

Table 5–3   **Nucleotides in Human Milk and Supplemented Formula**

	Human Milk[a]	Human Milk[b] (%)	Formula[a]
Nucleic acid	48	42	4
Nucleotides	36	52	81
Nucleotides	8	7	15
Total (μmol/L)	402	163	141

[a]See ref. 62.
[b]See ref. 63.

proteins just described, a significant number of the remaining proteins subserve a variety of functions, either other than or in addition to the nutritional support of the neonate. These proteins include carrier proteins, enzymes, hormones, growth factors, immunoglobulins, and cytokines (the latter two are discussed later under "Resistance to Infection"). Whether these proteins are still functional once they have been ingested by the neonate has not always been established, but it is clear that human milk supplies a mixture that is potentially far more complex than just nutritional substrate.

Carrier Proteins. A number of nutrients are supplied to the neonate bound to proteins found in human milk. This binding may play an important role in making these nutrients bioavailable. Lactoferrin is an iron-binding protein (a property that also may play a role in its bacteriostatic action) that is apparently absorbed intact by the infant.[72] Lactoferrin may be important in the improved absorption of iron by the infant from human milk compared with that from cow's milk preparations, which contain little lactoferrin.[26] Lactoferrin also may bind other minerals, including zinc and manganese, although the preferred mineral form appears to be the ferric ion.

A number of other proteins appear to be important as carriers of vitamins and hormones. Folate-binding, vitamin B_{12}–binding, and vitamin D–binding proteins all have been identified in human milk. These proteins appear to have some resistance to proteolysis, especially when they are saturated with the appropriate vitamin ligand.[73] Serum albumin acts as a carrier of a number of ligands, whereas α-lactalbumin acts as a carrier for calcium. Finally, proteins that bind thyroid hormone and corticosteroids have been reported to be present in human milk,[74,75] although serum albumin may in part fulfill this function.

Enzymes. The activity of more than 30 enzymes has been detected in human milk.[76] Most of these enzymes appear to originate from the blood, with a few originating from secretory epithelial cells of the mammary gland. Little is known about the role of these enzymes, other than lysozyme and the lipases, in human milk. The enzymes found in human milk range from ATPases to antioxidant enzymes, such as catalase, to phosphatases and glycolytic enzymes. Although these enzymes have important roles in normal body metabolism, it is not clear how many of them either function in the milk itself or survive ingestion by the infant to function in the neonate.

Lysozyme appears to have a part in the antibacterial function of human milk, whereas the lipases have a more nutrient-related role in modulating fat metabolism for the neonate. Two lipases have been identified in human milk, a lipoprotein lipase and a bile salt–stimulated lipase.[77] Lipoprotein lipase appears to be involved in determining the pattern of lipids found in human milk by regulating uptake into milk at the level of the mammary gland. Human milk bile salt–stimulated lipase is an acid-stable protein that compensates for the low activity of lipases secreted into the digestive tract during early development.[78] Thus, these two enzymes regulate both the amount and the pattern of lipid that appears in milk as well as the extremely efficient absorption of lipid by the infant. Human milk lipid is absorbed much more readily than lipid from commercial milk formulas despite the many adaptations that have been made to improve absorption, illustrating the effective mechanisms supported by the lipases.

Hormones and Growth Factors. Both peptide and steroid hormones, as well as growth factors, have been identified in trace amounts in human milk, although as with most of the enzymes, it is not clear to what degree they function in the neonate to whom they have been supplied. As discussed previously, binding proteins for corticosteroids and thyroxine have been identified in milk and, by extrapolation from observations of other milk components, may play a role in making these bioactive compounds more readily available to the infant.

Among the hormones identified in human milk are insulin, oxytocin, calcitonin, and prolactin. Most of these hormones appear to be absorbed by the infant, but their role in in vivo function remains unclear.[79] Breast-fed infants appear to have a different endocrine response from that of formula-fed infants, presumably reflecting the intake of hormones from human milk.[80] The advantages or disadvantages to the infant of these responses, however, are unknown.

Human milk also contains a rich mixture of growth factors, including epidermal growth factor (EGF) and nerve growth factor.[81] In addition, a variety of gastrointestinal peptides have been identified in human milk. Presumably, the supply of these various factors to the infant through the milk compensates for their possible deficiency in the infant during early development.

The composition of human milk provides a complex and complete nutritional substrate to the neonate. Human milk supplies not only individual nutrients but also enzymes involved in metabolism, carriers to improve absorption, and hormones that may regulate metabolic rates. Commercial formulas have not yet been developed to the point that they can provide an analogous complete nutritional system.

RESISTANCE TO INFECTION

Component Mechanisms of Defense: Origin and Distribution

Fresh human milk contains a wealth of components that provide specific, as well as nonspecific, defenses against infectious agents and environmental macromolecules (Table 5-4). These component factors include cells, such as T and B lymphocytes, polymorphonuclear neutrophils (PMNs) (i.e., polymorphonuclear leukocytes), and macrophages; soluble products, especially immunoglobulins; secretory immunoglobulin A (sIgA); immunomodulatory cytokines and cytokine receptors; components of the complement system; several carrier proteins; enzymes; and a number of endocrine hormones or hormone-like substances. Additional soluble factors that are active against streptococci, staphylococci, and tumor viruses also have been identified.[22] Other soluble milk factors with potential implications in host defense include the bifidus factor, which promotes growth of bifidobacteria, and an EGF, which promotes growth of mucosal epithelium and maturation of intestinal brush border. The developmental characteristics of sIgA have been studied more extensively than those of other components.[82-84]

On the basis of available information, it is clear that a majority of IgA-producing cells observed in milk have their origin in the precursor immunocompetent cells in the gut-associated lymphoid tissue (GALT) and *bronchus-associated*

Table 5–4   Immunologically and Pharmacologically Active Components and Hormones Observed in Human Colostrum and Milk

Soluble	Cellular	Hormones and Hormone-like Substances
Immunologically Specific Immunoglobulin sIgA (11S), 7S IgA, IgG, IgM, IgE, IgD, secretory component **T Cell Products** Histocompatibility Antigens **Nonspecific Factors** Complement Chemotactic factors Properdin Interferon α-Fetoprotein Bifidus factor Antistaphylococcal factor(s) Antiadherence substances Epidermal growth factor Folate uptake enhancer Antiviral factors(s) Migration inhibition factor **Carrier Proteins** Lactoferrin Transferrin B_{12}-binding protein Corticoid-binding protein **Enzymes** Lysozyme Lipoprotein lipase Leukocyte enzymes	**Immunologically Specific** T lymphocytes B lymphocytes **Accessory Cells** Neutrophils Macrophages Epithelial cells	Epidermal growth factors Prostaglandins Neurotensin Relaxin Somatostatin Bombesin Gonadotropins Ovarian steroids Thyroid-releasing hormone Thyroid-stimulating hormone Thyroxine and triiodothyronine Adrenocorticotropin Corticosteroids Prolactin Erythropoietin Insulin

Table 5–5   Specific Antibody or Cell-Mediated Immunologic Reactivity in Human Colostrum and Milk

Bacteria	Viruses	Other
Escherichia coli (O + K antigens and enterotoxin) *Salmonella* *Shigella* species *Vibrio cholerae* *Bacteroides fragilis* *Streptococcus pneumoniae* *Bordetella pertussis* *Clostridium tetani* and *Clostridium difficile* *Corynebacterium diphtheriae* *Streptococcus mutans* *Haemophilus influenzae* type B *Mycobacterium tuberculosis*[a]	Rotavirus Rubella virus Poliovirus types 1, 2, 3 Echoviruses Coxsackieviruses A and B Respiratory syncytial virus[a] Cytomegalovirus[a] Influenza A virus Herpes simplex virus type 1 Arboviruses Semliki Forest virus Ross River virus Japanese B virus Dengue virus Human immunodeficiency virus Hepatitis A and B viruses	*Candida albicans* *Giardia* species *Entamoeba histolytica* Food proteins

[a]Evidence of reactivity for both antibody and cellular immunity.

lymphoid tissue (BALT). Exposure of IgA precursor B lymphocytes in the GALT or BALT to microbial and dietary antigens in the mucosal lumen is an important prerequisite for their initial activation and proliferation. Such antigen-sensitized cells eventually are transported through the systemic circulation to other mucosal surfaces, including the mammary glands and, as plasma cells, initiate the synthesis of immunoglobulin against specific antigens previously experienced in the mucosa of the respiratory or alimentary tract.[82,83] It has been proposed that T cells observed in the milk also may be derived from GALT and BALT in a manner similar to that of IgA-producing cells. Little or no information is available regarding the site of origin of other cellular or soluble immunologic components normally present in human milk. Specific antibody and cellular immune reactivity against many respiratory and enteric bacterial and viral pathogens and ingested food proteins also are present in human breast milk (Table 5-5).

Soluble Products

Immunoglobulin A. As observed in other peripheral mucosal sites, the major class of immunoglobulin in human colostrum and milk is the 11S sIgA. Other isotypes—namely, 7S IgA, IgG, IgM, IgD, and IgE—also are present. The IgA exists as a dimer of two 7S IgA molecules linked together by a polypeptide chain, the J-chain, and is associated with a nonimmunoglobulin protein referred to as the secretory component. The sIgA protein constitutes about 75% of the total nitrogen content of human milk. The IgA dimers produced by plasma cells at the basal surface of the mammary epithelium are transported to specialized columnar epithelial cells, where they acquire the secretory component before their discharge into the alveolar spaces.[83,84]

Sequential quantitation of class-specific immunoglobulin in human colostrum and milk has demonstrated that the highest levels of sIgA and IgM are present during the first few days of lactation (Fig. 5-1). Levels of IgA are 4 to 5 times greater than those of IgM, 20 to 30 times greater than those of IgG, and 5 to 6 times greater than those of serum IgA.[84] As lactation progresses, IgA declines to levels that range from 20 to 27 mg per gram of protein, and IgM levels decline to 3.5 to 4.1 mg/g. IgG levels do not show any significant change during early and late lactation and usually are maintained in the range of 1.4 to 4.9 mg/g (see Fig. 5-1). Although a dramatic and rapid decline in milk IgA and IgM occurs during the first week of life, this decrease is more than balanced by an increase in the volume of milk produced as the process of lactation becomes established (see Fig. 5-1).
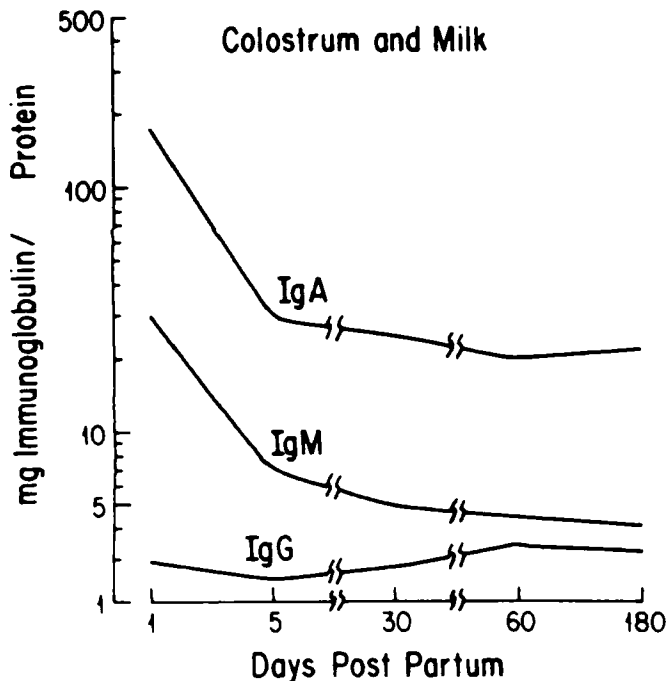
IgA antibodies found in milk possess specificity for infectious agents endemic to or pathogenic for the intestinal and respiratory tracts (see Table 5-4). These antibodies may be present in the milk in the absence of specific circulating IgA. In a study in which pregnant women were given oral feedings of *Escherichia coli* O83, development of IgA antibody in human milk was evident in the absence of detectable serum antibody-specific responses.[85] In another study, investigators have observed similar responses in animal models using intrabronchial immunization with *Streptococcus pneumoniae*. These and other studies[86-89] have strongly supported the concept of a bronchomammary, as well as an enteromammary, axis of immunologic reactivity in the breast.

Despite the elegance of studies that have defined the mechanisms of IgA cell trafficking from GALT and BALT to the mammary glands, it is clear that the actual number of B cells or IgA plasma cells in the mammary glands is sparse. At the same time, colostrum and milk may contain large amounts of IgA (as much as 11 g in colostrum and as much as 1 to 3 g per day in later milk), as shown in Table 5-6. The reasons underlying the apparent disparity between the content of immunoglobulin-producing cells and concentrations of immunoglobulin are not known. It may be related to the unique hormonal environment of the mammary glands. The hormones that have been consistently observed in human milk are listed in Table 5-4.

The effect of pregnancy- and lactation-related hormones on regulation of immunologic reactivity present in the resting and lactating breast has been examined.[90] In a study on immunoglobulin production in the nonlactating human breast, several interesting findings were noted.[91] Few mononuclear cells were present in the nonlactating breast of nulliparous and of parous women, although IgA-containing cells predominated. Synthesis of IgA appeared to be slightly increased in the parous women. IgA was found in the mammary tissues during the proliferative stage of the menstrual cycle in the nulliparous women and during the luteal phase in the parous women. The number of IgA-producing cells in the nonlactating breast was observed to increase with parity. These findings suggest that the immunologic makeup of the nonlactating, as well as the lactating, breast may be significantly influenced by the hormonal milieu. In another study of virgin mice given exogenously administered hormones,[92] an extended exposure to estrogen, progesterone, and prolactin was necessary for maximal increments in IgA-producing plasma cells in the breast. Similarly, castrated males exposed to these hormones became moderately receptive to mammary gland homing of cells specific for IgA synthesis. As would be expected, testosterone eliminated female breast receptivity to these cells. These studies suggest the existence of a hormonally determined homing mechanism in the mammary gland for class-specific, immunoglobulin-producing cells.

More recent studies have proposed another possible influence of lactational hormones on immunocompetent cells. In limited observations, combinations of prolactin with estrogen and progesterone (in concentrations observed normally at the beginning of parturition) appeared to have an amplifying effect on the synthesis and secretion of IgA from peripheral blood lymphocytes.[93] This observation raises the possibility that the high levels of sIgA observed in colostrum and milk may be the result of selective, hormonally mediated proliferation of antigen-sensitized IgA cells in the

Figure 5-1 Comparison of the mean levels of IgG, IgA, and IgM in colostrum and milk at different intervals after the onset of lactation in mothers who were breast-feeding. (Data from Ogra SS, Ogra PL. Immunologic aspects of human colostrum and milk. II. Characteristics of lymphocyte reactivity and distribution of E-rosette forming cells at different times after the onset of lactation. J Pediatr 92:550-555, 1978.)

peripheral blood. The immunoglobulin could acquire secretory component during its passage through the mammary epithelium and eventually appear in the colostrum or milk as mature sIgA. Although the appearance of sIga antibody in milk characteristically follows antigenic exposure in the GALT or BALT, the precise nature of the IgA content in milk appears to be determined by a variety of other factors operating in the mucosal lymphoid tissue. These include the regulatory T cell network in the GALT and possibly in the BALT,[94] the nature of antigens (soluble proteins versus particulate microbial agents),[95] and the route of primary versus secondary antigenic exposure.[96]

It has been estimated that the breast-fed infant may consistently receive an amount of about 1 g of IgA each day. Approximately $\frac{1}{100}$ of this amount each day is IgM and IgG.[97,98] The estimates of lactational immunoglobulin delivered to the breast-fed infant at different periods of lactation are presented in Table 5-6. Most ingested IgA is eliminated in the feces, although up to 10% may be absorbed from the intestine into the circulation within the first 18 to 24 hours after birth. Approximately 70% to 75% of ingested milk IgA survives passage through the gut and is excreted in the feces.[99] Feces of breast-fed infants contain functional antibodies present in the ingested milk.[100] Other studies also support the finding of prolonged survival of milk IgA in the gastrointestinal tract. Infants fed human milk have demonstrated the presence of all immunoglobulin classes in the feces. Fecal IgA content was three to four times greater than that of IgM after human milk feeding. Comparative studies on survival of human milk IgA and bovine IgG in the neonatal intestinal tract have suggested that the fecal content of IgA may be 14 to 20 times greater after human milk feeding than that of bovine IgG after feeding of bovine immune globulin.[101]

Direct information about the role of milk IgA in antimicrobial defense is available in several studies. Secretory IgA interferes with bacterial adherence to cell surfaces.[102] Colostrum and milk can inhibit the activity of E. coli and Vibrio cholerae enterotoxins in experimental settings.[103] The antitoxic activity of human milk appears to correlate well with its IgA content but not with its IgM and IgG content. Precoating of V. cholerae with specific sIgA protects infant mice from disease.[104] Similar results have been obtained by using specific purified milk sIgA in preventing E. coli– and Shigella dysenteriae–induced disease in rabbits.[105] Less definite, but suggestive, is a study conducted with human

milk feeding relative to the intestinal implantation of orally administered live poliovirus vaccine.[106] This study found that breast-feeding may reduce the degree of seroconversion for poliovirus antibody in the vaccinated infants. Because antipolio IgA is present in human milk and colostrum, the investigators concluded that specific IgA may bind poliovirus and influence viral replication in the intestinal mucosa. Extensive experience with oral polio immunization worldwide, however, has not found an association between breast-feeding and live vaccine failures. Other studies have clearly shown that the magnitude of poliovirus replication in the intestine is determined by the presence and level of pre-existing sIgA antibody. With high levels of intestinal IgA antibody, little or no replication of vaccine virus was observed in the gut. With lower levels, varying degrees of viral replication could be demonstrated.[107]

Indirect evidence, obtained from a more clinical perspective, suggests a protective role for milk against a variety of mucosal infections. Breast-feeding has been strongly implicated in supporting gastrointestinal homeostasis in the neonate and in establishing normal gut flora. Observations have shown the absence of diarrheal disease in breast-fed infants, even in the face of contamination of the fed milk with E. coli and Shigella species.[108] A preventive and therapeutic role for breast-feeding also has been suggested in nursery outbreaks of such disease due to enteropathogenic strains of E. coli[109] and diarrhea associated with rotavirus.[110] Breast-feeding plays an inhibitory role in the appearance of E. coli O83 agglutinins found in the feces of colonized infants. A decrease in the incidence of neonatal sepsis, specifically that associated with gram-negative bacilli and E. coli K1 serotypes, also has been linked to breast-feeding.[111,112] Milk IgA, possibly by limiting ingestion of foreign antigens by the neonate, or by binding of foreign proteins with specific antibodies to prevent absorption, or by both processes, may decrease the incidence of atopic-allergic diseases.[113-115] The frequency of IgE skin test–positive results has been described as being lower among breast-fed infants, possibly because of decreased exposure to cow's milk proteins or presence of maternal blocking antibodies.[116] Indirect epidemiologic data suggest that breast-feeding is protective against certain respiratory bacterial and viral infections.[117,118] Whereas the epidemiologic studies strongly support a protective role of breast-feeding, it is not possible in these studies to dissect the relative contribution of sIgA from that of other soluble or cellular components present in colostrum and milk.

Table 5-6   Level of Immunoglobulins in Colostrum and Milk and Estimates of Delivery of Lactational Immunoglobulins to the Breast-Feeding Neonate[a]

Day Post Partum	Percentage of Total Proteins Represented by Immunoglobulin			Output of Immunoglobulin (mg/24 hr)		
	IgG	IgM	IgA	IgG	IgM	IgA
1	7	3	80	80	120	11,000
3	10	45	45	50	40	2000
7	1-2	4	20	25	10	1000
7-28	1-2	2	10-15	10	10	1000
<50	1-2	0.5-1	10-15	10	10	1000

[a]Estimates based on the available data for total immunoglobulin and daily protein synthesis (see references 6, 83, 84).

Immunoglobulin G and Immunoglobulin M. Normal neonates exhibit characteristic paucity or lack of IgA during the first 7 to 10 days after birth. At that time, the presence of IgM and IgG in milk may be important to compensate for immunologic functions not present in the mucosal sites. For example, both IgG and IgM participate in complement fixation and specific bactericidal activity, functions not associated with IgA. Studies carried out after oral feeding of immune serum globulin (mostly IgG) have suggested that IgG may survive in the gastrointestinal tract of low-birth-weight infants.[119] Thus, other immunoglobulin isotypes in milk also may be able to serve as effective substitutes for IgA in the neonates of IgA-deficient mothers in prevention of infection with enteric or respiratory pathogens.

Immunoglobulin E and Immunoglobulin D. Studies on the distribution and role of IgE or IgD in colostrum and milk are few. Normal cord blood contains little or no IgE or IgD. The highest IgE concentrations observed in normal neonates usually are less than 5 ng/mL. Investigations have failed to demonstrate local synthesis of IgE in the breast.[120-122] Although IgE may be detected in up to 40% of colostrum and milk samples, the concentrations are extremely low, and many samples of colostrum and milk contain no IgE activity when paired samples of serum contain high IgE levels. On the other hand, IgD has been detected in most colostrum and milk samples. It has been suggested that nursing women with high serum IgD levels are more likely to have high IgD concentrations in their milk. The possibility of some local production of both IgE and IgD cannot be ruled out.[122]

Cellular Elements

Human colostrum and milk contain lymphocytes, monocytes-macrophages, neutrophils, and epithelial cells.[123] Early colostrum contains the highest concentration of cells, approximately 1×10^6 to 3×10^6 cells per mL. By the end of the first week of lactation, cell concentration is of the order of 10^5 cells per mL. Total cell numbers delivered to the newborn throughout lactation may, however, remain constant when adjustments are made for the increase in volume of milk produced.[124] The two major cell populations in human milk are difficult to distinguish by common staining methods because of the large number of intracytoplasmic inclusions, neutrophils, and macrophages. More accurate estimates made by flow cytometry analysis suggest that the relative percentages of neutrophils, macrophages, and lymphocytes in early milk samples are approximately 80%, 15%, and 4%, respectively.[125,126] The remaining cells are present in smaller amounts, especially in the absence of active suckling, engorgement, or local breast infection.

Macrophages. Histochemically, the milk macrophage differs from the blood monocyte in demonstrating decreased peroxidase staining, with increased lysosomes and significant amounts of immunoglobulin, especially IgA, in the cytoplasm.[127-129] The intracellular immunoglobulin in macrophages represents up to 10% of milk IgA.[130] Kinetic studies on the release of IgA by human milk macrophages suggest that immunoglobulin release by macrophages, unlike that by other phagocytic cells, is a time-dependent phenomenon and is not significantly influenced by the use of secretagogues or stimulants, such as phorbol myristate acetate.[130] Active phagocytosis, however, is associated with significant increase

in release of IgA.[131] In other studies, milk macrophages have been found to be efficient in release of superoxide anions after in vitro stimulation with phorbol myristate acetate.[132,133] Milk macrophages have the capacity to be primed by appropriate stimulation for greater release of superoxide anions.[133] It has been shown that milk macrophages obtained from preterm-delivered lactating mothers have a significantly higher phagocytic index than that for the macrophages in term milk. The bactericidal activity appears to be similar in pre- and full-term milk macrophages, however.[132] In neutrophils, milk macrophages appear to be activated, as demonstrated by the increased expression of CD11b and decreased expression of L-selectin.[125]

The precise functions of macrophages in colostrum or milk have not been fully explored. These cells have been suggested as potential transport vehicles for IgA.[128,129] Milk macrophages possess phagocytic activity against *Staphylococcus aureus*, *E. coli*, and *Candida albicans*, with possible cytocidal activity against the first two organisms.[134] Milk macrophages participate in antibody-dependent, cell-mediated cytotoxicity for herpes simplex virus type 1–infected cells.[135] Infection of milk macrophages by respiratory syncytial virus results in the production of the pro-inflammatory cytokines IL-1β, IL-6, and tumor necrosis factor-α (TNF-α).[136] These cells also are involved in a variety of other biosynthetic and excretory activities, including production of lactoferrin, lysozyme,[137] components of complement,[138] properdin factor B, epithelial growth factor(s), and T lymphocyte–suppressive factor(s).[82] Milk macrophages also have been suggested to be important in regulation of T cell function.[139,140]

Lymphocytes. Milk contains a small number of lymphocytes, 80% of which are T cells and 4% to 6% of which are B cells.[126] The small number of B cells reflects the sessile nature of these cells, which enter the lamina propria of the mammary gland to transform into plasma cells. Although several investigators have been unable to show in vitro antibody synthesis by milk lymphocytes, studies performed with colostral B cells transformed by Epstein-Barr virus have shown production of IgG, as well as J-chain–containing IgM and IgA.[141] A small population of CD16+ natural killer (NK) cells also can be identified in most milk samples but cannot be accurately quantitated.[126] In functional studies, however, colostral cells exhibit NK cytotoxicity, which is enhanced by interferon and IL-2. Colostral cells also elicit antibody- and lectin-dependent cellular cytotoxic responses. The NK, as well as the antibody- and lectin-dependent, responses in colostral cells, however, have been observed to be significantly lower than those of autologous peripheral blood cells. Reduced cellular cytotoxicity of colostral cells also has been observed against virus-infected targets and certain bacteria. In fact, with several specific virus-infected targets, colostrum and milk cells conspicuously lack cellular cytotoxicity when compared with autologous peripheral blood cells. There is also an apparent exclusion of cytolytic T cells in the milk that are specific for certain human leukocyte antigen (HLA) phenotypes.[142,143]

A majority of T lymphocytes in colostrum and milk are mature CD3+ cells. Both CD4+ (helper) and CD8+ (cytotoxic and suppressor) populations are present in human milk, with a proportion of CD8+ T cells higher than that found in human blood (Table 5-7). The CD4+/CD8+ ratio in milk is

Table 5-7   **Lymphocyte Subpopulations in Human Milk and Autologous Blood[a]**

Lymphocyte Subpopulation	Human Milk	Blood
CD3[+b]	83 ± 11	75 ± 7
CD3^{+} CD4[+b]	36 ± 13	44 ± 6
CD3^{+} CD8[+b]	43 ± 12	27 ± 4
CD4^{+}/CD8[+c]	0.88 ± 0.35	1.70 ± 0.45
CD19[+b]	6 ± 4	14 ± 5

[a]Expressed as mean ± standard deviation (SD).
[b]Expressed as percentage of total lymphocytes.
[c]Ratio of CD3^{+}/CD4^{+} to CD3^{+}/CD8^{+} lymphocytes.
Adapted from Wirt DP, Adkins LT, Palkowetz KH, et al. Activated-memory T lymphocytes in human milk. Cytometry 13:282-290, 1992.

significantly lower than that observed in peripheral blood and is not due to an increase of CD8^{+} cells in the peripheral blood of women during the postpartum period. Colostral and milk T lymphocytes manifest in vitro proliferative responses on stimulation with a number of mitogens and antigens. Several studies have shown a selectivity in lymphocyte stimulation responses in colostral and milk lymphocytes to various antigens when compared with peripheral blood lymphocyte responses.[124,144] Antigens such as rubella virus stimulate T lymphocytes in secretory sites and milk, as well as in systemic sites.[124] By contrast, E. coli K1 antigen whose exposure is limited to mucosal sites produces stimulation of lymphoproliferative responses only in milk lymphocytes. These findings support the concept of select T cell populations in the mammary gland.

In addition to antigen selectivity, a general hyporesponsiveness to mitogenic stimulation of milk lymphocytes relative to peripheral blood lymphocytes has been observed.[124,140] The decreased reactivity of milk lymphocytes to phytohemagglutinin (PHA) may be partly the result of a relative deficiency of certain populations of T cells in milk. Macrophage–T cell interactions also have been postulated as being responsible for this relative hyporesponsiveness,[84] although it is not known whether the effects are the result of decreased helper or increased suppressor function. Recent studies have shown that milk lymphocytes exhibit reduced responses to allogeneic cells but display good ability to stimulate alloreactivity.[142] Treatment of milk lymphocytes with monoclonal antibodies cytotoxic for T lymphocytes or with anti-HLA class II antigen–specific monoclonal antibodies has resulted in a substantial reduction in in vitro proliferative responses to bacterial antigens. It appears that, in general, the T cell proliferative responses to PHA and tetanus toxoid in breast-fed infants are significantly higher than those in bottle-fed infants, possibly secondary to the presence of maternally derived cell growth factors and other lymphokines present in human milk.[140,145]

Virtually all CD4^{+} and CD8^{+} T cells in milk bear the CD45 isoform CD45RO that is associated with immunologic memory.[126,146] In addition, the proportion of T cells that display other phenotypic markers of activation, including CD25 (IL-2R) and HLA-DR, is much greater than that in blood.[126,147] Consistent with their memory phenotype, T cells in human milk produce interferon-γ (IFN-γ).[146]

Furthermore, a significantly greater number of CD4^{+} T cells in colostrum express the CD40 ligand (CD40L) compared with autologous or heterologous blood T cells.[148] Cognate interaction between the CD40L on T cells and the CD40 on B cells is a necessary step for antibody production in vivo and is congenitally deficient in the newborn. The function of these memory T cells in the recipient human infant is currently unknown, however. Mucous membrane sites in the alimentary or respiratory tract, or both, of the recipient infant would seem to be potential entry sites for human milk leukocytes. Of considerable interest, very small numbers of memory T cells are detected in blood in infancy.[149] Thus, it is possible that maternal memory T cells in milk compensate for the developmental delay in their production in the infant. In this regard, the proportion of T lymphocytes bearing the T cell receptor-γδ (TCR-γδ) is approximately two times greater in colostrum than in blood.[150,151] Human TCR-γδ^{+} cells populate organized lymphoid tissues and represent half of the intraepithelial lymphocytes in the gut.[152] Thus, the intestinal epithelia may have a selective affinity for TCR-γδ^{+} cells and provide a favorable environment for maternal T cells in milk to be transferred to the breast-fed infant. Evidence from experimental animal studies indicates that milk lymphocytes enter tissues of the neonate,[153-156] but this has not been demonstrated in humans. In addition, the possible transfer of histocompatibility antigens and T cells to the neonate through breast-feeding has been examined by determining the fate of skin grafts in suckling rats fed by allogeneic mothers.[157] Such foster feeding of milk may result not only in increased allogeneic graft survival but also in development of "runting" syndrome, possibly as a result of a graft-versus-host–type reaction in the breast-fed animal. Effects of the transfer may be related to dosage of ingested allogeneic cells, in that increasing cell numbers transferred may prolong skin graft survival but may also increase the likelihood of a graft-versus-host reaction. Of note, the suckling rat gut has a higher degree of permeability to whole proteins than that characteristic of the human intestine. Furthermore, clinical experience in immunodeficient neonates has never supported the development of graft-versus-host reaction–like disease in the breast-fed human infant. In humans, possible transfer of maternal T cell reactivity to tuberculin protein from the mother to the neonate through the process of breast-feeding has been observed.[99,158,159] The implications of these observations are that maternal cellular products or soluble mediators of cellular reactivity may be transferred passively to the neonate through breast-feeding. Admittedly, however, the occurrence of such phenomena in humans has not been studied carefully. Thus, it must be emphasized that at present, evidence to suggest any T cell–mediated immunologic risks associated with breast-feeding in humans is lacking. On the other hand, it is still unknown whether milk T cells, either TCR-αβ[+] or TCR-γδ[+], play a role in the transfer of adoptive immunoprotection to the recipient infant.

Neutrophils. Milk contains large numbers of neutrophils. Although the absolute counts in actively nursing mothers exhibit considerable variability between different samples, highest numbers are generally observed during the first 3 to 4 days of lactation. The numbers of neutrophils decrease significantly after 3 to 4 weeks of lactation, and only rare

neutrophils are observed in samples collected after 60 to 80 days post partum. Leukocytes in human milk appear to be metabolically activated. Indeed, although the neutrophils are phagocytic and produce toxic oxygen radicals, they do not respond well to chemoattractants by increasing their adherence, polarity, or directed migration in in vitro systems.[160] This diminished response was found to be due to prior activation in that the neutrophils in milk displayed a phenotypic pattern that is typical of activated neutrophils. The expression of CD11b, the α chain subunit of Mac-1, was increased, and the expression of L-selectin was decreased.[125]

Epithelial Cells. On the basis of their anatomic distribution, epithelial cells in the human mammary gland can be classified into two main types: myoepithelial and luminal. Epithelial cells of both types, however, appear to be more heterogeneous on histologic and physicochemical testing.[142,161,162] They include secretory cells, which contain abundant rough endoplasmic reticulum, lipid droplets, and Golgi apparatus. The secretory cells appear to produce casein micelle. The squamous epithelial cells usually are seen in the regions of the cutaneous junction of the nipples, especially near the galactophores. The ductal or luminal cells, which exist in clusters, have many short microvilli, tight junctions, and remnants of desmosomes.[161,162] Studies using monoclonal antibodies have shown that in rodents, as many as 10 different types of epithelial cells in the adult mammary glands may exist. These cell types probably represent various stages of differentiation of mammary gland epithelium. These include, in the mammary end buds, the distinct cell types of the tip and the main compartment peripheral cell types I and II and, in alveoli as well as in the ducts of the mammary glands, the luminal cell types I and II and myoepithelial cells.[161] It is not known, however, whether similar epithelial cell differentiation occurs in the human mammary gland.

In human milk, relatively few epithelial cells are observed in the early phases of lactation. Most epithelial cells appear after 2 to 3 weeks and are seen in appreciable numbers, even as long as 180 to 200 days after the onset of lactation. With the possible exception of the synthesis of secretory component and casein and possibly other products, with which secretory epithelial cells have been associated in the stroma of the mammary gland, the role of epithelial cells in the milk remains to be defined.

Possible Functional Roles for Cellular Elements. The information reviewed thus far provides strong evidence for the existence of a number of dynamic cellular reactions in the mammary gland, colostrum, and milk. Unfortunately, the specific functional role, collectively or individually, for the epithelial cells, monocytes, neutrophils, or lymphocytes in the mammary gland or the milk remains to be defined. In view of the high degree of selectivity and the differences in the quantitative and functional distribution of cellular elements, it is suggested that the mammary gland, like mucosal surfaces, may function somewhat partitioned from the cellular elements in peripheral blood, in a manner similar to that for other peripheral sites (such as the genital tract) of the common mucosal system. It is, however, not known whether the characteristic proportions of macrophages, T lymphocytes, other cytotoxic cells, or epithelial cells are designed for any specific functions localized to the mammary

gland in the lactating mother or to epithelium or lumen of the intestinal or respiratory mucosa of the breast-feeding infant, or both. The observations on the transfer of delayed hypersensitivity reactions in human neonates and of graft-versus-host reactivity in the rat raise the possibility that milk cells may function as important vehicles in transfer of maternal immunity to neonates. The potential beneficial and harmful roles of such cell-mediated transfer through the mucosal routes, however, need to be investigated further. The paucity of NK and other cytotoxic cells in the colostrum may have a role for the breast-feeding neonate, especially in influencing the antigen processing and uptake of replicating microorganisms and their immune response at systemic or mucosal levels or both. Although colostral cells await further elucidation of their function in the mammary glands and the suckling neonate, it is likely that their presence in the milk represents a highly selective phenomenon and not a mere contamination with peripheral blood cells.

Other Possible Defense Factors

Human colostrum and milk contain all components of the complement system. Active production of C3 has been reported in vitro in breast milk cell cultures.[163,164] Interferon,[165] migration inhibition factor,[158] and α-fetoprotein[148] also are present in human milk, although their roles have not yet been fully elucidated (see Table 5-4).

Iron-binding proteins present in colostrum and milk, such as lactoferrin,[166] have bacteriostatic activity in vitro against E. coli, S. aureus, and C. albicans.[137] Some evidence suggests enhanced bactericidal activity of lactoferrin in association with IgA. Lysozyme and bifidus factor (a collection of glycosamides that promote growth of Lactobacillus and bifidobacterial species, whose growth in turn inhibits growth of enteric gram-negative aerobic bacilli) may function as ancillary inhibitors of gut and skin pathogens. Anti-staphylococcal factors appear to be active against experimental staphylococcal infections and may be important for local mammary gland protection.[166,167] Of particular interest is the demonstration of certain oligosaccharides that prevent attachment of S. pneumoniae to human epithelial cells[168] and of high-molecular-weight substances that inhibit virulence of enterotoxins of Enterobacteriaceae organisms (see Table 5-4).

Nonimmunoglobulin antiviral factors have been demonstrated in lipid and aqueous phases of human milk. These factors have shown activity against influenza A and B viruses, herpes simplex virus, Semliki Forest virus, Japanese B encephalitis virus, rubella virus, rhinovirus, and rotavirus (see Table 5-4). The milk-associated antiviral factors have been shown to have inhibitory functions only in vitro. Their in vivo role in neonatal and maternal infections remains to be elucidated. Recent studies also have demonstrated the presence of other substances in human milk that promote growth and maturation of intestinal epithelial tissue[169] and uptake of folate by the intestinal cells.[170]

Several recent studies have generated interest in the potential role of nonantibody proteins, bile salt lipases, whey proteins, and trace metals present in human milk in the control of enteric infections.[171-174] Several species of gram-positive and gram-negative bacteria frequently can be killed by incubation with human milk whey but not commercial infant formula.[171] The mechanisms responsible for such antibacterial activity are not known. The synergistic inter-

action among IgA, lactoferrin, and iron has been suggested to play a role in such defense.[171-172]

Concentrations of free fatty acid and possibly monoglycerides seem to increase during storage of milk because of spontaneous lipolysis generated by lipoprotein lipase.[175,176] Antibody-independent antiparasitic effect of stored, but not fresh, human milk against *Giardia lamblia* or *Entamoeba histolytica* has been attributed to such free fatty acids.[177] In additional studies conducted in vitro, bile salt–stimulated lipase, the major lipase in human milk, has been found to cause hydrolysis of milk triglycerides. It remains to be seen whether free fatty acids induce significant in vivo protection in the intestine against intestinal parasites. On the other hand, bile salts themselves may stimulate the growth of *G. lamblia*.[173]

Nonantibody proteins, several carrier proteins, and cellular enzyme proteins are present in milk in high concentrations. Concentrations of lysozyme range from 30 to 50 mg/100 mL in early colostrum to 5 to 10 mg/100 mL in late milk. The susceptibility of an organism to lysozyme depends on the availability of the peptidoglycan substrate. In certain situations in which the peptidoglycan may be blocked by lipoproteins, the organisms are relatively resistant to lysozymes.[173,174]

DIRECT-ACTING ANTIMICROBIAL AGENTS

General Features. The defense agents in human milk, although biochemically diverse, share certain features: (1) They usually are common to mucosal sites. (2) They are adapted to resist digestion in the gastrointestinal tract of the recipient infant. (3) They protect by noninflammatory mechanisms. (4) They act synergistically with each other or with factors produced by the infant. (5) Most components of the immune system in human milk are produced throughout lactation and during gradual weaning, but (6) there is often an inverse relationship between the production of these factors in the mammary gland and their production by the infant during the same time frames of lactation and postnatal development. Indeed, as lactation proceeds, the concentration of many factors in human milk declines. Concomitantly, the mucosal production of these factors rises in the developing infant. It is unclear whether the inverse relationship between these processes is due to feedback mechanisms or whether the processes are independent.

Carbohydrate Components. Human milk contains several oligosaccharides and glycoconjugates, including monosialogangliosides that are receptor analogues for heat-labile toxins produced by *V. cholerae* and *E. coli*[178]; fucose-containing oligosaccharides that inhibit the hemagglutinin activity of the classic strain of *V. cholerae*[179]; fucosylated oligosaccharides that protect against heat-stable enterotoxin of *E. coli*[180]; mannose-containing high-molecular-weight glycoproteins that block the binding of the El Tor strain of *V. cholerae*[178]; and glycoproteins and glycolipids that interfere with the binding of colonization factor (CFA/II) fimbriae on enterotoxigenic *E. coli*.[181] The inhibition of toxin binding is associated with acidic glycolipids containing sialic acid (gangliosides). Although the quantities of total gangliosides in human and in bovine milk are similar, the relative frequencies of each type of ganglioside in milk from these two species are distinct. More than 50 types of monosialylated

oligosaccharides have been identified in human milk, and new types are still being recognized.[182] Monosialoganglioside 3 constitutes about 74% of total gangliosides in human milk, but the percentage is much lower in bovine milk.[183,184] Also, the level of the enterotoxin receptor ganglioside G_{M1} is 10 times greater in human than in bovine milk.[184] This difference may be of clinical importance because G_{M1} inhibits enterotoxins of *E. coli* and *V. cholerae*.[185] It also is of interest that intact human milk fat globules, as well as the mucin from the membranes of these structures, inhibit the binding of S-fimbriated *E. coli* to human buccal epithelial cells.[186]

Oligosaccharides in human milk also interfere with the attachment of *H. influenzae* and *S. pneumoniae*.[187] In this regard, N-acetylglucosamine (GlcNAc) (1-3)Gal-disaccharide subunits block the attachment of *S. pneumoniae* to respiratory epithelium. Moreover, recent evidence indicates that human milk interferes with the binding of human immunodeficiency virus (HIV) envelope antigen gp120 to CD4 molecules on T cells.[188] Some evidence from animal models suggests that the oligosaccharides and glycoconjugates in human milk protect in vivo,[189-191] but relevant clinical data are scarce.[192]

In addition to the direct antimicrobial effects of the carbohydrates in human milk, nitrogen-containing oligosaccharides in human milk are growth promoters for *Lactobacillus bifidus* var. *pennsylvanicus*,[193] glycoproteins, and glycopeptides.[194,195] The bifidus growth promoter activity associated with caseins may reside in the oligosaccharide moiety of those complex molecules.[196] It appears that these factors are responsible to a great extent for the predominance of *Lactobacillus* species in the bacterial flora of the large intestine of the breast-fed infant. These bacteria produce large amounts of acetic acid, which aids in suppressing the multiplication of enteropathogens. It also has been reported that *Lactobacillus* species strain GG aids in the recovery from acute rotavirus infections[197] and may enhance the formation of circulating cells that produce specific antibodies of the IgG, IgA, and IgM isotypes, as well as serum levels of those antibodies.[198]

Generation of Antiviral, Antiparasitic Lipids from Substrata in Human Milk. Human milk supplies defense agents from fat as it is partially digested in the recipient's alimentary tract. Fatty acids and monoglycerides produced from milk fats by bile salt–stimulated lipase or lipoprotein lipase in human milk,[199] lingual/gastric lipase from the recipient from birth,[200] or pancreatic lipase after a few weeks of age are able to disrupt enveloped viruses.[201-205] These antiviral lipids may aid in preventing coronavirus infections of the intestinal tract[206] and also may defend against intestinal parasites such as *G. lamblia* and *E. histolytica*.[207,208]

Proteins. The principal proteins in human milk that have direct antimicrobial properties include the following.

α-Lactalbumin. α-Lactalbumin is a major component of the milk proteins and may possess some important functions of immunologic defense. This protein appears as large complexes of several α-lactalbumin molecules, which can induce apoptosis in transformed embryonic and lymphoid cell lines. A lower number of such aggregated α-lactalbumin molecules binding oleic acid as a co-factor can induce cytolysis of several types of malignant cells. Such preparations of *h*uman α-lactalbumin *m*ade *le*thal to *tu*mor

cells (HAMLET) are highly effective in inducing apoptosis. The antitumor cytolytic activity with HAMLET also has been observed against large numbers of human tumors.

Lactoferrin. Lactoferrin, the dominant whey protein in human milk, is a single-chain glycoprotein with two globular lobes, both of which display a site that binds ferric iron.[209] More than 90% of the lactoferrin in human milk is in the form of apolactoferrin (i.e., it does not contain ferric iron),[210] which competes with siderophilic bacteria and fungi for ferric iron[211-215] and thus disrupts the proliferation of these microbial pathogens. The epithelial growth–promoting activities of lactoferrin in human milk also may aid in the defense of the recipient infant.[216] The mean concentration of lactoferrin in human colostrum is between 5 and 6 mg/mL.[217] As the volume of milk production increases, the concentration falls to about 1 mg/mL at 2 to 3 months of lactation.[218,219]

Because of its resistance to proteolysis,[220-222] the excretion of lactoferrin in stool is higher in human milk–fed than in cow's milk–fed infants.[72,223-225] The mean intake of milk lactoferrin per day in healthy breast-fed, full-term infants is about 260 mg/kg at 1 month of lactation and 125 mg/kg by 4 months.[223] The quantity of lactoferrin excreted in the stools of low-birth-weight infants fed human milk is approximately 185 times that in stools of infants fed a cow's milk formula.[226] That estimate, however, may be too high because of the presence of immunoreactive fragments of lactoferrin in the stools of human milk–fed infants.[227]

In addition, a significant increment in the urinary excretion of intact and fragmented lactoferrin occurs as a result of human milk feedings.[227-229] Recent stable isotope studies suggest that the increments in urinary lactoferrin and its fragments are principally from ingested human milk lactoferrin.[230]

Lysozyme. Relatively high concentrations of lysozyme single-chain protein are present in human milk.[218,219,231-235] This 15-kDa agent lyses susceptible bacteria by hydrolyzing β-1,4 linkages between N-acetylmuramic acid and 2-acetylamino-2-deoxy-D-glucose residues in cell walls.[236] Lysozyme is relatively resistant to digestion by trypsin or denaturation due to acid. The mean concentration of lysozyme is about 70 μg/mL in colostrum,[218] about 20 μg/mL at 1 month of lactation, and 250 μg/mL by 6 months.[219] The approximate mean daily intake of milk lysozyme in healthy, full-term, completely breast-fed infants is 3 to 4 mg/kg at 1 month of lactation and 6 mg/kg by 4 months.[223]

Few studies have been conducted to examine the fate of human milk lysozyme ingested by the infant. The amount of lysozyme excreted in the stools of low-birth-weight infants fed human milk is approximately eight times that found in the stools of infants fed a cow's milk formula,[226] but the urinary excretion of this protein does not increase as a result of human milk feedings.

Fibronectin. Fibronectin, a high-molecular-weight protein that facilitates the uptake of many types of particulates by mononuclear phagocytic cells, is present in human milk (mean concentration in colostrum, 13.4 mg/L).[237] The in vivo effects and fate of this broad-spectrum opsonin in human milk are not known.

Complement Components. The components of the classical and alternative pathways of complement are present in human milk, but the concentrations of these components, except C3, are exceptionally low.[163,164]

ANTI-INFLAMMATORY AGENTS

Although a direct anti-inflammatory effect of human milk has not been demonstrated in vivo, a number of clinical observations suggest that breast-feeding protects the recipient infant from injury to the intestinal or respiratory mucosa.[238,239] This protection may be due in part to the more rapid elimination or neutralization of microbial pathogens in the lumen of the gastrointestinal tract by specific or broad-spectrum defense agents from human milk, but other features of human milk suggest that this is not the sole explanation. Phlogistic agents and the systems that give rise to them are poorly represented in human milk.[240] By contrast, human milk contains a host of anti-inflammatory agents,[241] including a heterogeneous group of growth factors with cytoprotective and trophic activity for the mucosal epithelium, antioxidants, antiproteases, cytokines and cytokine receptors and antagonists, and other bioactive agents that inhibit inflammatory mediators or block the selected activation of leukocytes. Like the antimicrobial factors, some of these factors are well adapted to operate in the hostile environment of the recipient's alimentary tract.

Growth factors in human milk include EGF,[169,242] the transforming growth factors TGF-α[243] and TGF-β,[244] lactoferrin,[216] mammary gland–derived growth factor,[245] and polyamines.[246,247] These and a host of hormones,[248] including insulin-like growth factor (IGF), vascular endothelial growth factor (VEGF), growth hormone–releasing factor (GHF), hepatocyte growth factor (HGF), prolactin, leptin, and cortisol,[249] may affect the growth and maturation of epithelial barriers, limit the penetration of pathogenic microorganisms and free antigens, and prevent allergic sensitization. Corticosterone, a glucocorticoid that is present in high concentrations in rat milk, speeds gut closure in the neonatal rat.[250] Although macromolecular absorption does not appear to be as marked in the human neonate,[251-253] the function of the mucosal barrier system in early infancy is important to host defense, and this system may be affected by factors in human milk. In this regard, the maturation of the intestinal tract as measured by mucosal mass, DNA, and protein content of the small intestinal tract appears to be influenced by milk, particularly early milk secretions.[254]

Antioxidant activity in colostrum has been shown to be associated with an ascorbate compound and uric acid.[255] In addition, two other antioxidants present in human milk, α-tocopherol[256,257] and β-carotene,[257] are absorbed into the circulation by the recipient gastrointestinal mucosa. Serum vitamin E concentrations rise in breast-fed infants from a mean of 0.3 mg/mL at birth to approximately 0.9 mg/mL on the fourth day of life.[256]

The pleiotropic cytokine IL-10, a potent suppresser of macrophage, T cell, and NK cell function, has been demonstrated at very high concentrations in samples of human milk collected during the first 80 hours of lactation.[258] IL-10 is present not only in the aqueous phase of the milk but also in the lipid layer. Its bioactive properties were confirmed by the finding that human milk samples inhibited blood lymphocyte proliferation and that this property was greatly reduced by treatment with anti-IL-10 antibody. Of interest, mice with a targeted disruption in the IL-10 gene, when

raised under conventional housing conditions, spontaneously develop a generalized enterocolitis that becomes apparent at the age of 4 to 8 weeks (time of weaning).[259] These observations suggest that IL-10 in human milk may play a critical role in the homeostasis of the immature intestinal barrier by regulating aberrant immune responses to foreign antigens. Soluble receptors and cytokine receptor antagonists also are potent anti-inflammatory agents. Human colostrum and mature milk have been shown to contain biologically active levels of IL-1 receptor antagonist (IL-1Ra) and soluble TNF-α receptors I and II (sTNF-αRI and sTNF-αRII).[260] The in vivo relevance of these observations also has been confirmed in a chemically induced colitis model of rats. Animals with colitis fed human milk had significantly lower neutrophilic inflammation than animals fed either chow or infant formula.[261] Similar "protective" effects were seen in rats with colitis fed an infant formula supplemented with IL-1Ra,[261] suggesting that this anti-inflammatory agent present in milk may contribute to the broad protection against different injuries provided by human milk feeding.

The presence in human milk of platelet-activating factor acetylhydrolase (PAF-AH), the enzyme that catalyzes the degradation and inactivation of PAF, is intriguing.[262] Indeed, elevated serum concentrations of PAF have been found in rat and human neonates with necrotizing enterocolitis (NEC), whereas the concentrations of PAF-AH were found to be significantly lower than in control (unaffected) neonates.[263,264] It also is of interest that serum concentrations of PAF-AH at birth are below those in adults and then gradually rise.[265] The enzyme is actively transferred from the mucosal to the serosal fluid in intestine of neonatal rats, particularly in the earliest postnatal period.[266] Other anti-inflammatory factors present in human milk include an IgE-binding factor, related antigenically to the FcεRII (the lower-affinity receptor for IgE), that suppresses the in vitro synthesis of human IgE,[267] and the glycophosphoinositol-containing molecule protectin (CD59) that inhibits insertion of the complement membrane attack complex (MAC) to cell targets.[268] The in vivo fate and effects of these anti-inflammatory factors in human milk are still poorly understood.

MODULATORS OF THE IMMUNE SYSTEM

Several seemingly unrelated types of observations suggest that breast-feeding modulates the development of the immune system of the recipient infant:

- Both prospective and retrospective epidemiologic studies have shown that breast-fed infants are at less risk for development of certain chronic immunologically mediated disorders later in childhood, including allergic diseases,[269] Crohn's disease,[270] ulcerative colitis,[271] insulin-dependent diabetes mellitus,[272] and some lymphomas.[273]
- Humoral and cellular immune responses to specific antigens (i.e., vaccines) given during the first year of life appear to develop differently in breast-fed and in formula-fed infants. Several studies have reported increased serum antibody titers to H. influenzae type b polysaccharide,[274] oral poliovirus,[275] tetanus,[276] and diphtheria toxoid[277] immunizations in breast-fed infants. In regard to cell-mediated immunity, breast-

fed infants given bacille Calmette-Guérin (BCG) vaccine either at birth or later show a significantly higher lymphocyte transformation response to purified protein derivative (PPD) than that in infants who were never breast-fed.[277] Moreover, maternal renal allografts survive better in persons who were breast-fed than in those who were not.[278-280] In this respect, the in vitro allogeneic responses between the blood lymphocytes of mothers (stimulating cells) and their infants (responding cells), as measured by an analysis of the frequencies of cytotoxic T lymphocyte (CTL) precursors directed against HLA alloantigens (CTL allorepertoire), are low in breast-fed infants.[281]

- Increased levels of certain immune factors in breast-fed infants, which could not be explained simply by passive transfer of those substances, also suggest an immunomodulatory activity of human milk. Breast-fed infants produce higher blood levels of interferon in response to respiratory syncytial virus infection.[282] It also was found that the increments in blood levels of fibronectin that were achieved by breast-feeding could not be due to the amounts of that protein in human milk.[237] In addition, it was found that human milk feeding led to a more rapid development in the appearance of sIgA in external secretions,[226,228,229,276,283] some of which, such as urine, are far removed anatomically from the route of ingestion.[228,229]

These and other observations suggest that the ability of human milk to modulate the development of the infant's own mucosal and systemic immune systems may be associated with immunoregulatory factors present in colostrum and in more mature milk. Several different types of immunomodulatory agents can be identified in human milk.[241] Among the numerous substances with proven or potential ability to modulate the infant immune response are prolactin,[284] α-tocopherol,[256] lactoferrin,[285] nucleotides,[67] anti-idiotypic sIgA,[286] and cytokines.[287] It is evident that many of these factors in milk have other primary biologic functions, as in the case of hormones or growth factors, and that their potential as immune regulatory agents overlaps with their antimicrobial or anti-inflammatory properties.[241]

Cytokines in Human Milk. In the 1990s, several cytokines, chemokines, and growth factors that mediate the effector phases of natural and specific immunity were discovered in human milk. These include IL-1β, IL-6, IL-7, IL-10, IL-12, IL-8, growth-related peptide-α (GRO-α), monocyte chemotactic protein-1 (MCP-1), granulocyte colony-stimulating factor (G-CSF), macrophage colony-stimulating factor (M-CSF), and TGF-β (Table 5-8). Human milk displays a number of cytokine-characteristic biologic activities, including the stimulation of growth, differentiation of immunoglobulin production by B cells,[288-290] enhancement of thymocyte proliferation,[291] inhibition of IL-2 production by T cells,[292] and suppression of IgE production.[267] IL-1β[293] and TNF-α[294] were the first two cytokines quantified in human milk. In colostrum, TNF-α is present mainly in fractions of molecular weight between 80 and 195 kDa, probably bound to its soluble receptors.[260] Milk TNF-α is secreted both by milk macrophages[294,295] and by the mammary epithelium.[296] IL-6 was first demonstrated in human milk by a specific bioassay.[297] In this study, anti-IL-6–neutralizing antibodies

Table 5–8   Cytokines, Chemokines, and
Colony-Stimulating Factors in
Human Milk

Cytokines	Chemokines	Colony-Stimulating Factors
IL-1β	IL-8	G-CSF
IL-6	GRO-α	M-CSF
IL-7		
IL-8		
IL-10	MCP-1	GM-CSF
IL-16[a]		TGF-β
IFN-γ	RANTES	
TNF-α	Eotaxin[a]	

G-CSF, granulocyte colony-stimulating factor; GM-CSF, granulocyte-macrophage colony-stimulating factor; GRO-α, growth-related peptide-α; IL, interleukin; MCP-1, monocyte chemotactic protein-1; M-CSF, monocyte colony-stimulating factor; RANTES, regulated upon activation, normal T cell expressed and secreted; TGF-β, transforming growth factor-β; TNF-α, tumor necrosis factor-α.

inhibited IgA production by colostrum mononuclear cells, suggesting that IL-6 may be involved in the production of IgA in the mammary gland. The presence of IL-6 in milk also has been demonstrated by immunoassays.[294,296,298,299] In like manner, IL-6 is localized in high-molecular-weight fractions of human milk.[298] The association of IL-6 with its own receptor has not been studied in milk, although the expression of IL-6 receptor by the mammary epithelium[296] and in secreted form in the milk[260] may explain the high molecular weight of this cytokine in human milk. The expression of IL-6 messenger ribonucleic acid (mRNA) and protein in milk cells and in the mammary gland epithelium suggests that both milk mononuclear cells and the mammary gland are likely major sources of this cytokine.[295,296,300] The presence of IFN-γ in human milk also has been reported,[151,296,299] although some investigators have found significant levels of IFN-γ only in milk samples obtained from mothers whose infants had been delivered by cesarean section. The significance of this observation is not clear at present. IFN-γ bioactivity as well as its association with specific subsets of milk T cells also remains to be determined.[151] (The presence and possible function of IL-10 in human milk are discussed in the section "Anti-inflammatory Agents.")

Chemokines are a novel class of small cytokines with discrete target cell selectivity that are able to recruit and activate different populations of leukocytes.[301] Two major subfamilies, the CXC and the CC chemokines, are defined by the splicing of the conserved cysteine residues, which are separated by either one amino acid (CXC chemokines) or adjacent amino acids (CC chemokines). IL-8 and GRO-α belong to the CXC family and are mainly chemotactic factors for neutrophils. On the other hand, CC chemokines, which include MCP-1, macrophage inflammatory protein-1α (MIP-1α), and RANTES (regulated upon activation, normal T cell expressed and secreted), are chemotactic factors for monocytes, basophils and eosinophils, and T lymphocytes.[302] The presence of both CXC and CC chemokines has been described in human milk (see Table 5-8).

IL-8 concentration was first determined in a small group of milk samples by Basolo and colleagues.[296] These investigators identified the expression and secretion of IL-8 by mammary epithelial cells, although milk cells also appear to produce this chemokine.[295,300] Another member of the CXC chemokine found in human milk is GRO-α, along with the two CC chemokines MCP-1 and RANTES.[300] Expression of MCP-1 and, to a lesser extent, RANTES mRNA was confirmed in studies of milk cells.[300] Recently, high levels of the CC chemokine eotaxin, a potent and specific chemotactic factor for eosinophils and subtype 2 helper T cells (T_H2), also have been demonstrated in human milk. [303]

Colony-stimulating factors—highly specific protein factors that regulate cell proliferation and differentiation in the process of hematopoiesis—were discovered relatively recently in human milk. Although colony-stimulating activity was demonstrated in milk in 1983,[304] G-CSF, M-CSF, and granulocyte-macrophage colony-stimulating factor (GM-CSF) were not specifically identified and measured in human milk until the 1990s.[151,305-307] The concentrations of M-CSF in particular appear to be 10- to 100-fold those in serum, and M-CSF evidently is produced by epithelial cells of the ducts and alveoli of the mammary gland under the regulatory activity of female sex hormones.[306]

Although it is tempting to speculate that cytokines present in milk may be able to interact with mucosal tissues in the respiratory and alimentary tracts of the recipient infant, the functional expression of specific receptors for cytokines on epithelial or lymphoid cells in the airway and gastrointestinal mucosa has not been fully explored.[241] A receptor-independent mechanism of cytokine uptake by the gastrointestinal mucosa during the neonatal period has not been demonstrated to date.

Milk and Altered Pregnancy

Several investigators have examined the effects of prematurity, early weaning, galactorrhea, and maternal malnutrition on the process of lactation. The immunologic aspects of these studies have focused largely on evaluation of the total content of sIgA and specific antibody activity. As described previously, the mammary secretions of nonlactating breast contain sIgA, although the amount appears to be much lower than in the lactating breast.[308] Mammary secretions of patients with galactorrhea appear to contain sIgA in concentrations similar to those of normal postpartum colostrum.[309] Although malnutrition has been associated with reduced secretory antibody response in other external secretions, maternal malnutrition does not seem to affect the total sIgA concentration or antimicrobial-specific antibody activity in the milk.[310]

The nutritional as well as immunologic composition of milk from mothers of premature infants appears to be significantly different from that of milk from mothers of infants born at term.[14,219,311,312] Comparative studies conducted during the first 12 weeks of lactation suggest that the mean concentrations of lactoferrin and lysozyme are higher in preterm than in term milk. Secretory IgA is the predominant immunoglobulin in preterm as well as in term milk, although the sIgA concentration appears to be significantly higher in preterm milk collected during the first 8 to 12 weeks of lactation. Secretory IgA antibody activity against certain organisms (E. coli somatic antigen) in the

preterm milk was observed to be somewhat less than, or at best similar to, that found in term milk. In addition, the number of lymphocytes and macrophages in milk appears to be lower at 2 weeks but significantly higher at 12 weeks in milk from mothers with preterm (born at 34 to 38 weeks of gestational age) infants than in milk from those with full-term infants.[311] The authors of these investigations have proposed that some of the observed changes may reflect the lower volume of milk produced by mothers delivered of preterm infants. The possibility remains that changes in the immunologic profile of preterm milk may be a consequence of inadequate stimulation by the preterm infant, alterations in the maternal hormonal milieu, or other factors underlying premature delivery itself.

BENEFITS AND RISKS OF HUMAN MILK

Benefits

Gastrointestinal Homeostasis and Prevention of Diarrhea

Development of mucosal integrity in the gut appears to depend on maturation of the mucosal tissue itself and the establishment of a normal gut flora. The former represents an anatomic and enzymatic blockage to invasion of micro-organisms and antigens, and the latter, an inhibition of colonization by pathogenic bacteria. Although permeability of the neonatal gut to immunoglobulin is rather short-lived or incompletely developed, unprotected or damaged neo-natal gut is permeable to a host of other proteins and macro-molecules for several weeks or longer. Large milk protein peptides and bovine serum albumin have been shown to enter the circulation and produce a circulating antibody response. The inflamed or ischemic gut is even more porous to both antigens and pathogens. A variety of proven and presumed mechanisms for the role of both IgA and the normal flora have been proposed to compensate for these temporary inadequacies. Evidence for gut-trophic substances in humans is still preliminary. Ample epidemiologic evidence exists for a positive effect of breast-feeding in establishing the normal gut flora. Most compelling are the observations in rural Guatemala of gross contamination of milk by potentially pathogenic aerobic, gram-negative bacilli, including E. coli and Shigella species, with an absence both of diarrheal illness and of significant numbers of these organisms in the feces of infants during the period of lactation. In addition, the diverse serotypes of aerobic, gram-negative bacilli present in the oropharynx and the gastro-intestinal tract of the neonate may serve as a source of antigen to boost the presensitized mammary glands, leading to a further modulation of specific bacterial growth in the mucosa.[313] The precise role of antibody that blocks adherence of these pathogens to the gut and the effects of other factors, such as lactoperoxidase, lactoferrin, lysozyme, and Bifido-bacterium bifidum, in those situations are undetermined.

Extensive epidemiologic evidence supports the "prophy-lactic value" of breast-feeding in the prevention or amelio-ration of diarrheal disease and is summarized in several reviews.[22,82,314,315] Ample experimental animal data on the value of specific colostral antibody in preventing diarrheal illness are available from studies of colostral deprivation.

These include colibacteriosis associated with E. coli K88 in swine; rotaviral gastroenteritis in cattle, swine, and sheep; and diarrheal illness associated with transmissible gastroenteritis of swine.[316] In humans, cholera is rare in infancy, especially in endemic areas where the prevalence of breast-feeding is high. The experience with an outbreak of cholera in the Persian Gulf lends support to the possibility that the absence of breast-feeding is an important variable in increasing the risk of cholera in infancy.

A few reports have claimed that nursery outbreaks of diarrhea associated with enteropathogenic strains of E. coli can be interrupted by use of breast milk. Conflicting data exist regarding prevention of human rotaviral infection and disease. Evaluation of nursery outbreaks of rotaviral disease has suggested that the incidence both of infection and of illness was lower in breast-fed infants, but the incidence of symptoms in formula-fed infants also was very low. Studies carried out in Japan have noted a fivefold decrease in incidence of rotaviral infection among breast-fed infants younger than 6 months of age. It must be emphasized that most rotavirus infections in neonates are asymptomatic, regardless of breast- or bottle-feeding.[317-321] On the basis of careful clinical observations, Bishop and co-workers[322] in Australia first questioned the positive effects of breast-feeding in rotavirus infection. More recent case-control studies of enteric viral infections in breast-fed infants have suggested that breast-feeding may protect infants from hospitalization rather than from infection itself.[323,324] Longitudinal follow-up of a large cohort of infants during a community outbreak of rotavirus has shown that attack rates of rotavirus infection were similar in breast-fed and in bottle-fed infants. The frequency of clinical disease with diarrhea, however, appeared to be significantly lower in breast-fed infants. Of interest, the protection observed in these patients was more a reflection of altered microbial flora from breast-feeding than of specific immunologic protection against rotavirus. Thus, it appears that breast-feeding provides significant protection against diarrheal disease, although the mechanism of such protection remains to be defined.[323,324]

Necrotizing Enterocolitis

NEC is a complex illness of the stressed premature infant, often associated with hypoxia, gut mucosal ischemia, and necrolysis and death.[325,326] Clinical manifestations have, on a few occasions, been associated with bacteremia and invasion by gram-negative bacilli, particularly Klebsiella pneumoniae, into the intestinal submucosa. Clinical manifestations include abdominal distention, gastric retention, and bloody diarrhea. Classic radiographic findings include air in the bowel wall (pneumatosis intestinalis), air in the portal system, and free infradiaphragmatic air (signifying perforation). Treatment involves decompression, systemic antibiotics, and, often, surgery.[327-329]

A number of studies have suggested a beneficial role of breast milk in preventing or modifying the development of NEC in high-risk human infants. Some pediatric centers have claimed virtual absence of NEC in breast-fed infants; however, many instances of the failure of milk feeding to prevent human NEC also have been reported. In fact, out-breaks of NEC related to Klebsiella and Salmonella species secondary to banked human milk feedings have been docu-

mented.[127,330,331] In an asphyxiated neonatal rat model of NEC, the entire syndrome could be prevented with feeding of maternal milk. The crucial factor in the milk appeared to be the cells, probably the macrophages.[127] It also is possible that antibody and nonspecific factors play a role, as does establishment of a gut flora. Prophylactic oral administration of immunoglobulin has been found to have a profound influence on the outcome of NEC in well-controlled studies.[331] Penetration of the gut by pathogens and antigens is increased with ischemic damage, and noncellular elements of milk may aid in blockage of this transit.[332]

The role of enteric anaerobic organisms has been seriously considered in the pathogenesis of NEC. Cytolytic toxins of *Clostridium difficile* and other clostridial species have been demonstrated in infants with NEC, often significantly more frequently than in normal infants.[333-336]

Clearly, NEC is a complex disease entity whose pathogenesis and cause remain to be defined. Although breast-feeding may be protective, a number of other factors are clearly related to the mechanism of mucosal injury and the pathogenesis of this syndrome.

Neonatal Sepsis

The incidence of bacteremia among premature infants fed breast milk has been suggested to be significantly lower than that among those receiving formula feedings or no feeding.[337-339] It has been shown that a high percentage of cases of neonatal bacteremia and meningitis caused by gram-negative bacilli are associated with the *E. coli* K1 serotype. Both antibody and compartmentalized cellular reactivity to this serotype have been demonstrated in human colostrum. High colostral antibody titers are associated most often with the colonization by the organism in the maternal gut. Other studies have, however, failed to demonstrate clear evidence of protection against systemic infection in breast-fed infants.[340-342]

Prevention of Atopy and Asthma

One of the most challenging developments in human milk research has been the demonstration in breast-fed infants of a reduced incidence of diseases with auto- or dysregulated immunity, long after the termination of breast-feeding.[269-273] Since the first report in 1936,[343] numerous published studies have addressed the effect of infant feeding on the development of atopic disease and asthma. Beneficial results of breast-feeding as prophylaxis against atopy have been observed in most of the studies; in others, however, beneficial effects were reported only in infants with a genetically determined risk for atopic disease. Finally, no beneficial effect at all or even an increased risk has been suggested in some breast-fed infants. Kramer, in an extensive meta-analysis of 50 studies published before 1986 that focused on infant feeding and atopic disease, has attempted to shed some light on the controversy.[344] Seven of the 13 studies on asthma included in this analysis claimed a protective effect of breast-feeding, whereas 6 claimed no protection. Several serious methodologic drawbacks, however, have been noted in this analysis. In a number of the studies analyzed, early infant feeding history was obtained months or years after the feeding period, ascertainment of the infant feeding history was obtained by interviewers who were aware of the disease outcome, or insufficient duration

and exclusivity of breast-feeding were documented; all were confounding variables that considered inappropriate "exposure standards." Nonblind ascertainment of disease outcome was found to be the most common violation of the "outcome standards."

Kramer's analysis also found that failure to control for confounding variables was a common violation in "statistical analysis standards" identified in several studies. Indeed, the effect of infant feeding on subsequent asthma may be confounded by other variables that are associated both with infant feeding and with unique investigational conditions. Factors that seem to have the greatest potential for confounding effects include the family history of atopic disease, socioeconomic status, and parental cigarette smoking. Only 1 of 13 studies on asthma included in the meta-analysis adequately controlled for these confounding factors. Moreover, 3 of the studies that did not demonstrate a protective effect of breast-feeding on asthma had inadequate statistical power. The effect of infant feeding on the severity of outcome and on the age at onset of the disease was virtually ignored in most of the studies.[344]

Although this extensive meta-analysis may suggest some uncertainty about the prophylactic benefit of breast-feeding, two recent studies strongly support a positive effect of breast-feeding on the development of atopic disease and asthma. The first study[269] consisted of prospective, long-term evaluation from infancy until the age of 17 years; the prevalence of atopy was significantly higher in those infants with short-duration (less than 1 month) or no breast-feeding, which increased to a demonstrable difference by the age of 17 years, than in the infants with intermediate-duration (1 to 6 months) or prolonged (longer than 6 months) breast-feeding. The differences in the prevalence of atopy persisted when the groups were divided according to positive or negative atopic heredity. Furthermore, the atopy manifestations in the different infant feeding groups did not remain constant with age. In particular, respiratory allergy, including asthma, increased greatly in prevalence up to the age of 17 years, with a prevalence as high as 64% in the group with short-duration or no breast-feeding.[269] In the second study, a prospective, longitudinal study of the prevalence and risk factors for acute and chronic respiratory illness in childhood, the investigators examined the relationship of infant feeding to recurrent wheezing at age 6 years and the association with lower respiratory tract illnesses associated with wheezing early in life.[345] Children who were never breast-fed had significantly higher rates of recurrent wheezing at 6 years of age. Increasing duration of breast-feeding beyond 1 month was not associated with significantly lower rates of recurrent wheezing. The effect of breast-feeding was apparent for children both with and without wheezing lower respiratory tract illnesses in the first 6 months of life. In contrast with the findings of the first study, however, the effect of breast-feeding was significant only among nonatopic children.[345]

The exact mechanisms by which breast-feeding seems to confer long-lasting protection against allergic sensitization are poorly understood. It is likely, however, that multiple synergistic mechanisms may be responsible for this effect, including (1) maturation of the recipient gastrointestinal and airway mucosa, promoted by growth factors present in human milk[242-244]; (2) inhibition of antigen absorption by

milk sIgA[346]; (3) reduced incidence of mucosal infections and consequent sensitization to bystander antigens[347]; (4) changes in the microbial flora of the intestine of breast-fed infants[325]; and (5) direct immunomodulatory activity of human milk components on the recipient infant.[241] A number of earlier and more recent studies have greatly contributed to the understanding of macromolecular transport across the immature gut and its consequences in terms of the generation of circulating antibody or immune complexes, the processes that are blocked predominantly by sIgA, the glycocalyx, and the intestinal enzymes. These mucosal immunologic events have been the basis for the concept of immune exclusion. Immune exclusion is not absolute, however, because uptake of some antigens across the gut may be enhanced rather than blocked by interaction with antibody at the mucosal surface. Beginning with the observations of IgA-deficient patients, it has become clear that the absence of the IgA barrier in the gut is associated with both an increased incidence of circulating antibodies directed against many food antigens and an increased occurrence of atopic-allergic diseases.[346] Some studies have noted complement activation in serum after feeding of bovine milk to children with cow's milk allergy. The neonate is similar in some respects to the IgA-deficient patient,[348] and increased trans-intestinal uptake of food antigen with consequent circulating antibody formation in the premature infant has been reported.[349] Other studies have suggested that early breast-feeding, even of short duration, is associated with a decreased serum antibody response to cow's milk proteins.[253] Prolonged breast-feeding not only may partially exclude foreign antigens through immune exclusion but may also, because the mother's milk is the infant's sole food, prevent their ingestion.[350] It must, however, be emphasized that intact bovine milk proteins and other food antigens and antibodies have been observed in samples of colostrum and milk.[6]

Other Benefits

As described previously, epidemiologic evidence suggests that bacterial and viral respiratory infections are less frequent and less severe among breast-fed infants in a variety of cultures and socioeconomic settings. Antibodies and immunologic reactivity directed against herpes simplex virus, respiratory syncytial virus, and other infectious agents[86,95,118,351,352] have been quantitated in colostrum and milk. Adoptive experiments in suckling ferrets have shown that protection of the young against respiratory syncytial virus can be transferred in colostrum containing specific antibody. The neonatal ferret gut, however, is quite permeable to macromolecules and permits passage of large quantities of virus-specific IgG. In the absence of either documented antibody or cellular transfer in the human neonate across the mucosa, any mechanisms of protection against respiratory syncytial virus and other respiratory pathogens remain obscure.

Data are lacking in humans regarding passive protection on other mucosal surfaces, such as the eye, ear, or genito-urinary tract. Some epidemiologic evidence suggests that recurrence of otitis media with effusion is strongly associated with early bottle-feeding and that breast-feeding may confer protection against otitis media with effusion for the first 3 years of life.[353] Foster feeding–acquired antibody to herpes simplex virus has been found to result in

significant protection against reinfection challenge in experimental animal studies.[351]

A number of other benefits have been associated with breast-feeding, including natural contraception during active nursing[354] and protection against sudden infant death syndrome,[355] diabetes,[356] obesity,[357] and high cholesterol level and ischemic heart disease later in life.[42] Of particular recent interest has been the association of breast-feeding with improved intellectual performance in older children. Several studies have demonstrated enhanced cognitive outcome in breast-fed children, although controversy exists regarding the mechanisms by which such improved performance may occur.[358-360] Health benefits for the mother also may be associated with breast-feeding: A reduced incidence of breast cancer has been noted in women who have lactated.[361]

Potential Risks

Noninfectious Risks

Several potentially harmful effects have been associated with breast-feeding. Some provocative data suggest that non-autologous human milk may, under certain conditions, be nutritionally inadequate for the premature infant.[22,23] The concentration of anti-Rhesus factor (anti-Rh) antibodies in milk appears to be too low to pose any threat to the incompatible neonate. Variable concentrations of medicinal products and their metabolites are excreted in colostrum and milk (Table 5-9). Environmental contaminants such as dichlorodiphenyl trichloroethane (DDT), polychlorinated biphenyls (PCBs), and mercury have been demonstrated in high concentrations in human milk.[22,362]

The failure to initiate lactation properly during early breast-feeding may present a risk of dehydration to the infant, because insufficient fluids may be ingested. Inappropriate introduction of bottles and pacifiers also may interfere with proper induction of lactation. Later in lactation, introduction of bottles may induce premature weaning as the result of a reduction in the milk supply.

Although human milk is the optimal form of nutrition for most healthy term infants, some circumstances have been identified in which breast-feeding is contraindicated and some in which continued breast-feeding should be conducted with caution to protect the infant. Infants with inherited metabolic diseases may be best nourished by treatment with alternative forms of nutrition. In particular, neonates with diagnosed galactosemia need to have galactose removed from their diet; in other words, they need to be switched to a milk containing lactose-free carbohydrate (because lactose is a glucose-galactose disaccharide). Infants diagnosed with phenylketonuria may receive some human milk to support their requirement for phenylalanine but often may be better managed by use of specially prepared commercial milks.

Management of hyperbilirubinemia associated with breast-feeding, so-called breast milk jaundice, has been an area of some controversy. The mechanism responsible for this form of jaundice is unknown but has been suggested to reflect inhibitors of glucuronidation, deficiency of related enzymes, excessive lipid breakdown, and insufficient milk intake.[363,364] Recent recommendations suggest that a more laissez-faire approach to this problem is appropriate.[365] Increasing milk

Table 5–9   **Drugs in Maternal Circulation Known to Pose Potential Health Problems for the Breast-Feeding Infant**

Drugs	Drugs	Environmental Contaminants
Anticoagulants	Autonomic drugs	Dichlorodiphenyl trichloroethane (DDT)
Ethyl biscoumacetate	Atropine	Polybromated biphenyls (PBBs)
Phenindione	Laxatives	Polychlorinated biphenyls (PCBs)
Anticonvulsants	Anthraquinone derivatives	Heptachlor
Mysoline	(Dialose Plus, Dorbane, Doxidon,	Mirex
Phenobarbital	Peri-Colace)	Lead
Phenytoin (diphenylhydantoin)	Aloe	Radioisotopes
Carbamazepine	Calomel	Caffeine
Antidepressants	Cascara	Food proteins
Lithium	Narcotics	Nicotine
Antihypertensives	Heroin	Cadmium
Reserpine	Methadone	Alcohol
Antimetabolites	Oral contraceptives	
Cyclophosphamide	Pain killers	
Methotrexate	Propoxyphene (Darvon)	
Antimicrobials	Sedatives	
Chloramphenicol	Barbiturates	
(Chloromycetin)	Bromides	
Metronidazole (Flagyl)	Chloral hydrate	
Tinidazole	Diazepam (Valium)	
Nalidixic acid	Steroids	
Nitrofurantoin[a]	Prednisone	
Sulfonamides[a]	Prednisolone	
Antithyroid drugs	Miscellaneous	
Iodide	Dihydrotachysterol (DHT)	
Thiouracil	Ergot alkaloids	
Radioactive iodine	Gold thioglucose	

[a]This drug causes problems mainly in infants suffering from the inherited deficiency of glucose-6-phosphate dehydrogenase.

Adapted from Packard VS. Human milk and infant formula. In Stewart GE (ed). Food and Science Technology Series. New York, Academic Press, 1982, p 118, with permission.

volume by increasing the number of feedings may be the most appropriate approach to breast milk jaundice; however, severe cases may necessitate phototherapy. Increased intake of fluids in breast-feeding infants appears to be effective in many cases.[366]

Several instances of specific nutrient deficiencies in breast-fed infants have been described, specifically related to lack of vitamin K, vitamin D, vitamin B_{12}, folic acid, vitamin C, and carnitine. In each of these instances, several case reports have appeared warning against deficiencies that have resulted in clinical consequences to the neonate. For example, hemorrhagic disease reported in a few breast-fed infants was successfully treated with vitamin K.[367] These infants did not receive vitamin K at birth. Mothers who practice unusual dietary habits, such as strict vegetarianism, may have reduced levels of vitamin B_{12} and folic acid in their milk, and deficiencies in breast-fed infants of such mothers have been reported.[368,369] Cases of rickets in breast-fed infants have been reported, particularly during winter among infants not exposed to the sun.[32,370] Deficiency of carnitine, a nutrient responsible for modulating fat absorption, also has been reported to result in clinical symptoms in breast-fed infants in mothers ingesting unusual diets.[61,371]

These various clinical expressions of nutrient deficiency in milk are of concern, but they also should be put in the context of nutrient deficiencies observed in formula-fed infants. Clearly, millions of infants in developing countries are at severe risk of malnutrition when they are formula-fed because of the economic stress of supplying sufficient

formula. Even in developed countries, large numbers of nutrient deficiencies and associated clinical symptoms have occurred as a result of accidents in the manufacture of formulas.[372] The most notable of these accidents have taught us the effects of early vitamin B_6 deficiency, folic acid deficiency, and chloride deficiency. Formula feeding also has been associated with an increased incidence of diabetes.[373]

Thus, some situations arise in which breast-feeding must be carefully considered as an appropriate feeding modality for the infant. Commercial formulas also represent risks, however. The infant is best served by observant pediatricians and mothers who promptly respond to any clinical signs in the neonate.

Infectious Risks

The presence of microbial contamination in milk is of serious concern. Contaminated milk has been implicated in neonatal infection with S. aureus, group B hemolytic streptococci, mycobacteria, and, possibly, Salmonella species (Table 5-10). Mastitis and breast abscess have been associated with the presence of bacterial pathogens in human milk. Such inflammation of the breast will often resolve even with continued breast-feeding. Resolution of the inflammation may be related to the presence of antisecretory factor (AF), a factor induced in the milk by enterotoxin-producing bacteria that appears to promote recovery from acute bacterial mastititis. In general, feeding an infant from a breast affected by an abscess is not recommended.[1] Infant feeding on the affected breast may be resumed, however, once the mother

Table 5-10   **Spectrum of Infectious Agents[a] Recovered in Human Milk and Their Possible Role in Infections in the Neonate**

		Effect on Breast-Fed Neonate[b]	
Agent in Milk	Seroconversion	Replication of Agent with Illness	Replication of Agent without Illness
Rubella virus	++ (25-30)	0	++ (56)
Cytomegalovirus	+	±	++ (58)
Hepatitis B virus	−	?	++
Hepatitis C virus	−	−	−
Varicella-zoster virus	?	?	?
West Nile virus	±	±	±
Herpes simplex virus	−	+	−
Human immunodeficiency virus (HIV)	+	±	++
Tumor viruses	−	−	+
HTLV-1	+	±	+
HTLV-2	+	±	+
Coxiella burnetii	−	−	−
Streptococcus species	−	±	+
Staphylococcus species	−	±	+
Enterotoxin	−	−	−
Mycobacterium species	−	−	−
Salmonella species	−	−	++
Escherichia coli	−	−	+

[a]All agents listed can be rendered noninfectious by heat inactivation at 62.5° C.
[b]+ to ++, modest to strong evidence; ±, presumptive evidence; ?, inconclusive data; −, not known; 0, absent; ( ), percentage of subjects reported.
HTLV, human T-lymphotropic virus.

has received adequate treatment. Furthermore, breast-feeding may continue on the unaffected breast. Mothers with active tuberculosis should refrain from breast-feeding for at least 2 weeks or longer after institution of appropriate treatment if they are considered contagious.[1]

Viral contaminants of maternal origin in the milk include rubella virus, herpes simplex virus, hepatitis B virus (HBV), cytomegalovirus (CMV), HIV-1, human T-lymphotropic virus type 1 (HTLV-1), and, possibly, HTLV-2 (see Table 5-10). For most viruses, although transmission has been documented as evidenced by seroconversion, no serious illness in the neonate, with the possible exception of CMV infection–related illness secondary to breast-feeding, has been reported.[22,314] Occasional reports of possible neonatal herpes simplex virus infection associated with presence of the virus in the mother's milk may just as easily have been caused by an infant-to-mammary gland rather than a mammary gland-to-infant route of inoculation.[374] Both the RNA-dependent DNA polymerase and structural proteins of C-type tumor viruses, possibly related to mouse mammary tumor and Mason-Pfizer viruses, have been identified in human breast tissue and products of lactation.[82] An association between breast-feeding of female infants and the development of breast cancer has been hypothesized in families with a strong history of carcinoma of the breast. Epidemiologic evidence to support such an association is lacking, however. Breast-feeding may in fact be a protective factor relative to maternal risk of such neoplastic disease.[375] Therefore, with adherence to reasonable maternal hygiene and in the absence of intense chemical contamination, generally few proven or well-defined contraindications to natural breast-feeding exist. Current recommendations regarding the transmission of infectious virus in human milk and their implications for the breast-fed infant are summarized next.

Human Immunodeficiency Virus Infection. Recently, serious concern has been voiced regarding the potential risk of the transmission of HIV from infected mothers to their suckling neonates through the process of breast-feeding. The possibility of postnatal transmission of this virus from mother to child has been considered in a large number of infants breast-fed in the United States and in other parts of the world. In some of these infants, breast-feeding has been implicated as one of the major risk factors for acquisition of HIV infection. Since 1985, small but significant numbers of infants with HIV infection possibly acquired through the process of breast-feeding have been reported.[376] In virtually all reported cases, maternal seroconversion for HIV antibody probably occurred after delivery of the infant. More than 50% of these mothers acquired the infection through heterosexual transmission, and about 30% through blood transfusion. Few of the mothers were judged to be intravenous drug users. Although acquisition of HIV infection before delivery cannot be ruled out with certainty, the likely route of transmission in these infants has been presumed to be through breast-feeding. The most convincing observations are based on several maternal-infant pairs in whom maternal seroconversion to HIV antibody occurred 4 months or longer after delivery.[377]

A number of studies have demonstrated HIV in milk.[378-382] The findings include isolation of HIV from milk supernatants collected from symptom-free women and from cellular fractions of maternal milk, recovery of HIV virions in the histiocytes and cell-free extracts of milk by electron microscopy, and detection of viral DNA by polymerase chain

Table 5-11   **Comparisons of HIV-I Transmission Rates in Infants Born to HIV-Infected Mothers Relative to Breast- and Bottle-Feeding**

Country of Study Population	Percentage of Infected Infants	
	Breast-Fed (N = 353)	Bottle-Fed (N = 108)
Haiti	25	0
USA	0	29
USA	28	33
Congo	52	0
Zaire	18	25

HIV, human immunodeficiency virus.
Adapted from Ruff AJ, Halsey NA, Coberly J. Breast-feeding and maternal-infant transmission of human immunodeficiency virus type 1. J Pediatr 121:325-329, 1992.

reaction (PCR) assay in greater than 70% of samples from HIV-seropositive lactating women. Limited epidemiologic studies carried out to date, however, have failed to demonstrate the magnitude of risk of HIV infection in breast-fed infants. Cohort studies[383] in different populations have suggested increased, reduced, or similar transmission rates in breast-feeding and in non–breast-feeding (bottle-feeding) infants of seropositive mothers (Table 5-11). Thus, it appears that although precise epidemiologic data are still lacking, a majority of breast-fed infants born to HIV-seropositive mothers remain uninfected despite the presence of HIV DNA in the milk in a high proportion of such mothers. Nevertheless, the risk of acquisition of HIV infection through breast-feeding must not be ignored. On the basis of meta-analysis of available data, it has been estimated that the additional risk of HIV infection through breast-feeding may be as high as 22%.[384] Some studies have suggested that breast-feeding contributes up to a 50% increase in the overall vertical transmission of HIV infection.[385]

Despite the potential risk of HIV infection in infants of HIV-infected breast-feeding mothers, consideration of cessation of breast-feeding must be balanced against other beneficial effects as outlined in this chapter. In a 1990 study, breast-fed HIV-infected children progressed to acquired immunodeficiency syndrome (AIDS) at a slower rate than that noted for bottle-fed children.[386]

Current estimates indicate the overall risk of acquiring HIV infection from breast-feeding to be about 16%. Of all HIV-infected infants, 47% may be infected by means of breast-feeding. Among those breast-fed for 3 months or longer, the rate of infection was estimated to be approximately 21%, and among those breast-fed for 2 months or less, the rate of infection was approximately 13%.[1] It is, however, important to realize that a number of other risk factors contribute to the increased transfer of HIV through breast-feeding. Associated maternal factors include younger age, multiple deliveries, high virus load, lower number of CD4$^+$ lymphocytes, and maternal mastitis. Other risk factors associated with the maternal milk include high viral load in the milk, long duration of breast-feeding, especially mixed formula feeding and breast-feeding, low levels of antiviral

factor in the milk (low CTL, sIgA, lactoferrin, lysozyme). Evidence of oral candidiasis in the breast-feeding neonate also appears to be a risk factor for development of breast-feeding–associated HIV infection.[1]

Current recommendations from the American Academy of Pediatrics state that in populations such as that of the United States, in which the risk of death from infectious diseases and malnutrition is low and in which safe and effective alternative sources of feeding are readily available, HIV-infected women should be counseled not to breast-feed their infants nor to donate milk. All pregnant women in the United States should be counseled and encouraged to be tested for HIV infection. Data are not available about the safety of breast-feeding by mothers receiving highly active antiretroviral therapy (HAART).

In geographic areas in which infectious diseases and malnutrition are important causes of death early in life, the feeding decision may be more complex. The World Health Organization (WHO) states that if a mother is infected with HIV, replacement of human milk to decrease the risk of HIV transmission may be preferable to breast-feeding, provided that the risk associated with replacement feeding is less than the potential risk of HIV transmission. Implementation of this suggestion has many obstacles. The WHO policy stresses the need for continued support for breast-feeding by mothers who are HIV negative or of unknown HIV serostatus, improved access to HIV counseling and testing, and government efforts to ensure uninterrupted access to nutritionally adequate human milk substitutes.[1]

Cytomegalovirus Infection. CMV infection is a common perinatal infection. The virus is shed in the milk in about 25% of infected mothers. Although breast-feeding from infected mothers may result in seroconversion in up to 70% of breast-feeding neonates, the infection often is not associated with clinical symptoms of disease. Low-birth-weight infants (born at less than 1500 g), however, may exhibit evidence of clinical disease, with thrombocytopenia, neutropenia, or hepatosplenomegaly seen in 50% of breast-feeding–infected babies. The decision to breast-feed a premature baby by an infected mother should be based on weighing the potential benefits of human milk versus the risk of CMV transmission. [1]

Hepatitis B Virus Infection. Hepatitis B surface antigen (HBsAg) has been detected in milk of HBV-infected mothers. Nevertheless, breast-feeding does not increase the risk of HBV infection among these infants. Infants born to HBV-positive mothers should receive hepatitis B immune globulin (HBIG) and the recommended series of hepatitis B vaccine without any delay in the institution of breast-feeding.[1]

Hepatitis C Virus Infection. The RNA of hepatitis C virus (HCV) and antibody to HCV have been detected in the milk from infected mothers. Transmission by means of breast-feeding, however, has not been documented in anti-HCV–positive, anti-HIV–negative mother. According to current guidelines, HCV infection does not contraindicate breast-feeding.[1]

Rubella. Rubella virus has been recovered from milk after natural as well as vaccine-associated infection. It has not been associated with significant disease in infants, however,

although transient seroconversion has been frequently demonstrated. No contraindication to breast-feeding exists in women recently immunized with currently licensed rubella vaccines.

West Nile Virus Infection. The RNA of west Nile virus has been detected in human milk, and seroconversion in breast-feeding infants also has been observed. Although West Nile virus can be transmitted in milk, its extent of transmission in humans remains to be determined. Most infants and children infected with the virus to date have been asymptomatic or have had minimal disease.[1]

Infection Due to Human T-Lymphotropic Viruses 1 and 2. Epidemiologic studies strongly suggest the possibility of mother-to-infant transmission of HTLV-1 by breast-feeding. In the United States, currently it is recommended that HTLV-1–infected women should not breast-feed. On the other hand, the status of maternal-infant transmission of HTLV-2 through the process of breast-feeding has not been well established, and until additional information is available, breast-feeding should not be recommended in seropositive women.[1]

Summary

It is apparent that human colostrum and milk are richly endowed with a wide variety of cellular and soluble components that participate in many nutritional, immunologic, and anti-infective processes of specific benefit to the neonate. The function of the products of lactation and maternal breast-feeding best characterized to date is nutritional support, and modulation and/or compensation for the transient mucosal immune deficiency against infectious and dietary macromolecules in the autologous infant.

In general, it is quite safe for the mother to collect her milk for later feeding or to directly breast-feed her own neonate. Nevertheless, increasing concerns regarding contamination of human milk by infectious agents have resulted in the limited use of either milk banks or wet nursing. Because of the transfer of infectious agents from maternal blood to milk (see Table 5-10), several national advisory committees have recommended that patients who have known transmissible infectious viral or bacterial diseases should not breast-feed.[388]

Other clinical situations in which withholding breast-feeding is appropriate because of high metabolite content in the milk include presence of galactosemia (galactose from lactose), phenylketonuria (phenylalanine), and other amino acid disorders in the infant.

As shown in Tables 5-9 and 5-10, many drugs, infectious agents, and environmental agents can be transferred to the infant in maternal milk. Rather than stopping breast-feeding, a nursing mother should avoid use of any drug unless it is absolutely essential. Many organohalides and fat-soluble environmental products, such as DDT and PCBs, may be present in higher concentrations in human milk.[22] Although not much is known about their risk to the infant, it is generally agreed that unless the degree of exposure in the mother is extremely high, the benefits of breast-feeding outweigh the possible risks associated with environmental contaminants. Caffeine, alcohol, and nicotine also present potential hazards to the infant (see Table 5-9). It is advisable to reduce or preferably discontinue the intake of tobacco, caffeine-containing products, and alcoholic beverages during lactation and nursing.

CURRENT TRENDS IN BREAST-FEEDING

Both international[389] and national[1,390,391] organizations have endorsed breast-feeding as the optimal means of feeding for the healthy term infant. In general, the percentage of mothers initiating breast-feeding in developing countries is 80% or higher and often 90% or more.[392] The health and economic consequences for bottle-fed infants in these countries are severe, however. In the United States, at one point in the early 1970s, the rate of breast-feeding initiation was as low as 25%. This low point was followed by an increase to a high of 61.9% in 1982. After 1982 a slow decline was observed (to 52.2% in 1989), after which a modest increase has been observed since the early 1990s.[393]

The pattern of breast-feeding initiation is accompanied by concomitant changes in maintenance of breast-feeding to 6 months, from 24% (1984) to 18% (1989) to 21.6% (1995).[393,394] These changes took place despite goals set by the U.S. Surgeon General for 75% of infants to be breast-feeding in the first week of life and 35% at 6 months.[395] These goals were reestablished for the year 2000.[396]

Within the United States, a variety of demographic patterns appear to be associated with breast-feeding behavior. Older mothers, mothers with a college education, and higher-income mothers all are more likely to breast-feed. By contrast, black and Hispanic mothers, mothers of lower socioeconomic status who are participants in the Women, Infants, and Children (WIC) program of the U.S. Department of Health and Human Services and mothers who live in the southern regions of the United States are much less likely to breast-feed. The low rate of breast-feeding for mothers enrolled in WIC is of particular concern, as that agency has a specific policy to encourage breast-feeding. Many states, however, now depend on formula manufacturer rebates to fund part of their WIC programs, creating something of a conflict of interest. The disturbing part of the demographic pattern of breast-feeding in the United States is that the infants of lower-socioeconomic-status mothers, who would accrue the greatest health and economic benefits from breast-feeding, are those least likely to be breast-fed.

Although demographic studies indicate who is breast-feeding, they do not explain the behavioral differences among groups of mothers. One of the more complete models designed to explain breast-feeding behavior includes components that address maternal attitudes and family, societal, cultural, and environmental variables.[397] Individual studies have shown that the maternal decision-making process is closely related to the social support and influence that come from the family members surrounding the mother.[398] The husband in particular appears to have a strong positive influence, whereas the mother's mother may have a negative influence on the breast-feeding decision. Social support appears to be different among ethnic groups, as are maternal attitudes; such differences may provide one explanation for differences in breast-feeding behavior among ethnic groups.[399,400]

SUMMARY AND CONCLUSIONS

Clearly, human milk contains a wide variety of soluble and cellular components with a diverse spectrum of biologic functions. The major milk components identified to date exhibit antimicrobial, anti-inflammatory, pro-inflammatory, and/or immunoregulatory functions; cytotoxicity for tumor cells; ability to repair tissue damage; and receptor analogue functions, as well as other metabolic effects. The relative contributions of different milk components to these biologic effects are summarized in Table 5-12.

The bulk of antimicrobial effects are associated with milk immunoglobulin, especially the sIgA isotype, which makes up to 80% of all immunoglobulins in the human body. Clinical observations have demonstrated that milk antibodies protect against a large number of intestinal pathogens such as Campylobacter, Shigella, E. coli, V. cholerae, Giardia, rotavirus, and respiratory pathogens such as respiratory syncytial virus. The milk antibodies also effectively neutralize toxins and a variety of human viruses. The role of small amounts of IgG and IgM in milk has not been fully examined. Recently, it has been suggested that milk IgG may hydrolyze nucleotides and DNA.[401] In general, milk IgA antibodies induce antimicrobial protection in the absence of any inflammation, a characteristic of other complement-binding immunoglobulins such as IgG and IgM.

Significant numbers of PMNs, macrophages, and epithelial cells are observed in the milk. Their precise function in the milk remains to be determined. It is possible that their primary task is the defense of the mammary gland itself. The lymphocytes present in the milk transfer immunologic information and may offer significant T cell–mediated immunologic defense to the suckling neonate. Breast-fed infants seem to become tolerant to their mothers' HLA, which may have important implications regarding immune responsiveness and allograft rejection.[402]

Lactoferrin, a major milk protein, also may play an important role in antimicrobial defense. It can kill bacteria, fungi, and viruses without causing inflammation. Lactoferrin also has been found to block mechanisms that result in the expression of pro-inflammatory cytokines (TNF-α, IL-6, IL-1β) by inhibiting nuclear transcription factor (NF$\kappa\beta$) activation mechanisms.[403]

Recent observations have suggested that α-lactalbumin may exist in human milk as large complexes binding to oleic acid. Such complexes, referred to as human α-lactalbumin made lethal to tumor cells (HAMLET), induce apoptosis of all malignant cells tested to date. These complexes have remarkably little to no effect on normal cells.[404,405]

Additional recent studies have suggested that milk contains large numbers of cytokines, chemokines, and growth factors. Although their precise role in the milk remains to be determined, some of these may act as signals for recruitment of pro-inflammatory and or immunoregulatory cells to the mucosal sites. TGF-β may be important in downregulating immune response and induction of tolerance, thereby decreasing the risk of allergic disease. IL-7 may promote development of a $\gamma\delta$ T cell population in cryptopatches which are small aggregates of lymphocytes in the intestinal crypts, and in the maintenance of thymus size.[406,407] It also has been shown that increased levels of IL-1β, TNF-α, IL-6,

and possibly other cytokines increase the level of leptin, an appetite-regulating hormone that is present in significant quantities in mammary epithelial cells, milk fat globules, and milk.[407,408] Leptin has several immunologic effects, including differentiation and proliferation of hematopoietic cells and regulation of monocyte-macrophage function. It also influences T cell response by enhancing IL-2 and IFN-γ production (for T_H1 cells) and IL-4 and IL-10 production (for T_H2 cells). Leptin is absorbed by the breast-feeding infant[408,409] and may be associated with prevention of obesity observed in breast-fed infants.

Another important recent observation with milk relates to the presence of antisecretory factor (AF). It has been observed in samples of milk, possibly induced by exposure to enterotoxin-producing bacteria.[410] It possesses significant effect on intestinal fluid secretions. The precise function of AF remains to be determined. Preliminary observations, however, suggest that AF may be highly effective in treatment of inflammatory bowel disease.[411,412]

Human milk has been found to possess large quantities of soluble CD14 and soluble Toll-like receptors TLR-2 and TLR-4, important elements for innate immunity.[413,414] CD14 promotes differentiation in expression of B cell function and anti-inflammatory effect of lactoferrin. Intestinal epithelium does not possess CD14, and it is possible that milk CD14 facilitates phagocytosis of organisms that require expression of this ligand. The TLR-2 and TLR-4 bind to a variety of microorganisms and may play an important role in downregulating inflammatory responses in the mucosal sites.

It is beyond the scope of this chapter to explore in any detail the reasons for and possible benefits of the evolution of mammalian life forms. Nevertheless, the passive transfer of the diversity of maternal biologic experiences to the neonate through the process of breast-feeding represents an essential component of the survival mechanism in the mammalian neonate. For millions of years, maternal products of lactation delivered through the process of breast-feeding have been the sole source of nutrition and immunity during the neonatal period and early infancy for all mammals, including the human infant. During the past 150 to 300 years, however, the human societal culture has undergone remarkable changes in rapid succession, which have had a major impact on the basic mechanisms of maternal-neonatal interaction and breast-feeding. Such changes include introduction of sanitation and nonhuman milk and formula feeds for neonatal nutrition, use of antimicrobial agents, introduction of processed foods, and exposure to newer environmental macromolecules and dietary antigens. The introduction of such manmade changes in the neonatal environment has had a profound impact on human homeostatic mechanisms and at the same time allowed new insights into the role of breast-feeding in the developing human neonate.

Comparative analysis of natural (traditional) forms of breast-feeding and artificial feeding modalities of modern times has demonstrated clearly that natural breast-feeding is associated with significant reduction in infant mortality and morbidity, protection against acute infectious diseases (both in the acute phase of the disease and with long-term re-exposure), and possible protection against allergic disorders and autoimmune disease, acute and chronic inflammatory disorders, obesity, diabetes mellitus and other metabolic

Table 5–12   Possible Role of Soluble and Cellular Factors Identified in Human Milk

Factor	Antimicrobial	Anti-inflammatory	Pro-inflammatory	Immunoregulatory	Antitumor	Receptor Blockade	Tissue Maturation	Other
Immunoglobulin (sIgA)	+++	++	—	++				++
Other immunoglobulins	+++	+	++	+				
T lymphocyte products	+++	++	++					
PMNs, macrophages	++		+	++				
Lactoferrin	+++	+++						
α-Lactalbumin (HAMLET)		++			+++			
Carbohydrates								
Oligosaccharides	++	++				++		
Glycoconjugates	++	++				++		
Glycolipids								
Lipid and fat globules	++							
Nucleotides	+			++			++	
Defensins	+			+				
Lysozymes	±							
Cytokines, chemokines								
TGF-β		++	++	++(↓)				
IL-10		++	++	++(↓)				
IL-1β		++	++	++(↓)				
TNF-α				++				
IL-6				↑++				
IL-7				(thymus)				
Others				++				
Prostaglandins		++						
Antisecretory factor		+++		++				
Leptin[a]							++	++
Antiproteases		++						
Growth factors		++		+++				++
(TLR-2, TLR-4) CD14		+++		+++				

[a]IL-1β, TNF-α, IL-6 are associated with increased levels of leptin.
HAMLET, human α-lactalbumin made lethal to tumor cells; IL, interleukin; PMNs, polymorphonuclear neutrophils [leukocytes]; sIgA, secretory immunoglobulin A; TGF, transforming growth factor; TLR, Toll-like receptor; TNF, tumor necrosis factor; + to +++, minimal to moderate effect; —, no effect.

disorders, allograft rejection, and development of a number of malignant conditions in childhood or later in life. Newer evidence suggests a protective role of breast-feeding in modulating many respiratory, intestinal, and urinary tract infections, otitis media, and NEC in the neonate. This information has been recently reviewed by Hanson in an elegant monograph.[415] Despite the overwhelmingly protective role attributed to natural breast-feeding and the evolutionary advantages related to the development of lactation, several infectious agents have acquired, during the course of evolution, the ability to evade immunologic factors in milk and to use milk as the vehicle for maternal-to-infant transmission. The potential for the acquisition of infections such as those due to HIV, HTLV, CMV, and possibly other pathogens highlights potential hazards of breast-feeding in some clinical situations.

Thus, it is reasonable to conclude that the development of lactation, the hallmark of mammalian evolution, is designed to enhance the survival of the neonate of the species, and that breast-feeding may have a remarkable spectrum of immediate and long-term protective functions.

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