Mother’s milk delivered naturally through breastfeeding 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. Such products provide an alternative to breastfeeding that is useful in certain situations. Nonetheless, compelling evidence demonstrates that breastfeeding is an ideal source of infant nutrition whose use is associated with lower rates of postnatal infant mortality in the United States and in other parts of the world.1-3 Human milk helps to protect the infant against a wide variety of infections and to reduce the risk for allergic and autoimmune diseases, the risk of obesity and its complications, and the risk for certain types of neoplasms later in life, and it has been associated with slightly better performances on tests of cognitive development in some studies.3 For these reasons, the American Academy of Pediatrics (AAP) and the World Health Organization (WHO) recommend that in the absence of specific contraindications (see “Benefits and Risks of Human Milk”) healthy term infants should be exclusively breastfed or fed expressed breast milk beginning within the first hour after birth through 6 months of age and supported with breastfeeding until at least 1 year of age.1-3 This chapter reviews existing information on major aspects of the physiologic, nutritional, and bioactive components of human milk.
Table 5-1 Possible Endocrine Factors in Growth of Human Female Mammary Glands

<table>
<thead>
<tr>
<th>Clinical State</th>
<th>Growth Characteristics</th>
<th>Maturational Hormones</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prenatal</td>
<td>Rudimentary</td>
<td>None</td>
</tr>
<tr>
<td>Infancy</td>
<td>Rudimentary</td>
<td>None</td>
</tr>
<tr>
<td>Puberty</td>
<td>Growth and budding of milk ducts</td>
<td>Growth hormone, prolactin, estrogen, corticosteroids, prolactin (high doses)</td>
</tr>
<tr>
<td>Pregnancy</td>
<td>Growth of acinar lobules and alveoli</td>
<td>Estrogen, progesterone, prolactin, growth hormone, corticosteroids</td>
</tr>
<tr>
<td>Parturition</td>
<td>Alveolar growth</td>
<td>Prolactin, corticosteroids</td>
</tr>
<tr>
<td>Lactational growth of tissue</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Secretory products</td>
<td>Casein, α-lactalbumin</td>
<td>Prolactin, insulin, corticosteroids</td>
</tr>
</tbody>
</table>

of placental and transplacentally acquired maternal hormones, with transient development of the excretory and lactiferous ductal systems. Such growth, differentiation, and secretory activities are transient and regress soon after birth.  

POSTNATAL DEVELOPMENT

Postnatal development occurs in five distinct stages: puberty, pregnancy, lactation, involution, and menopause. Each stage requires a specific set of morphogenetic changes in glandular structure and function. Cycles of cell proliferation, differentiation, and involution may recur until menopause. 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. Estrogens appear to be important for the growth and development of the ductular system, and progesterone for lobuloalveolar development. 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.

Prolactin secretion from the pituitary gland is under neural control and the increasing innervation of the breast throughout pregnancy is regulated by estrogens. 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 breastfeeding 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 pre-pregnant 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.

Physiology of Lactation

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. 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. The postpubertal mammary gland undergoes cyclic 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. 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 lobuloalveolar 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 advanced 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 breastfeeding; this response helps to restore the uterus to pre-pregnancy 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 the 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, growing as they fuse with one another. Some whole cells also are found in milk, including leukocytes, macrophages, lymphocytes, and 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-specific 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.

Colostrum is the first postpartum milk that is produced and is generally very dense in protein and fat content and has an enriched amount of immunologic factors compared with mature milk. Colostrum gives way to a transitional milk during the first week of life, where water content increases. Mature milk is relatively constant during the next ensuing months until weaning. Mode of delivery appears to also influence macronutrient composition of colostrum, with vaginal delivery versus cesarean section being associated with higher protein content. In addition, there are strong influences of maternal diet on fatty acid profiles in colostrum and later milk. Maternal age may also positively influence fat content in colostrum and may be related to increased fat synthesis or reduced water production. 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 likely to inhibition is the mother-child interaction. Early and frequent attachment of the infant to the breast is mandatory to stimulate 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. This rapid attachment to the mother may reflect olfactory stimuli from the breast received by the infant at birth. 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 breastfeeding properly and to cope with problems, and reduction of environmental hindrances. Such hindrances may include lack of rooming-in in the hospital, use of supplemental formula feeds, and lack of convenient daycare 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 one or two 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. In some cases where breast milk has not come in and newborns have dropped greater than 5% weight, discrete amounts of supplemental formula may improve breastfeeding success.

There are physiologic consequences to women who never lactate. Parous women aged 50 years or younger who had never lactated had higher prevalence of hypertension, obesity, and diabetes. Long-term cancer risk for breast and ovarian cancer are also reduced in women who have lactated.

Components of Human Milk and Their Potential Benefits for the Breastfed Infant

Human milk contains a rich diversity of nutrients, including proteins and peptides, lipids, carbohydrates, vitamins, minerals, electrolytes, and trace metals. The distribution and
relative content of various nutritional substances found in human milk are presented in Box 5-1. The chemical composition often exhibits considerable variation among lactating women and in the same woman at different times of lactation, as well as between samples obtained from mothers of preterm infants and from mothers of full-term infants. The appropriate amounts of each nutrient must be considered within these constraints.

**Box 5-1 Distribution of Secretory Products in Human Milk**

- **Water:** 86%-87.5%
- **Total solids:** 11.5 g

**Nutritional Components**
- **Lactose:** 6.9-7.2 g
- **Fat:** 3.0-4.4 g
- **Oligosaccharides:** 0.5-2 g
- **Protein:** 0.9-1.03 g
- **α-Lactalbumin:** 150-170 mg
- **β-Lactoglobulin:** trace
- **Serum albumin:** 50 mg

**Nitrogen-Containing Components**
- **Total protein:** 0.9-1.2 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:** (B1): 12-15 μg
- **Niacin:** 183.7 μg
- **B6:** 11-14 μg
- **B12:** <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 mg
- **A (retinol):** 54-56 μg
- **D:** <0.42 IU
- **E:** 0.56 μg
- **K:** 1.5 μg

**Electrolytes, Minerals, and 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

*Estimates based on amount per deciliter.

**PROTEINS, PEPTIDES, AND NONPROTEIN NITROGEN**

The total protein concentration in mature, term human milk is approximately 0.9 to 1.2 g/dL. Protein concentrations are significantly higher in the milk of women with preterm deliveries. Protein concentrations are generally not affected by maternal diet but increase with maternal body weight for height and decrease in women who produce higher milk volumes.

Human milk proteins can largely be classified into three groups: caseins, whey proteins, and mucins (milk fat globule membrane proteins). Caseins and whey proteins contribute the largest part, and their ratio and composition vary over the course of lactation. 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). In contrast, mucins constitute a smaller percentage of total milk protein, and their concentration changes little during lactation.

Human milk proteins provide the thriving infant with a source of essential amino acids (nutritional proteins) but also exert physiologic activities to further benefit infant health (bioactive proteins). Most milk proteins are both nutritional and bioactive. Recent technical advances in proteomics have dramatically expanded our knowledge about human milk proteins. In 2010, 285 unique proteins were identified from human milk; in 2012, the inventory was updated to 761 entries. In addition, human milk contains peptides that are either originally present in the milk or released from milk proteins after proteolytic cleavage in the infant’s gastrointestinal system (protein-derived peptides). Furthermore, approximately 25% of the human milk nitrogen comes in the form of nonprotein nitrogen and includes compounds such as urea, uric acid, creatine, creatinine, amino acids, and nucleotides. Milk proteins, peptides, and nonprotein nitrogen compounds will be reviewed in the following sections.

**Nutritional Proteins**

Caseins are a family of phosphoproteins that make up 20% to 45% of the protein in human milk. In contrast, caseins constitute greater than 80% of the total protein in bovine milk. β-Casein is the major protein found in human milk. The protein binds to calcium at its phosphorylated regions, which enables the caseins to form micelles that improve casein digestibility for the infant. The soluble κ-casein is believed to stabilize the casein micelles.

The major whey protein in human milk is α-lactalbumin, comprising about 25% to 35% of the total protein. It has a well-balanced amino acid composition with a high content of essential amino acids. α-Lactalbumin fragments have not been found in the stool of term or preterm infants, suggesting that it is well used. However, in vitro proteolysis generates α-lactalbumin fragments with bifidogenic or bactericidal properties as described in the section “Protein-Derived Peptides.” Although 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). 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. Most of the serum albumin, however, probably is synthesized outside the mammary gland. In addition to α-lactalbumin, the whey protein fraction contains all of the proposed functional proteins in human milk (immunoglobulins, lysozyme, lactoferrin, enzymes, cytokines, peptide hormones) that are described in the sections that follow.

**Bioactive Proteins and Peptides**

Whereas a major proportion of human milk protein is composed of the nutritional proteins just described, a significant number of the remaining proteins serve 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. 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 that is apparently absorbed intact by the infant. 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. Lactoferrin also may bind other minerals, including zinc and manganese, although the preferred mineral form appears to be the ferric ion. However, greater than 90% of the lactoferrin in human milk is in the iron-unsaturated form (apo-lactoferrin). The apo-form has very high affinity for iron, thereby withholding iron from iron-dependent pathogens. For example, the iron-sequestering, bacteriostatic activity of lactoferrin has been shown to inhibit the growth of *Cronobacter* (previously *Enterobacter*) sakazakii, a foodborne pathogen known to cause diarrhea in infants. Lactoferrin also has bactericidal activity, killing a variety of infant disease-relevant pathogens such as *Vibrio cholerae*. Oral lactoferrin prophylaxis has been recently shown to reduce the incidence of late-onset sepsis in infants weighing less than 1500 g and most effectively in infants weighing less than 1000 g. A statistically significant reduction in late-onset sepsis was observed in infants weighing less than 1500 g who received lactoferrin (relative ratio [RR], 0.34; 95% confidence interval [CI], 0.17 to 0.70); more research is currently underway to determine the utility of lactoferrin in clinical use.

A number of other proteins appear to be important as carriers of vitamins and hormones. Folate-binding, vitamin B12-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. 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, although serum albumin may in part fulfill this function.

**Enzymes.** The activity of several dozen enzymes has been detected in human milk. 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. Lysozyme is present in human milk at relatively high concentrations and is known to degrade the cell walls of gram-positive bacteria. Lactoferrin may in some contexts promote bacterial killing by lysozyme. A key feature of this defense is structural antimicrobial peptide motifs on the N-terminus of the protein.

Two main lipases have been identified in human milk, a pancreatic lipase–related protein 2 (PLRP2) and a bile salt–stimulated lipase (BSSL). PLRP2 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 BSSL is an acid-stable protein that compensates for the low activity of lipases secreted into the digestive tract during early development. 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. Pasteurization of human milk inactivates these enzymes and decreases lipid use.

**Hormones and Growth Factors.** Human milk contains several growth factors with potential effects on the intestine, vasculature, nervous system, and endocrine system. Epidermal growth factor (EGF) is critical to maturation and repair of the intestinal mucosa. EGF is resistant to low stomach pH and digestive enzymes and reaches the intestine. There, it may stimulate intestinal cells and alter DNA and protein synthesis, cell division, and absorbance of water and glucose. EGF concentrations are highest in colostrum and decrease over the course of lactation. Even in mature milk, average EGF concentrations are 100-fold higher than in maternal serum. Preterm milk contains higher EGF concentrations than term milk. Human milk also contains insulin-like growth factor (IGF)-1 and -2 as well as IGF-binding proteins and IGF-specific proteases. Concentrations are highest in colostrum and decrease over the course of lactation. Breastfed infants have higher serum IGF-1 concentrations than formula-fed infants, but the exact physiologic role and function of human milk IGF and IGF-related proteins remains unclear. In addition, human milk contains transforming growth factor-β (TGF-β), vascular endothelial growth factor (VEGF), erythropoietin...
Adiponectin concentrations in the mother’s milk correlate inversely with infant weight and body mass index, but whether or not this is a causal relationship whereby adiponectin contributes to a reduced incidence of overweight and obesity later in life remains unclear.61,62

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.

**Immunoglobulins.** As observed in other peripheral mucosal sites, the major class of immunoglobulin in human colostrum and milk is the 11S secretory immunoglobulin A (sIgA). Other isotypes, namely, 7S IgA, IgG, IgM, IgD, and IgE, also are present. The 11S 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 bound by the polymeric immunoglobulin receptor on the basolateral surface of mammary epithelial cells, which transports them through these cells, where they are released into the alveolar spaces as an 11S IgA dimer associated with a portion of the polymeric immunoglobulin receptor referred to as the secretory component.64

Sequential quantitation of class-specific immunoglobulin in human colostrum and mature 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.65 As lactation progresses, IgA declines to levels that range from 20 to 27 mg/g of protein, and IgM levels decline to 3.5 to 4.1 mg/g of protein. 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 of protein (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 (Table 5-2; see Fig. 5-1).

It has been estimated that the breastfed infant may consistently receive an amount of about 1 g of IgA each day and approximately 1% of this amount for IgM and IgG.66,67 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.68 Feces of breastfed infants contain functional antibodies present in the ingested milk.69 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.70

Endogenous production of secretory IgA by the infant’s mucosal immune system increases progressively in the postnatal period.71 Nonetheless, breastfed infants have

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**Table 5-2 Level of Immunoglobulins in Human Milk and Estimates of Delivery of Lactational Immunoglobulins to the Breastfeeding Neonate Over the Course of Lactation**

<table>
<thead>
<tr>
<th>Day Postpartum</th>
<th>PERCENTAGE OF TOTAL PROTEINS REPRESENTED BY IMMUNOGLOBULIN</th>
<th>OUTPUT OF IMMUNOGLOBULIN (mg/24 hr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Postpartum</td>
<td>IgG IgM IgA IgG IgM IgA</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>7 3 80 80 120 11,000</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>10 45 45 50 40 2,000</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>1-2 4 20 25 10 1,000</td>
<td></td>
</tr>
<tr>
<td>7-28</td>
<td>1-2 2 10-15 10 10 1,000</td>
<td></td>
</tr>
</tbody>
</table>

*Estimates based on the available data for total immunoglobulin and daily protein synthesis.

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**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 breastfeeding.** (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)
substantially greater concentrations of fecal sIgA than formula-fed infants. Prentice and colleagues found approximately 10-fold and approximately 4-fold higher concentrations of sIgA in the stools of breastfed than formula-fed infants at 6 and 12 weeks of postnatal age, respectively, even though only approximately 15% to 20% of ingested sIgA appeared in the feces.

IgA antibodies found in milk possess specificity for infectious agents endemic to or pathogenic for the intestinal and respiratory tracts. 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 O8:3, development of IgA antibody in human milk was evident in the absence of detectable serum antibody-specific responses. In another study, investigators have observed similar responses in animal models by using intrabronchial immunization with Streptococcus pneumoniae. These and other studies have strongly supported the concept of a bronchomammary as well as an enteromammary axis of immunologic reactivity in the breast.

Maternal immunity can be transferred to the infant via antigen-specific sIgA and other immunoglobulins in the mother’s milk and thereby prevent adherence and penetration of both bacterial and dietary antigens that would otherwise provoke inflammation in the intestinal mucosa. Colostrum and milk can inhibit the activity of E. coli and V. cholerae enterotoxins in experimental settings. The anti-toxic activity of human milk appears to correlate well with its IgA content but not with its IgM and IgG content. Pre-coating of V. cholerae with specific sIgA protects infant mice from disease. Similar results have been obtained by using specific purified milk sIgA in preventing E. coli– and Shigella dysenteriae–induced disease in rabbits.

**Cytokines.** In the 1990s, several cytokines, chemokines, and growth factors that mediate the effector phases of natural and specific immunity were discovered in human milk, and many more have been identified subsequently. Moreover, human milk displays a number of biologic activities characteristic of cytokines, including the stimulation of growth, differentiation of immunoglobulin production by B cells, enhancement of thymocyte proliferation, inhibition of interleukin-2 (IL-2) production by T cells, and suppression of IgE production. IL-1β and tumor necrosis factor-α (TNF-α) were the first two cytokines quantified in human milk. In colostrum, TNF-α is present mainly in fractions of molecular weight 80 to 195 kDa, probably bound to its soluble receptors. Milk TNF-α is secreted both by milk macrophages and by the mammary epithelium.

IL-6 was first demonstrated in human milk by a specific bioassay and also has been demonstrated by immunooassays. In like manner, IL-6 is localized in high-molecular-weight fractions of human milk. 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 and in secreted form in the milk may explain the high molecular weight of this cytokine in human milk. The expression of IL-6 messenger ribonucleic acid 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. The presence of interferon-γ (IFN-γ) in human milk also has been reported 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. Whether this IFN-γ is bioactive, as well as its source, also remains to be determined.

Chemokines are a novel class of small cytokines with discrete target cell selectivity that are able to recruit and activate different populations of leukocytes, and they are grouped into families defined by the spacing between cysteine residues (see Chapter 4). In CXC, cysteine pairs are separated by one amino acid, whereas in CC chemokines, paired cysteines are adjacent to each other. Certain CXC chemokines that contain an ELR (glutamic acid–leucine-arginine) motif, including CXCL8 (also known as IL-8) and CXCL1 (GRO-α), predominantly attract neutrophils, whereas basophils, eosinophils, dendritic cells, monocytes, and specific subsets of T and B lymphocytes are attracted by specific CC chemokines and non-ELR CXC chemokines. The presence of a number of CXC and CC chemokines has been described in human milk.

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. A receptor-independent mechanism of cytokine uptake by the gastrointestinal mucosa during the neonatal period has not been demonstrated to date.

Whether and to what extent cytokines in human milk contribute to the beneficial effects of human milk in the gut and elsewhere is largely unknown. Indirect evidence suggests that IL-7, which is a growth factor for T-cell progenitors (and for memory T cells), in human milk may support thymic growth. Thymus size was found to be larger in breastfed than formula-fed 4-month-old infants in Denmark, and thymus size and IL-7 content of breast milk were directly correlated with each other in exclusively breastfed infants in The Gambia. In the latter study, reduced human milk content of IL-7 was observed in the “hungry season” in association with reduced thymus size and thymic production of T cells; however, whether IL-7 in breast milk was absorbed intact and causally related to greater thymus size or was merely a surrogate for other factors cannot be determined from this study.

**Protein-Derived Peptides**

In addition to the proteins and peptides already present in human milk, a number of bioactive peptides have been identified that are derived from milk proteins. These protein-derived peptides are encrypted within the sequence of parental milk proteins and are released after enzymatic digestion in the infant’s stomach and intestine. The size of these bioactive protein-derived peptides are in the range of 2 to 20 amino acids, and some of them are known to have multiple functions. Some of them exert their bioactive function directly in the gastrointestinal tract (pepsin-absorptive bioactive peptides); others are absorbed in the intestine and function at peripheral sites (postabsorptive bioactive peptides). These bioactive peptides can be derived from both caseins and whey proteins.
The open and flexible structure of caseins allows for easy access of digestive enzymes to release bioactive peptides. For example, caseinophosphopeptides (CPPs) are casein-derived phosphopeptides that are thought to facilitate calcium ion absorption through vitamin D–independent mechanisms. Glycamacropeptide (GMP) is derived from the highly glycosylated C-terminus of κ-casein. GMP has bifidogenic effects and promotes the growth of *Bifidobacterium bifidum* and *Bifidobacterium infantis*; bifidobacteria are more prevalent in exclusively breastfed infants and have other effects that are thought to be beneficial, suggesting that GMP may help to promote a beneficial gut microbiota. GMP was also shown to have antiadhesive properties against certain pathogens, including *Helicobacter pylori*, enteropathogenic *E. coli*, *Salmonella typhimurium*, and *Shigella flexneri*. Although α-lactalbumin is one of the major nutritive whey proteins in human milk that provides the neonate with essential amino acids, it is also a considerable source of bioactive peptides. Lactoferrin has a variety of bioactive functions; some are only exerted after enzymatic digestion in the intestine and the release of lactoferrin-derived bioactive peptides. For example, the antimicrobial potency of some of the lactoferrin-derived peptides is higher than that of the intact, undigested lactoferrin itself. Most of the studies on protein-derived peptides stem from bovine milk, but a growing body of work suggests that human milk proteins also deliver bioactive peptides. Future research is needed to fully describe the role of these human milk protein–derived bioactive peptides in the context of infant health and disease.

**Nonprotein Nitrogen**

Nonprotein nitrogen contributes up to 15% to 30% of the total nitrogen in human milk and consists of free amino acids, peptides, urea, ammonia, carnitine, polyamines, nucleic acids, and nucleotides. 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 or of soy formulas that contain little carnitine, 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, whereas carnitine appears to be important for appropriate fatty acid metabolism.

Nucleotides constitute 2% to 5% of nonprotein nitrogen and serve as structural backbones of DNA and RNA and play important roles in cell replication and metabolism, store cellular energy, mediate intracellular metabolic processes, and support protein synthesis. Levels of nucleotides in human milk are much higher than those found in previously made infant formulas. Some infant formulas now are supplemented with nucleotides to levels closer to those in breast milk. The level of nucleotides in human milk varies greatly between individuals and may be related to maternal diet and ethnicity. Requirements for nucleotides are higher during periods of stress, and it has been proposed that nucleotides are conditionally essential to infants. Infants fed formula with nucleotide supplementation had lower risk of diarrhea, higher natural killer–cell activities, and produced higher antibody responses to *Haemophilus influenzae* type b, diphtheria toxoid, and oral polio vaccines when compared with infants who did not receive supplementation. There is still controversy as to the presence of any growth advantage with nucleotide supplementation in infant formulas. However, a recent randomized controlled study demonstrated a significant advantage in nucleotide-supplemented infants regarding growth in head circumference, with a mean difference in Z scores at 8 weeks of 0.4 (95% CI, 0.1 to 0.7; *P* = .006), which remained significant after adjustment for potential confounding factors (*P* = .002), and weight at 8 weeks of age was also greater. Nucleotide supplementation was associated with a relative increase in abundance of *Bifidobacterium* spp. compared with *Bacteroides* spp., *Porphyromonas* spp., or *Prevotella* spp. in infants’ fecal microbiota, but whether these changes in the microbiota are causally related to the improved growth is not known. An older study reported improved rates of growth in rapidly growing infants born small for gestational age, but additional work is required to validate the beneficial effects of dietary nucleotides in infants with specific clinical conditions.

**LIPIDS**

Lipids (fats) provide about half of the calories in human milk and are supplied in the form of fat globules enclosed in plasma membranes derived from the mammary epithelial cells. The lipid content is highest in colostrum and decreases from transitional to mature milk. There is a diurnal variation in lipid content but an even larger variation between milk collected at the beginning (foremilk) and the end (hindmilk) of the feeding. During the feeding, the lipid content increases so that hindmilk has a higher fat content than foremilk.

Most of the lipids are provided as triglycerides and with a minor part as phospholipids, cholesterol, and glycolipids. The fatty acid pattern differs in breast milk from mothers delivering very preterm, preterm, or term and further—from colostrum to transitional to mature milk. Most of the fatty acids are saturated, with a predominance of palmitic acid (16:0). Besides saturated and monounsaturated fatty acids, breast milk supplies the neonate with essential fatty acids (EFA) and long-chain polyunsaturated fatty acids (LCPUFA). Humans cannot synthesize EFA because the desaturases for introducing a double bond at carbons 3 and 6 (counted from the methyl end) are missing. The essential fatty acid linoleic acid (18:2ω6) supplies about 10% of the calories derived from the lipid fraction. Several different elongases and desaturases transform EFAs to LCPUFAs, such as arachidonic acid (AA), docosahexaenoic acid (DHA), and eicosapentaenoic acid (EPA), which serve as substrates for a wide range of bioactive metabolites, including eicosanoids, lipoxins, resolvins, and protectins. Different fatty acids compete as substrates for the different elongases and desaturases, and different substrates lead to different bioactive metabolites with sometimes opposing effects. The EFA and LCPUFA contents in human milk mainly depend on the mother’s diet. The mother’s long-term diet affects the mother’s lipid profile stored in her adipose tissue, which then feeds into the lipid composition of her milk. The mother’s short-term diet affects milk lipid composition directly. For instance, there is a high correlation between the mother’s fish intake and the DHA concentrations in her...
plasma and her breast milk. Therefore the maternal diet should receive special attention during both pregnancy and lactation.

DHA is important for neurologic development, including visual acuity. The brain is mainly built of lipids, and DHA constitutes 40% of the fatty acids in the gray matter, concentrated in the synaptic membranes. The retina is the organ with the highest DHA content, especially in the rod outer segment. Sensibility of the retina is very susceptible to low DHA concentrations. Fatty acids also influence gene expression, which might affect long-term neurodevelopment and metabolism. The most vulnerable to inadequate specific fatty acid intake are preterm infants, who have limited capacity to generate enough LCPUFAs on their own despite having the synthetic enzymes present. As a result, infant formulas for both term and preterm formulas are now supplemented with LCPUFAs because, originally, designed formulas were based on plant lipids and therefore did not contain appreciable amounts of LCPUFAs, although systematic reviews have concluded that insufficient evidence is present to recommend LCPUFA supplementation for term and preterm formulas. However, in preterm infants, levels of DHA two to three times the levels found in current preterm formulas or average breast milk have resulted in improved neurodevelopmental outcomes. Therefore the exact dosing for preterm infants still needs to be determined. For the term infant, there is some recent long-term evidence that LCPUFA supplementation may be beneficial. This includes improved visual acuity by 12 months of age in those infants who received LCPUFA supplementation in their infant formula. Early infant formula fatty acid supplementation also led to faster information processing in children followed up to age 6 years compared with those who only received unsupplemented formula.

Cholesterol is an important lipid constituent of human milk (12 mg/dL) even if it is a small fraction of total fat content (0.5%). Breastfed infants consume very high amounts of cholesterol per kg of body weight compared with adults (more than 10-fold). Cholesterol is found in only trace amounts in commercial formulas. It has been suggested that early breastfeeding associated with high measured total blood serum cholesterol may actually prevent some of the risks of developing cardiovascular diseases later in life. Additional research is required to determine the potential role that early cholesterol consumption may play in later cardiovascular health.

**CARBOHYDRATES**

Lactose

Lactose is the primary sugar found in human milk and usually is the carbohydrate chosen for the preparation of commercial formulas. Lactose supplies approximately one third 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 DHA in the retina. Therefore the maternal diet should receive special attention during both pregnancy and lactation.

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There are strong interpersonal and intraper 12
sonal variations in the amount and composition of cells in 
vid body. Human milk contains maternal cells that are live in fresh 
emly, individual HMOs are becoming available in quantities and qualities for the use in clinical 
tudies to test their efficacy in human infants.

VITAMINS

Vitamin D

Human milk typically contains a vitamin D concentration of 25 IU (international units) per liter or less, which alone does not provide infants with an adequate intake. Although most breastfed infants are able to synthesize additional vitamin D through routine sunlight exposure, avoidance of direct sunlight has limited this source of vitamin D. The low content of vitamin D in human milk has been related to the development of rickets in a few breastfed infants. Therefore the AAP and Institute of Medicine (IOM) currently recommend that all breastfed infants, including preterm infants, receive 400 IU of oral vitamin D drops daily, beginning during the first 2 months of life and continuing until adequate amounts of vitamin D are provided through daily consumption by the infant of fortified formula or milk.

Vitamin E

The vitamin E content (mainly α-tocopherol) is higher in human milk than in bovine milk. α-Tocopherol concentrations in colostrum are higher than that of transitional and mature milk. In well-nourished populations, supplementing the mother’s diet with vitamin E has only limited effects on vitamin E concentration in human milk. α-Tocopherol functions mostly as a chain-breaking antioxidant that prevents propagation of lipid peroxidation, modulates immune function, affects cell signaling, regulates gene expression, and contributes to several metabolic processes in the human body.

Vitamin K

Human milk contains 1 to 4 μg/L of vitamin K1. Bleeding in infants caused by vitamin K deficiency is rare (0.25%−1.7% incidence), but can be severe. Thus the AAP recommends 0.5 to 1.0 mg vitamin K1 administered to all infants shortly after birth.

Vitamin B

Poor maternal status of vitamins such as thiamine (B1), riboflavin (B2), vitamin B6, and vitamin B12 causes concentrations of these nutrients to be low in breast milk and the infant to become deficient. In contrast, in maternal folate depletion, breast-milk folate concentrations are maintained. The prevalence of maternal deficiency of these vitamins is uncertain but certainly higher in developing countries with limited availability of high-quality food sources.

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 presents a fairly low solute load to the developing kidney. The levels of major minerals tend to decline during lactation, with the exception of magnesium, but with considerable variability among women tested. 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. Dietary influences may affect levels of calcium and copper but not other minerals. Preterm infants fed human milk require supplements of calcium, phosphorus, sodium, and iron.

CELLS

In addition to its nutritive and bioactive biochemical components, milk contains maternal cells that are live in fresh milk and that might have specific functions for the breastfed infant. There are strong interpersonal and intrapersonal variations in the amount and composition of cells in human milk. Human milk can contain between 10,000 and 13,000,000 cells/mL. Colostrum often contains more cells than transitional and mature milk. At least three different cell types have been reported in human milk: leukocytes, epithelial cells, and stem cells. These three categories are reviewed in more detail as follows.

Leukocytes

In addition to the many biochemical immune modulators already described, human milk contains blood-derived leukocytes that are believed to be transported into the milk via the paracellular pathway. Depending on the stage of lactation and the health of the mother or the infant, leukocytes may constitute the majority of the cells in human milk. Leukocyte content in colostrum varies within a wide range (13.2%−70.4%). Infection of the mother or the infant is associated with an increase in milk leukocytes, which decreases when the infection resolves. The predominant leukocyte types in colostrum are macrophages (40%−50%), followed by polymorphonuclear neutrophils (40%−50%), and lymphocytes (5%−10%). Greater than 80% of the lymphocytes are T cells, and only 5% to 6% are B cells. In fresh milk, many of these cells are live, activated, and motile.

Data
from animal models suggest that they cross the infant’s intestinal epithelial barrier and are engrafted in different organs and tissues, including mesenteric lymph nodes, liver, and spleen.\textsuperscript{184-187} Milk leukocytes are thought to protect the mother’s lactating mammary gland from infections and may provide a benefit to the infant, although this remains to be demonstrated.\textsuperscript{188} In contrast to colostrum, leukocyte content in mature milk is rather low but also increases in response to infections in the breastfeeding mother or the breastfed infant.

**Epithelial Cells**

Human milk contains both ductal and alveolar, luminal-epithelial, and myoepithelial cells. Over the years and based on newly acquired data, three different hypotheses developed about why epithelial cells occur in milk. At first, it was thought that epithelial cells are the product of apoptosis. Later, evidence showed that the majority of epithelial cells in freshly expressed milk are viable and can be propagated in primary culture to form functioning mammospheres.\textsuperscript{189,190} This led to the second hypothesis, that epithelial cells passively detached from ducts and alveoli in the process of milk synthesis and secretion, for instance, because of the vacuum applied to the ducts during suction.\textsuperscript{191} The most recent data supports a third hypothesis, that epithelial cells are detached in an active process that is driven by differential gene expression. As a result, the cells become more motile, leading to an active detachment. Milk epithelial cells often occur as clusters that can be isolated and cultured for further analysis.\textsuperscript{192,193} Whether the presence of these cells is biologically useful or merely reflects physiologic turnover is not known.

**Stem Cells**

Cell preparations from fresh human milk are able to expand in culture and form different colony types that can be maintained through multiple passages.\textsuperscript{192-195} This observation led to the hypothesis that human milk contains breast-milk stem cells (hBSCs). The hypothesis was confirmed when researchers discovered that some human milk cells express the stem cell markers CK5 and nestin, as well as the stem/progenitor cell markers p63 and CD49f.\textsuperscript{196,197} These cells were able to self-renew and differentiate into luminal and myoepithelial cells. Most recently, it was discovered that some of these hBSCs express pluripotency markers, such as the transcription factors OCT4, SOX2, and NANOG.\textsuperscript{189} As with most milk components, there is wide interpersonal and intrapersonal variation in hBSC content and expression levels of stem/progenitor cell and pluripotency markers. What factors influence this variation has not yet been determined. hBSCs are able to differentiate into cells of the mammary gland lineage that synthesize and secrete milk-specific proteins and other components. Most intriguing, however, are results that demonstrated the ability of hBSCs to also differentiate into cells of other lineages, including cells with properties of adipocytes, cardiomyocytes, chondrocytes, osteoblasts, neurons, hepatocytes, and pancreatic beta cells.\textsuperscript{189} The discovery of pluripotent hBSCs that are capable of differentiating into cells from all three germ layers opened an entirely new area of human milk research. Future studies will have to show how hBSCs impact the breastfed infant and whether they have the potential to be used in the context of regenerative medicine.\textsuperscript{177}

Overall, the presence of viable cells in human milk, whether they are leukocytes, epithelial cells, or stem cells, raises the question of how they affect the breastfed infant. Considering the concentration of cells in human milk and the milk volume consumed, it can be estimated that thousands or even millions of viable cells are ingested by the breastfed infant with each and every feed. Research in animals has shown that some of these cells are able to cross infant epithelial barriers and are engrafted in infant tissues and organs.\textsuperscript{184-187} This has mainly been shown for leukocytes but may also be possible for epithelial cells and stem cells, causing a microchimerism with maternal cells (i.e., detected as cells containing only maternal DNA) engrafted in infant tissues (i.e., infant DNA with maternal and paternal contribution). Potential lifelong consequences are unknown and need further investigation.\textsuperscript{197}

**BACTERIA**

Traditionally, bacteria in human milk had been associated with infections and perceived as harmful for the breastfeeding mother and the breastfed infant. Several studies, however, reported the presence of bacteria even in the milk of healthy women without any clinical or subclinical signs of infection. Newer culture-independent techniques based on 16S ribosomal RNA sequencing led to a more comprehensive description of the human milk microbiome.\textsuperscript{198-200} The human milk microbiome tends to be stable over time and is highly personalized.\textsuperscript{199} Although the bacterial composition varies between mothers, most milk samples contain a set of core bacteria that include Streptococcus, Staphylococcus, Pseudomonas, Corynebacteria, Propionibacterium, Sphingomonas, and Bradyrhizobiaeae. Although it is now generally accepted that milk from healthy women is not sterile, the origin of the human milk microbiome remains uncertain.\textsuperscript{198,201,202} One hypothesis is that human milk microbes are derived from the mother’s skin and/or the infant’s oral flora. The second hypothesis is that the microbes stem from the mother’s gastrointestinal tract. It suggests that permeability in the mother’s intestine is increased, which allows bacteria to enter the bloodstream and reach the mammary gland. The third hypothesis is that microbes in the mother’s intestine are sampled by specialized dendritic cells that transport selected bacteria to the mammary gland. If the second or third hypothesis proves to be correct, it will be intriguing to study how a manipulation of the mother’s intestinal microbiome with prebiotics, probiotics, or antibiotics might impact the human milk microbiome and affect not only maternal but also infant health. Bacteria in human milk could protect the infant against infections and contribute to the maturation of the immune system.\textsuperscript{202} On the other hand, dysbiosis may lead to mammary gland infections (mastitis), which remains one of the main reasons for women to stop breastfeeding.

**Benefits and Risks of Human Milk**

**BENEFITS**

**Gastrointestinal Homeostasis**

The 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
anatomic and enzymatic blockades to invasion of microorganisms and antigens, and the latter, an inhibition of colonization by pathogenic bacteria. Although permeability of the neonatal gut to immunoglobulin is rather short-lived, damaged neonatal gut is permeable to a host of other proteins and macromolecules for several weeks or longer. Peptides derived from proteins and bovine serum albumin can enter the circulation and induce 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 sIgA and the normal flora have been proposed to compensate for these temporary inadequacies.\textsuperscript{204}

**Prevention of Diarrhea**

Extensive epidemiologic evidence supports a “prophylactic value” of exclusive breastfeeding in the first 6 months of life, with the addition of complementary feeding thereafter, in the prevention or amelioration of diarrheal disease in infants and young children in developed and developing nations and is summarized in several reviews.\textsuperscript{1,203–208} These earlier findings are supported by a recent Cochrane analysis that concluded that infants who continue exclusive breastfeeding for 6 months or more appear to have a significantly reduced risk of gastrointestinal and respiratory infection, at least in the Iranian and Nigerian studies, compared with those who breastfeed for less than 4 months. Historically, a preventive and therapeutic role for breastfeeding has been suggested in nursery outbreaks of diarrheal disease caused by enteropathogenic strains of *E. coli* and rotavirus.\textsuperscript{209} Earlier observations have shown a reduced rate of diarrhea in breastfed infants, even in the face of milk contamination with *E. coli* and *Shigella* spp.\textsuperscript{211}

Cholera is rare in infancy, especially in endemic areas where the prevalence of breastfeeding is high. The experience with an outbreak of cholera in Guinea-Bissau lends support to the possibility that breastfeeding is an important variable in preventing against cholera in infancy. In this study, the risk of diarrheal disease caused by cholera was reduced from 29% to 7% in breastfed children (RR for breastfed children, 0.19; 95% CI, 0.04 to 0.91, adjusted for age) and in those breastfed children who developed disease, maternal milk contained lower concentrations of anti-cholera toxin IgA/total IgA (median, 2.0 units/mL) than in the breastfed children who did not develop diarrheal disease (median, 17.4 units/mL).\textsuperscript{212}

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; rotavirus gastroenteritis in cattle, swine, and sheep; and diarrheal illness associated with transmissible gastroenteritis of swine.\textsuperscript{213} In addition, the diverse serotypes of aerobic, gram-negative bacilli present in the oropharynx and the gastrointestinal 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.\textsuperscript{214}

Case-control studies of enteric viral infections in breastfed infants suggest that breastfeeding may more often protect infants from severe disease and hospitalization rather than from infection itself.\textsuperscript{215,216} This is also the case for the protection provided by rotavirus vaccines.\textsuperscript{217} Notable in this regard are conflicting data regarding the impact of breastfeeding on risk for human rotaviral infection and disease. A community-based study in Germany, Switzerland, and Austria showed a protective effect of breastfeeding on the incidence of acute rotavirus gastroenteritis.\textsuperscript{218} However, in developed countries, most rotavirus infections in neonates are asymptomatic, regardless of breastfeeding or bottle feeding,\textsuperscript{219–221} and on the basis of careful clinical observations, Bishop and co-workers\textsuperscript{222} in Australia questioned the positive effects of breastfeeding in rotavirus infection. Consistent with this point, the risk of rotavirus-associated diarrheal disease in hospitalized infants in Uganda was not lower in those who were breastfeeding (OR, 1.08; 95% CI, 0.52 to 2.25; *P* = 0.8).\textsuperscript{223} Thus, although breastfeeding protects against many causes of diarrheal disease, the magnitude of the benefit can vary, and the mechanisms of such protection remain to be more fully defined.\textsuperscript{215,216}

**Necrotizing Enterocolitis**

Necrotizing enterocolitis (NEC) is a complex gastrointestinal inflammatory condition that occurs principally in very premature infants and is often associated with hypoxia, gut mucosal ischemia, and necrosis.\textsuperscript{224,225} Evidence supports the notion that NEC is associated with an exaggerated immunologic or inflammatory response in the host.\textsuperscript{226,227} A role for a gestational age window of susceptibility to dysregulated inflammation in the timing of NEC may be present as NEC occurs after several weeks in the smallest preterm infants compared with the first week for older preterm infants.\textsuperscript{228} Outbreaks of NEC related to *Klebsiella* and *Salmonella* spp. secondary to banked human milk feedings have been documented.\textsuperscript{229–231} However, although NEC has been associated in some cases with a specific pathogen, most cases are not caused by a single organism.

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). A breakdown in the mucosal defense leads to dysregulated inflammation from unchecked invasion of pathogenic bacteria. Treatment involves decompression, systemic antibiotics, and, often, surgery.\textsuperscript{257,232–236}

The prevention of NEC is the primary management for this condition. A number of studies have demonstrated a beneficial role of human milk in preventing or modifying the severity of NEC in high-risk preterm infants, in particular the potential for an exclusively human milk diet with human milk versus bovine-based fortifiers to lead to a 50% reduction of all NEC and 90% reduction of surgical NEC.\textsuperscript{237–243} And although some other pediatric centers have claimed a virtual absence of NEC in human milk–fed infants with slowly advancing feeding protocols, others have not.\textsuperscript{244,245}

In an asphyxiated neonatal rat model of NEC, the condition could be prevented with feeding of maternal milk. Although cellular components in human milk are obvious candidates, most preterm diets have limited mammalian cells because of storage, freezing, or use of donor human milk. It also is possible that antibody and nonspecific factors play a role, including establishment of a physiologic gut microbiota with associated beneficial effects on intestinal integrity and homeostasis and gut intrinsic immune processes (see Chapter 4).\textsuperscript{111,246,247}
Use of broad-spectrum antibiotics is standard therapy when NEC has developed, and this is based on the assumption that reducing pathogenic bacterial burden is supportive of gut restitution. The most effective combination of antibiotics has not been determined clearly for the treatment of NEC. Past strategies of enteral antibiotic regimens have been associated with reduced NEC incidence, but concerns for antibiotic resistance limited this approach. Conversely, however, retrospective data from the National Institute of Child Health and Human Development Neonatal Research Network and others have associated longer initial empirical antibiotic use after birth with NEC incidence. Presumably, initial antibiotics may significantly alter gut microflora and increase the chances for NEC to occur. Antenatal antibiotic use has also been a risk factor for the development of NEC.

Thus NEC continues to present a medical challenge whose etiopathogenesis remains to be more fully defined. Although breastfeeding may be protective, a number of other factors are clearly related to the mechanism of mucosal injury and the pathogenesis of this condition.

**Neonatal Sepsis**

There is a body of evidence suggesting that the incidence of bacteremia among preterm infants fed breast milk is significantly lower than that among those receiving formula feedings or no feeding. Other studies have, however, failed to demonstrate protection against systemic infection in breastfed infants. These controversies notwithstanding, there is substantial support for the notion that the use of human milk for very-low-birth weight (VLBW) neonates reduces the risk of late-onset sepsis.

**Prevention of Atopy and Allergy**

Since the first report in 1936, numerous publications have addressed the effect of infant feeding on the development of atopic disease and asthma. Breastfeeding has been reported to have a prophylactic benefit on the development of atopic disease and asthma. The first prospective long-term study, with extended follow-up from infancy until the age of 17 years, found that the prevalence of atopy was significantly higher in those infants with short-duration (<1 month) or no breastfeeding than in infants with intermediate-duration (1-6 months) or prolonged (>6 months) breastfeeding. The differences in the prevalence of atopy persisted when the groups were divided according to positive or negative atopic heredity. Furthermore, the atopic 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 at this age as high as 64% in the group with short-duration or no breastfeeding. In another prospective, longitudinal study of the prevalence and risk factors for acute and chronic respiratory illness in childhood, investigators examined the relationship of infant feeding to recurrent wheezing at age 6 years and the association of wheezing early in life with lower respiratory tract illnesses. Children who were never breastfed had significantly higher rates of recurrent wheezing at 6 years of age, but in this study, increasing the duration of breastfeeding beyond 1 month was not associated with significantly lower rates of recurrent wheezing. The beneficial effect of breastfeeding 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 beneficial effect of breastfeeding was significant only among nonatopic children.

How might breastfeeding confer long-lasting protection against allergic sensitization? Multiple synergistic mechanisms may contribute (1) maturation of the recipient gastrointestinal and airway mucosa, promoted by growth factors present in human milk; (2) inhibition of antigen absorption by milk sIgA; (3) reduced incidence of mucosal infections and consequent sensitization to bystander antigens; (4) impact of breast milk on the infant’s microbiota; and (5) direct immunomodulatory activity of human milk components on the recipient infant and differences in exposure to potential allergens.

Secretory IgA, along with the intestinal glycocalyx and intestinal enzymes, might impede the development of allergic sensitization, in part via a process of immune exclusion, whereby immune processes help to impede foreign macromolecular transport across the immature gut and its consequences in terms of the generation of circulating antibody or immune complexes. 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. The neonate is similar in some respects to the IgA-deficient patient and increased transintestinal uptake of food antigen with consequent circulating antibody formation in the premature infant has been reported. Immune exclusion is not an absolute rule, however, because uptake of some antigens across the gut may be enhanced rather than blocked by interaction with antibody at the mucosal surface.

In addition to immune exclusion, prolonged breastfeeding could also protect against atopy by direct exclusion—if the mother’s milk is the infant’s sole food. Ingestion by the infant is precluded unless the mother consumes the food and food antigens are transported into her milk. In this regard, intact bovine milk proteins and other food antigens and antibodies have been observed in samples of colostrum and milk. In any case, such direct exclusion is not required because other studies have suggested that early breastfeeding, even of short duration, is associated with a decreased serum antibody response to cow’s milk proteins.

These potential biologic mechanisms and the studies noted above notwithstanding, there is considerable debate regarding the protective immune effects of early breastfeeding on the development of atopy and allergy, the duration and exclusivity of breastfeeding that results in benefit, the duration of that benefit after the termination of breastfeeding, and the importance of the act of breastfeeding in addition to the constituents of breast milk. For example, one retrospective Korean study showed an increased risk for asthma in infants who were breastfed beyond 1 year. Another reported that the protective effect of breastfeeding was not found for infants fed breast milk by bottle.

Differences in conclusions may be related, at least in part, to methodologic issues. Kramer conducted an extensive meta-analysis of 50 studies published before
A number of other benefits have been associated with breastfeeding, including natural contraception during active nursing, protection against sudden infant death syndrome, diabetes, obesity, high cholesterol level, and ischemic heart disease later in life. A reduced risk of breast cancer has also been shown in several studies, and a recent meta-analysis reported a pooled odds ratio of 0.72 (95% CI, 0.58 to 0.89) for women who ever breastfed, although reduced risk was not found in another recent cohort study. Several studies have demonstrated enhanced cognitive outcome in breastfed children, although controversy exists regarding the mechanisms by which such improved performance may occur.

The 2012 Cochrane analysis, based on the only study (from Belarus) that met their review criteria, concluded that 6 months of exclusive breastfeeding conferred no benefit (vs. 3 months of exclusive breastfeeding, followed by continued partial breastfeeding through 6 months) on cognitive ability or behavior at 6.5 years of age.

NONINFECTIOUS RISKS

Human milk is the optimal form of nutrition for healthy term infants in almost all situations. The failure to initiate lactation properly during early breastfeeding 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. The Baby Friendly Hospital Initiative (BFHI) is a worldwide effort by the WHO and United Nations Children’s Fund to promote best breastfeeding practices in delivery hospitals by following 10 important steps (www.unicef.org/programme/breastfeeding/baby.htm).

There are very few absolute contraindications to breastfeeding. The most important reasons for cessation of breastfeeding include certain maternal medications and infection. Some circumstances have been identified in which continued breastfeeding should be conducted with caution to protect the infant. Infants with inherited metabolic diseases may also require alternative forms of nutrition instead of breast milk: neonates with galactosemia caused by deficiency of galactose-1-phosphate uridyltransferase should receive lactose-free milk (lactose is a glucose-galactose disaccharide); infants with phenylketonuria may receive some human milk to support their requirement for phenylalanine, and this may be best with concurrent blood levels of phenylalanine with alternative support of specially prepared commercial milks. Mothers who have received radionuclides for diagnostic or therapeutic purposes should use alternative forms of nutrition for the days to weeks required for these compounds to be eliminated, as should mothers receiving certain chemotherapeutic and immunosuppressive agents and actively using drugs of abuse, including amphetamines, cocaine, heroin, and phencyclidine. Breastfeeding and the use of cannabis substances is concerning, given reports of neurodevelopmental impairment, but it is not clear when the critical windows of exposure are.

Low-level maternal exposure to environmental chemicals and tobacco smoking should
be avoided as much as possible but is not a contraindication to breastfeeding.

Antimicrobial agents taken by mothers only rarely represent a contraindication to breastfeeding. As first principles, antimicrobials that may be safely given to infants may be safely given to their lactating mothers, and blood concentrations that may be achieved through breast-milk ingestion are lower than therapeutic doses used in infants. Breastfeeding by mothers receiving chloramphenicol is contraindicated because its use may be associated with fatal complications in newborn infants. The effects of metronidazole are uncertain, but to minimize exposure to this drug, which is mutagenic in bacteria, mothers receiving single-dose therapy should discontinue breastfeeding for 12 to 24 hours. Excretion of antibiotics in human milk is also discussed in Chapter 37. The reader is also referred to the leading source of up-to-date information on maternal medications and breastfeeding, called LactMed (see LactMed tab at http://toxnet.nlm.nih.gov/).

Several instances of specific nutrient deficiencies in breastfed infants have been described, specifically related to lack of vitamin K, vitamin D, vitamin B12, 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 is almost exclusively reported in breastfed infants who did not receive vitamin K or received only one dose at birth. This can be successfully treated with vitamin K. Endogenous vitamin D production versus dietary vitamin D is the primary means by which humans naturally obtain vitamin D. Breastfed infants are dependent on their maternal vitamin D stores. Cases of rickets in breastfed infants have been reported, particularly during winter among infants not exposed to the sun. Vitamin D requirements can be best met with infant supplementation with oral vitamin D, although maternal high-dose vitamin D supplementation has also been suggested. Mothers who practice unusual dietary habits, such as strict vegetarianism, may have reduced levels of vitamin B12 and folic acid in their milk, and deficiencies in breastfed infants of such mothers have been reported. Deficiency of carnitine, a key amino acid responsible for fat metabolism, also has been reported to result in clinical symptoms in breastfed infants in mothers ingesting unusual diets. However, these concerns can best be addressed in almost all cases by counseling mothers regarding nutritional practices and by the provision of supplemental vitamins and other micronutrients when appropriate; this is the case in the developed world and even more so in the developing world, where the untoward consequences of not breastfeeding are particularly great.

Management of hyperbilirubinemia associated with breastfeeding has been an area of some controversy in the past. Two primary mechanisms occur in the development of jaundice with breastfeeding. Not enough breast-milk jaundice occurs early in lactation when milk supply does not meet fluid requirements of the infant. This can be accompanied by slower gastrointestinal transit that exacerbates the uptake of bilirubin through the enterohepatic circulation. Breast-milk jaundice occurs later in the first week of lactation and is suggested to be due to the several components in human milk, including progesterone metabolites, free fatty acids, inflammatory cytokines, and growth factors. Some recent evidence suggests a role for commensal bacteria, such as bifidobacteria, in protecting against the development of breast-milk jaundice. Present recommendations are for continued breastfeeding with efforts to increase the volume of milk ingested, with the provision that with severe hyperbilirubinemia, a brief interruption of breastfeeding might be appropriate.

INFECTIOUS RISKS

Human milk may contain infectious agents that are secreted into the milk, enter milk during lactation, or are acquired when milk is improperly collected, stored, and later fed to her infant. Formal training and evaluation of breastfeeding practices by trained caregivers is the best way to reduce these risks; routine culture or heat treatment of a mother’s milk even when it is stored and later used to feed her infant is not cost-effective.

Stored milk is now routinely used to feed infants when their mothers are not able to breastfeed directly because of work or travel constraints or when an infant is premature or otherwise unable to breastfeed effectively. Inadvertent feeding of stored milk from other than the birth mother has occurred in nurseries. If this occurs, the AAP recommends that this be handled in the same manner as if accidental exposure to blood or other body fluids has occurred (see Chapter 35 for additional information).

In the United States, the Human Milk Banking Association of North America (www.hmbana.org/) collects human donor milk for the purpose of administration to infants whose mother’s milk is not available or adequate. Members of this association follow guidelines formulated in consultation with the U.S. Food and Drug Administration (FDA) and Centers for Disease Control and Prevention (CDC). These guidelines help to assure that donors are screened for transmissible infectious agents and that the milk is carefully collected, processed, and stored. Using these practices, donor milk is collected and pooled, subjected to Holder pasteurization (62.5° C) for 30 minutes, which reliably kills bacteria and inactivates HIV and cytomegalovirus (CMV), and eliminates or substantially reduces the amounts of other viruses. The pooled milk is then tested to assure that it meets standards and frozen for later distribution and use.

Bacterial Infections

Transmission of bacterial pathogens, including Staphylococcus aureus, group B streptococci, mycobacteria, and other species may occur through breastfeeding (Table 5-3) Mastitis and breast abscesses may be associated with substantial concentrations of bacteria in the mother’s milk. In general, feeding an infant from a breast affected by an abscess is not recommended. Infant feeding on the affected breast may be resumed, however, 24 to 48 hours after drainage and the initiation of appropriate antibiotic therapy. Mastitis usually resolves with appropriate antimicrobial therapy and with continued lactation, even if feeding from the affected breast is temporarily interrupted. In both of these conditions, feeding from the unaffected breast need not be interrupted.

Mothers with active tuberculosis should refrain from breastfeeding for at least 2 weeks or longer after institution of appropriate treatment if they are considered contagious.
This recommendation also applies to the uncommon situation where mastitis or breast abscess is caused by Mycobacterium tuberculosis.  

**Viral Infections**

Viruses that have been detected in human milk include CMV, hepatitis B (HBV) and hepatitis C (HCV) viruses, herpes simplex virus (HSV), HIV-1, human T-lymphotropic virus type 1 (HTLV-1) and type 2 (HTLV-2), rubella virus, and West Nile virus (see Table 5-3). Whether varicella virus is secreted into human milk is unknown. Although some of these viruses present a risk to infant, for most but not all, the benefits of breastfeeding to the infant are greater than the risk.

**Cytomegalovirus Infection.** Cytomegalovirus infection is a common perinatal infection. The virus is shed in the milk in about 25% of infected mothers. Although breastfeeding from infected mothers may result in seroconversion in up to 70% of breastfeeding neonates, the infection often is not associated with clinical symptoms of disease. Very-low-birth weight infants (born at <1500 g), however, may exhibit evidence of clinical disease, with thrombocytopenia, neutropenia, or hepatosplenomegaly seen in 50% of those infected through breastfeeding. The decision to breastfeed a premature baby by an infected mother should be based on weighing the potential benefits of human milk versus the risk of CMV transmission.

**Hepatitis B Virus Infection.** Hepatitis B surface antigen (HBsAg) has been detected in milk of HBV-infected mothers. Nevertheless, breastfeeding 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 initial dose of hepatitis B vaccine within 12 hours of birth, followed by the recommended series of hepatitis B vaccine without any delay in the institution of breastfeeding.

**Hepatitis C Virus Infection.** The RNA of HCV and antibody to HCV have been detected in the milk from infected mothers. Transmission by means of breastfeeding, however, has not been documented in anti-HCV–positive, anti-HIV–negative mothers but is a theoretical possibility about which these mothers should be informed before deciding whether they will breastfeed. According to current guidelines, HCV infection is not a contraindication to breastfeeding, although mothers with cracked or bleeding nipples should consider refraining from breastfeeding until they have healed.

**Herpes Simplex Virus.** Herpes simplex virus transmission directly from maternal breast lesions to their infants has been demonstrated. Women with lesions on one breast may feed from the other unaffected breast, making sure that lesions on the other breast or on other parts of the body are covered and using careful hand hygiene.

**Human Immunodeficiency Virus.** A number of studies have demonstrated HIV in milk. 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 reaction in greater than 70% of samples from HIV-seropositive lactating women. Transmission of HIV through breastfeeding may account for up to one third of all HIV infections in infants globally, with risk of transmission being approximately 1.5% when breastfeeding continues beyond the first year of life. The risk of postnatal HIV transmission appears to be constant throughout the first 18 months of life; thus risk is cumulative as duration of breastfeeding increases. Risk of transmission via breast milk is greater when maternal HIV infection is acquired during lactation; when viral load is greater or maternal disease is more advanced; when babies are both breastfed and formula fed; when the mother has bleeding or cracked nipples, mastitis, or a breast abscess; and when the infant has thrush or certain other coinfections (see Chapter 21).

Current recommendations from the AAP and other authorities 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 breastfeed their infants nor to donate milk. A recent report found that highly active antiretroviral therapy (HAART) administered during pregnancy or postpartum suppresses HIV RNA, but not DNA, in breast milk. At present, the AAP recommends that infants of HIV-infected mothers in the United States receiving HAART should not be breastfed.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Transmission</th>
<th>Disease</th>
<th>Intervention</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytomegalovirus</td>
<td>+</td>
<td>VLBW infants</td>
<td>Consider risk/ benefit</td>
</tr>
<tr>
<td>Hepatitis B virus</td>
<td>+</td>
<td>+</td>
<td>HBIG/HB vaccine</td>
</tr>
<tr>
<td>Hepatitis C virus</td>
<td>HIV-positive</td>
<td>only</td>
<td>See text</td>
</tr>
<tr>
<td>Herpes simplex virus</td>
<td>+</td>
<td>+</td>
<td>See text</td>
</tr>
<tr>
<td>Human immunodeficiency virus</td>
<td>+</td>
<td>United States: Do not</td>
<td>See text</td>
</tr>
<tr>
<td>HTLV-1</td>
<td>+</td>
<td>±</td>
<td>United States: Do not</td>
</tr>
<tr>
<td>HTLV-2</td>
<td>+</td>
<td>±</td>
<td>United States: Do not</td>
</tr>
<tr>
<td>Rubella virus</td>
<td>+</td>
<td>0</td>
<td>None</td>
</tr>
<tr>
<td>West Nile virus</td>
<td>±</td>
<td>±</td>
<td>None</td>
</tr>
<tr>
<td>Group B streptococci</td>
<td>+</td>
<td>±</td>
<td>See text</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>+</td>
<td>±</td>
<td>See text</td>
</tr>
<tr>
<td>Mycobacterium tuberculosis</td>
<td>+</td>
<td>+</td>
<td>See text</td>
</tr>
</tbody>
</table>

HB, Hepatitis B; HBIG, hepatitis B immunoglobulin; HTLV, human T-lymphotropic virus; VLBW, very low birth weight.

*In many other parts of the world, the benefits of breastfeeding often outweigh the risks of alternative methods of infant feeding. See text for discussion of risk versus benefit in other parts of the world.
Despite the potential risk of HIV infection in infants of HIV-infected breastfeeding mothers, consideration of cessation of breastfeeding must be balanced against the other beneficial effects described in this chapter. Thus, in areas of the world where infectious diseases and malnutrition are important causes of death early in life, the beneficial effects of breastfeeding often outweigh the potential risk of HIV transmission through breastfeeding. Studies in such settings have shown that HIV-free survival at 7 months of age is similar in exposed infants who were breastfed or formula fed from birth. Studies in Africa demonstrate that exclusive breastfeeding in the first 6 months of life reduces the risk of HIV transmission compared with mothers who supplement breastfeeding with other foods and milk sources. Thus, in areas of the world where the burden of infectious diseases and malnutrition is high and where alternatives to breast milk that provide adequate nutrition are not acceptable, affordable, feasible, and safe, the WHO recommends exclusive breastfeeding for the first 6 months of life, followed by complementary foods and breastfeeding through 12 months of age, for women whose HIV status is unknown and for women known to have HIV, accompanied in the latter group by postnatal infant or maternal antiretroviral prophylaxis to reduce HIV transmission during breastfeeding. The WHO policy also stresses the need for continued support for breastfeeding by mothers who are HIV-negative, improved access to HIV counseling and testing, and government efforts to ensure uninterrupted access to nutritionally adequate human milk substitutes.

**Human T-Lymphotrophic Viruses Types 1 and 2.** Human T-lymphotropic virus type 1 is endemic in Japan, the Caribbean, and parts of South America. This infection can be transmitted from mother to infant, and this transmission occurs primarily through breastfeeding. HTLV-2 infection has been identified in some Native Americans and Native Alaskans and in some injection drug abusers in the United States and Europe. Mother-to-infant transmission of HTLV-2 has been demonstrated, although the frequency with which this occurs and the route of transmission are uncertain. Women in the United States who are known to be seropositive for HTLV-1 or HTLV-2 should not breastfeed. However, routine screening for HTLV-1 and HTLV-2 is not recommended.

**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 breastfeeding 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 breastfeeding infants also has been observed. Although West Nile virus can be transmitted in milk, the extent of transmission in humans remains rare. Most infants and children infected with the virus to date have been asymptomatic or have had minimal disease. Because the risk is uncertain, the AAP recommends that women in endemic areas may continue to breastfeed.

### Current Trends in Breastfeeding

Both international and national organizations have endorsed breastfeeding as the optimal means of feeding for the healthy term infant. In general, the percentage of mothers initiating breastfeeding in developing countries is 90% or greater, which both reflects the lack of other options and the severe health and economic consequences for bottle feeding their infants. In the United States, at one point in the early 1970s, the rate of breastfeeding initiation was as low as 25%. The rate of initiation has improved dramatically since that time. In the Infant Feeding Practices Survey (IFPS) II survey conducted by the FDA from 2005 to 2007, 83% of respondents initiated breastfeeding. More recent establishment of benchmarks on a new Perinatal Care Core Measure Set by the Joint Commission on Accreditation of Health Care Organizations (JCAHO) has led to their mandatory reporting. In 2012, California reported that any breastfeeding during hospitalization occurred at an average of 92.2% of healthy term infants. The CDC 2013 Breastfeeding Report Card found that 77% of new mothers breastfed their babies, up from 71% about a decade ago. In 2010, 49% of mothers were still breastfeeding when their child was 6 months of age, and 27% were still doing so when their child was 1 year of age. In 2000, rates were 35% and 16%, respectively. From 2007 to 2011, the percentage of facilities with at least 90% of infants receiving skin-to-skin contact after vaginal birth increased from 40.8% to 54.4%, and the percentage of facilities with at least 90% of mothers and babies staying together in the same room throughout the stay increased from 30.8% to 37.1%.

The growth of the BFHI worldwide has expanded to include more than 16,000 institutions (www.who.int/nutrition/topics/bfhi/en/), and this initiative has been shown to improve the establishment of exclusive breastfeeding. In the United States as of 2014, there are 175 hospitals in 41 states that have the BFHI designation. This represents an increase from 2.9% to 6.9% of births occurring in a Baby Friendly status.

Healthy People 2020 is a national program introduced by the U.S. Department of Health and Human Services that provides science-based, 10-year national objectives for improving the health of all Americans (www.healthypeople.gov). It has set a reachable target of 8.1% of births taking place in a Baby Friendly hospital by 2020.

Within the United States, a variety of demographic patterns appear to be associated with breastfeeding behavior. Older mothers, mothers with a college education, and higher-income mothers all are more likely to breastfeed. 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 breastfeed. The low rate of breastfeeding for mothers enrolled in WIC is of particular concern because that agency has a specific policy to encourage breastfeeding. Approximately half of all mothers of infants born
in the United States receive services through the WIC. In 2009, a new program called Special Supplemental Nutrition Program was introduced by WIC to encourage more breastfeeding by including greater food packages for those mothers who continue to breastfeed. Some data would suggest that these programs are associated with improved breastfeeding success.\textsuperscript{[335]} Many states continue to depend on formula manufacturer rebates to fund part of their WIC programs, creating an apparent conflict of interest. The disturbing part of the demographic pattern of breastfeeding in the United States is that the infants of lower socioeconomic status mothers, who would accrue the greatest health and economic benefits from breastfeeding, are those least likely to be breastfed.\textsuperscript{[336,337]}

Although demographic studies indicate who is breastfeeding, they do not explain the behavioral differences among groups of mothers. One of the more complete models designed to explain breastfeeding behavior includes components that address maternal attitudes and family, societal, cultural, and environmental variables.\textsuperscript{[338]} 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.\textsuperscript{[339]} The husband, in particular, appears to have a strong positive influence, whereas the mother’s mother may have a negative influence on the breastfeeding decision. Social support appears to be different among ethnic groups, as are maternal attitudes; such differences may provide one explanation for differences in breastfeeding behavior among ethnic groups.\textsuperscript{[340,341]}

### Summary and Conclusions

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, antiinflammatory, proinflammatory, and/or immunoregulatory functions; cytotoxicity for tumor cells; ability to mature and repair tissue damage; and receptor analogue functions, as well as other metabolic effects. The biologic activities of different milk components are summarized in Table 5-4.

The major antimicrobial effects of human milk are associated with milk immunoglobulin, especially the sIgA isotype, which makes up to 80% of all immunoglobulins in the human body (see Figure 5-1 and Table 5-2). Milk antibodies appear to provide protection against many intestinal pathogens, such as \textit{Campylobacter}, \textit{Shigella}, \textit{E. coli}, \textit{V. cholerae}, \textit{Giardia}, and rotavirus, and against respiratory pathogens such as respiratory syncytial virus. Milk antibodies also effectively neutralize toxins and a variety of human viruses. The role of small amounts of IgG and IgM in milk is uncertain. Lactoferrin, lysozyme, \(\alpha\)-lactalbumin, and other milk proteins,

<table>
<thead>
<tr>
<th>Table 5-4 Possible Role of Soluble and Cellular Factors Identified in Human Milk</th>
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<tr>
<td><strong>Factor</strong></td>
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<td>----------------------</td>
</tr>
<tr>
<td>Immunoglobulin (sIgA)</td>
</tr>
<tr>
<td>Other immunoglobulins</td>
</tr>
<tr>
<td>PMNs, macrophages</td>
</tr>
<tr>
<td>Lactoferrin</td>
</tr>
<tr>
<td>(\alpha)-Lactalbumin</td>
</tr>
<tr>
<td><strong>CARBOHYDRATES</strong></td>
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<tr>
<td>Oligosaccharides</td>
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<tr>
<td>Glycoconjugates</td>
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<tr>
<td>Glycolipids</td>
</tr>
<tr>
<td>Lipid and fat globules</td>
</tr>
<tr>
<td><strong>NUCLEOTIDES</strong></td>
</tr>
<tr>
<td>Defensins</td>
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<tr>
<td>Lysozymes</td>
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<tr>
<td><strong>CYTOKINES, CHEMOKINES</strong></td>
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<tr>
<td>TGF-(\beta)</td>
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<td>IL-10</td>
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<td>IL-1</td>
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<td>TNF-(\alpha)</td>
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<td>IL-6</td>
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<tr>
<td>IL-7</td>
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<tr>
<td>Prostaglandins</td>
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<tr>
<td>Leptin</td>
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<tr>
<td>Antiproteases</td>
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<tr>
<td>Other growth factors</td>
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<tr>
<td>sTLR2, sCD14</td>
</tr>
</tbody>
</table>

+ to ++, Minimal-to-moderate effect; –, no effect; IL, interleukin; PMNs, polymorphonuclear neutrophils (leukocytes); sCD14, soluble CD14 (protein marker); sIgA, secretory immunoglobulin A; TGF-\(\beta\), transforming growth factor-\(\beta\); sTLR2, soluble Toll-like receptor 2; TNF-\(\alpha\), tumor necrosis factor-\(\alpha\).
along with lipids, also contribute to the antimicrobial and immunomodulatory properties of human milk. Milk carbohydrates, particularly milk oligosaccharides, assist in modulating the microbial milieu of the infant’s gut, acting largely as complex and diverse prebiotics. Milk also contains large numbers of cytokines, chemokines, growth factors, soluble Toll-like receptors, and CD14, which may modulate inflammatory and immunologic responses in the gut, although this remains to be clearly established. Polymorphonuclear neutrophils, macrophages, lymphocytes, and epithelial cells are observed in human milk, but their functions in milk are unknown; it is possible that their primary task is the antimicrobial defense of the mammary gland itself.

The passive transfer of the diversity of maternal biologic experiences to the neonate through the process of breastfeeding represents an essential component of the survival mechanism in the mammalian neonate. For millions of years, maternal products of lactation delivered through breastfeeding have been the sole source of nutrition and immunity during the neonatal and early infancy period for all mammals, including the human infant. During the past few centuries, however, human societies have undergone remarkable changes that have had a major impact on basic maternal-infant interaction, breastfeeding, and on our environment. 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. These changes have had a profound impact on human homeostatic mechanisms that, at the same time, are opening up new insights into the importance of breastfeeding in the developing human neonate.

Comparative analysis of natural (traditional) forms of breastfeeding and artificial feeding modalities has demonstrated clearly that natural breastfeeding is associated with significant reduction in infant mortality and morbidity, protection against acute infectious diseases, and possible protection against allergic disorders and autoimmune disease: acute and chronic inflammatory disorders; obesity, diabetes mellitus, and other metabolic disorders; and development of certain malignancies later in life. This information has been reviewed by Hanson in an elegant monograph and by others. Despite the overwhelmingly protective role attributed to natural breastfeeding 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-1, HTLV-2, CMV, and possibly other pathogens highlights potential hazards of breastfeeding in some clinical situations. Nevertheless, we conclude that the development of lactation, a hallmark of mammalian evolution, is designed to enhance the survival of the neonate of the species through a remarkable spectrum of immediate and long-term protective functions.

References


