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Immunology of Human Milk and Host Immunity

In 1891, it was discovered that immunity could be transmitted through breast-feeding in experimental animals.[1] In the 1920s,[2-4] the incidence and severity of diarrheal diseases were found to be much lower in breast-fed than cow's milk–fed infants. Those clinical observations were confirmed repeatedly,[5-8] and it was ascertained that breast-feeding protected against many bacterial and viral enteric pathogens.[7-13] Three explanations for the protection were advanced. The first explanation: Because human milk is less contaminated with enteropathogens than formula feedings, breast-fed infants are exposed to fewer infectious agents. The second: Increased birth-spacing as a result of the contraceptive effects of lactation decreases the number of children who transmit common contagious agents to susceptible siblings.[14] The third: Breast-fed infants are rarely in group-care facilities and are thus less exposed to communicable infections.

These propositions did not, however, entirely explain the protection provided by breast-feeding, for breast-fed infants are asymptomatic even when they are exposed to bacterial enteropathogens such as *Shigella* that contaminate the mother's nipples, colostrum, and milk.[5] Further, breast-fed infants are more resistant to common respiratory infections.[15-18] Much of the protection is provided by a complex immunologic system in human milk. Furthermore, antimicrobial agents, which were the first parts of the immunological system to be recognized,[19-21] have certain shared features (Box 158-1). The inverse relationship between the quantities of many agents in human milk and the production of these agents by the infant suggested a relationship between the development of the infant's immune system and the ability of the lactating mammary gland to produce the immune factors.[22-24]

After the concept of an immune system in human milk was formed,[19] antiinflammatory[21,25,26] and immunomodulating agents[21,26] were discovered to be part of that system. Thereafter the evolutionary relationships between the immune system in human milk and the development of the immune system in the infant were appreciated.[22-24]

1. Certain postnatal developmental delays in the infant's immune system are compensated by the transmission of the same agents in human milk.
2. Other postnatal delays in components of the immune system in the infant are compensated by dissimilar agents in human milk.
3. Some agents in human milk initiate or augment functions poorly expressed in the infant.
4. Many antimicrobial agents in human milk act synergistically.
5. Some agents in human milk alter the physiological state of the alimentary tract from one suited for fetal life to one that is appropriate for extrauterine life.
6. Antibodies in human milk are produced by plasma cells that transformed from B cells that originate in the maternal intestines and bronchi.
7. Specialized living leukocytes are found in human milk.
8. Defense agents in human milk protect against microbial pathogens without provoking inflammation in the infant.
9. Some agents in human milk inhibit inflammation.
10. Some agents in human milk are immunoregulators.
11. Some agents in human milk are antineoplastic.
12. Defense agents in human milk resist enzymatic digestion and thus function in the recipient's GI tract.
13. Certain defense agents are created in the infant's GI tract by partial digestion of substrates in milk.
14. When defense agents in human milk interact with some pathogens, the infant develops specific adaptive immune responses but no symptomatic infections. Such a sheltered immunization is similar to immunizing with an attenuated microbial pathogen.
15. Agents in human milk augment the growth of commensal enteric bacteria adapted to infants that produce compounds that protect against bacterial pathogens and convey other immunologic benefits.[27,28]
16. There is often a reciprocal relationship between the defense agents that are transmitted in milk and those transmitted during fetal life via the placenta.

BOX 158-1

Features of Antimicrobial Agents in Human Milk

- Heterogeneous array of biochemical agents and live leukocytes
- Agents not well represented in other mammalian milks used to feed human infants
- Common to mucosal sites
- Adapted to persist in the gastrointestinal tract
- Often inhibit or kill microbial pathogens synergistically
- Often multifunctional
- Do not trigger inflammation
- Production often inversely related to the production in the infant

ANTIMICROBIAL FACTORS

The physical features, functions, and quantities of antimicrobial agents in human milk are summarized in Table 158-1. The proteins will first be considered.

Antibodies

IgM, IgG, IgD, and IgA are in human milk. IgM concentrations are much lower in human milk than in serum.[29] IgM molecules in blood and milk are pentamers. However, unlike serum IgM, human milk IgM is bound to secretory component.

The concentrations of IgG in human milk are lower than IgM in human milk and are much less than IgG in human serum.[29] All IgG subclasses are in human milk,[30] but the relative proportion of IgG4 is higher in human milk than serum.[30] Very little IgD is present in human milk.[31] IgE, the immunoglobulin responsible for immediate hypersensitivity, is essentially absent.[32] In contrast, IgA in the form of secretory IgA comprises more than 95% of human milk immunoglobulins.[29] Although some trimers and tetramers of IgA are in human milk, most secretory IgA consists of two identical IgA monomers united by a 15-kD polypeptide called the joining chain and complexed to a 75-kD glycoprotein, the secretory component.[33-35] Secretory IgA is assembled when dimeric IgA produced by plasma cells in the stroma of the mammary gland binds to the first domain of polymeric immunoglobulin

TABLE 158-1

Principal Antimicrobial Agents in Human Milk

Agents	Main Functions
Proteins and peptides	Microbiostatic and microbiocidal
Lactoferrin	Lyses siderophilic pathogens by chelating Fe^{+++}
Lysozyme	Lyses certain bacteria by degrading exposed cell wall peptidoglycans
SIgA	Binds adherence sites, toxins, virulence factors on intestinal and respiratory pathogens
α-Lactalbumin	Kills *Streptococcus pneumoniae*
CCL28	Kills *Candida albicans* and many gram-positive and gram-negative bacteria
MUC1	Blocks binding of S-fimbriated *Escherichia coli* to epithelium
Lactadherin	Blocks attachment of rotavirus to mucosa
C3 and fibronectin	Augment phagocytosis of pathogens
β-Defensin-1 and α-Defensin-1,2, and 3	Lyses bacteria and inhibits HIV-1, respectively
Oligosaccharides	Receptor analogues inhibit binding to epithelium
Glycoconjugates	
GM_1 gangliosides	*Vibrio cholerae* and *E. coli*
Globotriaosylceramide Gb3	Shigatoxin β subunits
Fucosyl oligosaccharides	*E. coli* stable toxin, *Campylobacter jejuni*
G1cNAc(β1-3) Gal-disaccharide	*Streptococcus pneumoniae*
Sulfated glycolipids	HIV-1
Glycoaminoglycans	HIV-1
Lewis X component	HIV-1
Monoglycerides and fatty acids from digested milk lipids	Inactivate enveloped viruses, certain bacteria, *Giardia lamblia* and *Entameoba histolytica*

TABLE 158-2

Antigen-binding Repertoire of SIgA Antibodies in Human Milk

Bacteria and Their Products	Viruses	Fungi	Parasites	Autoantigens
Escherichia coli	Adenoviruses	*Candida* sp	*Giardia lamblia*	DNA
Helicobacter pylori	Cytomegalovirus			RNA
Clostridium botulinum	Polioviruses and other enteroviruses			CCR5
Klebsiella pneumoniae	Rotaviruses			
Campylobacter sp	RSV			
Shigella sp				
Salmonella sp				
Vibrio cholerae				
Streptococcus pneumoniae				
Group B *Streptococcus*				
Haemophilus influenzae				

receptors on the basolateral surface of epithelial cells.[35] The complex is internalized, the original cytoplasmic part of the receptor is cleaved off, and the remaining assembled protein is transported across the cell into milk.

Secretory IgA antibodies in human milk are directed principally against enteric and respiratory pathogens (Table 158-2). The precursors of the cells that generate those antibodies originated at those mucosal sites. In fact, those precursors are released from the maternal mucosal sites because of immunogen-triggered events.[36] Hormonal stimulation during lactation causes antigen-stimulated B cells from Peyer's patches of the lower small intestinal tract to switch from the IgM to the IgA isotype and migrate to the mammary gland.[37,38] A similar B-cell pathway links bronchial lymphoid tissues to the mammary gland.[39] The details are as follows.

After antigenic stimulation, cytokines from mononuclear leukocytes in Peyer's patches induce local B cells to switch from IgM to IgA.[40,41] The IgA^+ B cells then migrate sequentially into local intestinal lymphatic channels and lymph nodes, the cisterna chyli, the thoracic duct, and the vascular circulation. During lactation, they home to the stroma of the mammary gland. Experimental evidence suggests that the chemokine CCL28 and its receptor CCR10 are crucial to this process: (1) CCL28 is up-regulated in the murine mammary gland epithelium during lactation;[42] (2) most IgA^+ B cells in the enteromammary gland pathway display CCR10;[43] and (3) IgA^+ B cells that are $CCR10^+$ move toward CCL28, which is expressed by mammary gland epithelium during lactation.[43]

IgA^+ B cells in the mammary gland differentiate into IgA-producing plasma cells that remain in the lamina propria of the organ. IgA dimers produced by mammary gland plasma cells principally contain λ-light chains, whereas κ-light chains predominate in serum immunoglobulins.[44] IgA dimers released from those plasma cells bind to polymeric immunoglobulin receptors on the basolateral external membranes of mammary gland epithelial cells.[33,34,45,46] The resultant receptor-dimeric IgA complex is transported to the apical side of the cell where the intracytoplasmic portion of the receptor is removed. The remaining molecule, secretory IgA, is secreted into milk. Thus enteromammary and bronchomammary pathways protect the immunologically immature infant against pathogens that the mother encounters (see Table 158-2). This is important since secretory IgA antibodies and the antigen-binding repertoire of immunoglobulins are developmentally delayed during early infancy.[47] Also, some secretory IgA molecules in human milk are antiidiotypic antibodies that function as immunogens.[48]

Certain secretory IgA antibodies in human milk are directed against the autoantigen CCR5, which is not only a chemokine receptor but is also the co-receptor for R5-tropic strains of HIV-1 that permits macrophages and immature dendritic cells to become infected with HIV-1.[49] The antibodies from uninfected as well as HIV-1 infected women bind to the second extracellular loop of CCR5 and thus block infection of macrophages and dendritic cells by HIV-1. In addition, secretory IgA antibodies to DNA enzymatically cleave DNA.[50,51] Therefore the DNA is recognized as an autoantigen and a substrate by the antibody enzyme and may hydrolyze free nucleic acids in the recipient's intestinal and respiratory tracts.

Secretory IgA in human milk also facilitates mucosal immunization against enteric microorganisms.[52] Secretory IgA adheres selectively to cells in Peyer's patches. Subsequently, the antibodies are transported to the underlying lymphoid tissue. There they bind to and are internalized by dendritic cells in the subepithelial dome region of Peyer's patches. If foreign enteric protein antigens are coupled to these antibodies, they will be processed by dendritic cells and then presented to T cells. Once the processed antigens are presented to TcRs on helper T cells, the T cells are activated. The activated T cells secrete cytokines that activate local cytotoxic T cells or stimulate local IgM^+ B cells to proliferate and switch their isotype to IgA. That eventually leads to the production of secretory IgA at mucosal sites.

The quantity of secretory IgA in human milk declines as lactation proceeds, but considerable secretory IgA is transmitted to the infant throughout breast-feeding.[54-57] Concentrations of secretory IgA in human milk are highest in colostrum[50] and gradually plateau later in lactation to about 1 mg/mL.[56] The approximate mean daily intake of secretory IgA in healthy full-term

breast-fed infants is 125 mg/kg/day at 1 month and 75 mg/kg/day by 4 months.[57]

The pattern of immunoglobulins in human milk differs from the pattern in other mammals except closely related primates.[25] For example, the dominant immunoglobulin in bovine colostrum, IgG, is absorbed into the calf's blood. IgG production is developmentally delayed in those newborns. Without colostrum, they remain IgG-deficient and very susceptible to intestinal infections.

Secretory IgA resists intestinal proteases such as pancreatic trypsin.[58] Bacterial proteases attack the hinge region of IgA1,[59] but the second subclass, IgA2, is resistant to those proteases and is disproportionally increased in human milk.[30] Furthermore, secretory IgA antibodies against bacterial IgA proteases are present in human milk.[59] In that respect, the amount of secretory IgA excreted in stools of low birth weight infants fed human milk is about 30 times that in infants fed a cow's milk formula.[60] In addition, urinary excretion of secretory IgA rises in infants fed human milk.[61,62] It is unlikely that the antibodies are from human milk because there is no mechanism for the their transport from the gastrointestinal tract to the blood. Thus human milk may stimulate the infant to produce the antibodies and transport them into the urinary tract.

Free Secretory Component

Free secretory component is also secreted into human milk, and this peptide inhibits the adherence of certain enterobacteria to epithelial cells.[63,64]

Lactoferrin

Lactoferrin is a single-chain glycoprotein with two globular lobes, each of which displays a binding site for ferric iron.[65] In 90% of lactoferrin in human milk,[66] iron-binding sites are available to compete with siderophilic bacteria and fungal enterochelin for ferric iron.[67-69] The iron chelation disrupts the proliferation of those pathogens. Lactoferrin also kills by damaging outer membranes of many gram-positive and gram-negative bacteria.[70-72] This process is conducted by a peptide, lactoferricin, which is usually comprises 18 amino acid residues from the N-terminal region of lactoferrin that are formed by gastric pepsin digestion.[71,72] Furthermore, lactoferrin inhibits certain viruses by a chelation-independent mechanism[73-76] and interferes with the adhesion of *Escherichia coli* to epithelial cells.[68]

The mean concentration of lactoferrin in human colostrum is between 5 and 6 mg/mL.[54] As the volume of milk production increases, the concentration falls to about 1 mg/mL at 2 to 3 months of lactation.[55] The mean intake of milk lactoferrin in healthy breast-fed full-term infants is about 260 mg/kg/day at 1 month and 125 mg/kg/day by 4 months.[57] Because human lactoferrin resists proteolysis[77] and the concentration of lactoferrin is much greater in human than bovine milk,[25] the excretion of lactoferrin in the stools is higher in infants fed human milk than in those fed a cow's milk formula.[60,78] The quantity of lactoferrin excreted in stools of low-birth-weight infants fed human milk is approximately 185 times that excreted by infants fed a cow's milk formula.[60] That estimate, however, may be too high because of immunoreactive fragments of lactoferrin in the stools of human milk-fed infants.[79] There is also a significant increment in urinary excretion of intact and fragmented lactoferrin as a result of human milk feedings.[62,79] Although the transport mechanism is unknown, the increase is a result of absorbed human milk lactoferrin and it fragments.[80]

Lysozyme

Lysozyme, a 15-kD single chain protein, lyses susceptible bacteria by hydrolyzing β-1,4 linkages between N-acetylmuramic acid and 2-acetylamino-2-deoxy-D-glucose residues in cell walls.[81] High concentrations of lysozyme are in human milk throughout lactation.[54-56] Longitudinal changes in lysozyme during lactation are unlike most other immune factors in human milk. The mean concentration of lysozyme is about 70 μg/mL in colostrum,[54] 20 μg/mL at 1 month and 250 μg/mL by 6 months of lactation.[55] The high content of lysozyme in human milk and its resistance to proteolysis lead to an eightfold increase in lysozyme in the stools of low-birth-weight infants fed human milk.[60] The urinary excretion of this protein is not increased in infants fed human milk.[62]

α-Lactalbumin

α-Lactalbumin is expressed only in the lactating mammary gland. A folding variant of the protein kills *Streptococcus pneumoniae* in vitro.[82] Furthermore, a molecular complex consisting of partially unfolded α-lactalbumin and oleic acid kills many types of tumor cells in vitro by several mechanisms including apoptosis and macroautophagy.[83,84] The protein invades tumor cells, depolarizes mitochondrial membranes, releases cytochrome c, exposes phosphatidyl serine, reduces caspase responses, and activates 20S proteasomes. α-Lactalbumin also translocates to tumor cell nuclei to cause chromatin disruption, loss of transcription, and nuclear condensation. The protein also kills certain skin papillomas and bladder cancers in situ. However, the in vivo antineoplastic action of the protein in human milk upon the recipient infant is undetermined.

CCL28

CCL28 is not only a chemokine; it also kills *Candida albicans* and many gram-positive and gram-negative bacteria. The killing is mediated by the 28 amino acid C-terminus of the molecule.[85]

MACROPHAGE MIGRATION INHIBITORY FACTOR

Macrophage inhibitory factor (MIF), a constituent of human milk,[86] is a proinflammatory cytokine that also up-regulates TLR-4[87] and aids in killing *Mycobacterium tuberculosis* in human macrophages.[88]

Fibronectin

Fibronectin facilitates the uptake of many particulates by mononuclear phagocytes. The in vivo effects of this opsonin in human milk[89] are unknown.

Complement

All components of the classical and alternative pathways of complement are in human milk, but their concentrations are much lower than those in serum.[90-92] Based upon in vitro experiments,[93] degradation products of C3 (C3b or C3bi) in human milk may augment phagocytosis of microbial pathogens in the infant's gastrointestinal or respiratory tracts.

Human Milk Mucin

Milk mucins are high-molecular-weight, highly glycosylated proteins.[94] Two thirds of mucin in human milk is bound to milk fat globule membranes. The concentration of mucin in human milk ranges between 50 and 90 mg/mL. Human milk fat globules and mucin from their membranes inhibit the binding of S-fimbriated *E. coli* to human epithelial cells.[94]

The most prominent mucin, MUC1, resists intragastric digestion in preterm infants.[95] Major fragments of MUC1 are in feces of breast-fed infants,[96] and mucins from such feces inhibit the adhesion of S-fimbriated *E. coli* to epithelial cells.[97]

Lactadherin

Human milk mucin was thought to defend against rotavirus in experimental mice,[98] but the protection turned out to be lactadherin,[99] a 49-kDa glycoprotein on milk fat globules that resists intragastric digestion.[95]

A protein analogous to human lactadherin, MFG-E8, is found on murine milk fat globules.[100] MFG-E8 on murine macrophages binds to phosphatidyl serine exposed on the outer membranes of apoptotic cells.[101,102] It also binds to α1β3 and α1β5 integrins on macrophages in the spleen and lymph nodes and elicited peritoneal macrophages via a tripeptide (RDG) motif within the second of its two EGF repeats. Because of the double linkage, apoptotic cells are phagocytized without inducing inflammation. Human lactadherin may have similar effects.

Low-Molecular-Weight Antimicrobial Peptides

In addition to antimicrobial peptides generated by partial digestion of lactoferrin, cysteine-rich, cationic low–molecular-weight peptides are in human milk including β-defensin-1,[103] which disrupts *E. coli,* and the α-defensins -1,-2, and -3 (HNP -1, -2, and -3)[104] and α-defensins, which inhibit HIV-1 replication and may interfere with postpartum transmission of HIV-1.[104]

Oligosaccharides and Glycoconjugates

Oligosaccharides in human milk are produced by mammary gland glycosyltransferases. Their concentrations in colostrum and mature milk are about 20 mg/dL and 12 mg/dL, respectively.[105] Many oligosaccharides are in human milk,[106] and they differ from those found in cow's milk. Although the quantities of total gangliosides in human and bovine milk are similar, the frequencies of each type of ganglioside in the milk of the two species are different. For example, greater amounts of monosialoganglioside 3 and GM_1 are found in human than in bovine milk.[106,107]

Because these agents resist enzymatic digestion, it was predicted that they would have nonnutritional functions. Indeed, some are receptor analogues that inhibit the in vitro binding of certain enteric or respiratory bacterial pathogens and their toxins to epithelial cells.[106,108,109] The chemistry of these compounds dictates the specificity of their binding. For example, GM_1 gangliosides are receptor analogues for *Vibrio cholerae* and *E. coli* toxins,[109] whereas the globotriaosylceramide Gb3 binds to b subunits of shigatoxin.[110] A fucosyloligosaccharide inhibits the stable toxin of *E. coli*,[111] whereas a different one inhibits *Campylobacter jejuni*.[112] Human milk oligosaccharides interfere with the attachment of *Haemophilus influenzae* and *S. pneumoniae* to respiratory epithelium,[113] and G1cNAc(β1-3) Gal-disaccharide subunits block attachment of *S. pneumoniae* to respiratory epithelium.[113]

The severity of *Campylobacter* or calcivirus enteritis in breast-fed infants is inversely proportional to concentrations of oligosaccharides in maternal milk that consist mainly of α-(1→2) oligosaccharides.[114] It is unclear whether the risk to other enteropathogens is related to quantitative variations in other oligosaccharides in human milk.

In addition, sulfated glycolipids, glycosaminoglycans,[115] and Lewis X component[116] in human milk inhibit in vitro infection by HIV-1. Polymers of Lewis X component interact with a dendritic cell–specific ICAM3-grabbing nonintegrin that facilitates the transfer of HIV-1 from dendritic cells to $CD4^+$ T cells. Consequently, gp120 on the envelope of HIV-1 is unable to bind to those T cells.

Animal experiments suggest that oligosaccharides and glycoconjugates in human milk protect against certain enteric bacterial infections.[117] In that regard, certain human milk oligosaccharides survive passage through the alimentary tract[118] and some are absorbed and then excreted into the urinary tract.[119] This may account for some protection by human milk against urinary tract infections.[120] Sugars in the glycoconjugates mucins, lactadherin, and secretory IgA also interfere with binding of bacterial pathogens to epithelial cells.[93,94,121]

In addition to the direct antibacterial effects of the carbohydrates in human milk, nitrogen-containing oligosaccharides, glycoproteins, and glycopeptides in human milk are growth promoters for lactobacilli and bifidobacilli.[122,123] For example, the growth-promoter activity associated with caseins may reside in the oligosaccharide moiety of those complex molecules.[123] These factors are responsible for the predominance of lactobacilli and bifidobacilli in the bacterial flora of the large intestine of breast-fed infants. The commensal bacteria produce large amounts of acetic acid, which suppress the multiplication of enteropathogens. The *Lactobacillus* strain GG may also aid in the recovery from acute rotavirus infections[124] and may enhance the formation of specific circulating antibodies during enteric infections.[29] In addition, enteric commensal bacteria may stimulate the production of IL-12[125] and low-molecular-weight antibacterial peptides such as defensins.[126] These latter defense mechanisms may contribute to the comparative paucity in stools of breast-fed infants of bacterial pathogens most often found in urinary tract infections such as P-fimbriated *E. coli*.[127]

Lipids

Fatty acids and monoglycerides generated by the enzymatic digestion of lipid substrates in human milk disrupt enveloped viruses.[128,129] These antiviral lipids may aid to prevent coronavirus infections of the intestinal tract[130] and defend against intestinal parasites such as *Giardia lamblia* and *Entameoba histolytica*.[131,132] Monoglycerides from milk lipid hydrolysis also inactivate certain gram-positive and gram-negative bacteria.[133] These lipids may act synergistically with one another and with antibacterial peptides.[134] Hydrolysis of milk lipids occurs in infants because of lingual lipase and the activation of human milk bile-salt stimulated lipase in the duodenum. Thus the products of lipid digestion may help defend breast-fed infants against enteric infections in the proximal gastrointestinal tract.

LEUKOCYTES IN HUMAN MILK

Living leukocytes are found in human milk,[135] and virtually all of them are activated.[136,137] In contrast to B cells that transform into plasma cells that remain sessile in the mammary gland, other leukocytes attracted to the site traverse the mammary epithelium and become part of milk secretions. The highest concentrations of leukocytes in human milk occur in the first few days of lactation ($1–3 \times 10^6$/mL). The several types of leukocytes and their major features are as follows.

Lymphocytes

The relative frequencies of T cells and B cells among lymphocytes in early human milk secretions are 83% and 6%, respectively.[136] The small number of natural killer cells in human milk[136] is in keeping with the low cytotoxic activity of human milk leukocytes.[138] The numbers of B cells in human milk are small because most B cells that enter the lamina propria of the mammary gland transform into sessile plasma cells.

Both $CD4^+$ (helper) and $CD8^+$ (cytotoxic/suppressor) T-cell subpopulations are present in human milk.[136] But compared with human blood T cells, the proportion of $CD8^+$ T cells in human milk is greater.[136] $CD4^+$ and $CD8^+$ T cells in human milk bear markers of cellular activation including CD45RO and HLA-DR.[136] Moreover, a greater percentage of human milk $CD8^+$ T cells express the intestinal homing receptor, CD103, and the mucosal homing receptor, CCR9, than those found in blood.[139]

T cells in human milk produce certain cytokines such as interferon-γ.[140] Additional cytokines are produced by human milk leukocytes,[141] but the extent of their production and secretion is undetermined.

Neutrophils and Macrophages

Neutrophils and macrophages are the dominant leukocytes in human milk. Both types of cells are laden with milk fat globules and other membranes that have been phagocytized. Because of

these intracytoplasmic bodies, they are difficult to recognize by common staining methods. They can be identified however by the presence of myeloperoxidase in neutrophils),[141,142] nonspecific esterase in macrophages,[141,142] or CD14 or MHC class II molecules in macrophages.[141] Both types of cells in human milk are phagocytic. A respiratory burst occurs in milk macrophages after stimulation.[142,143] Superoxide anion generation by those cells is more marked after exposure to mannose-receptor ligands.[143] The macrophages also process and present antigens to T cells.[144]

In contrast to blood neutrophils, human milk neutrophils do not increase adherence, polarity, directed migration,[145] or deformability after exposure to chemoattractants.[146] These alterations may be due to agents in human milk. For example, the decreased calcium influx found in human milk neutrophils is duplicated by incubating blood neutrophils in human milk.[147]

Unlike human milk neutrophils, the motility of macrophages in human milk is increased compared with blood monocytes.[148] The features of these cells in human milk are likely due to cellular activation, because they display phenotypic features of activation, including an increased expression of CD11b/CD18 and a decreased expression of CD62L.[137] The activation may be due in part to ingestion of milk fat globules or other membranous materials in human milk.[137]

Potential In Vivo Effects

The in vivo fate and role of human milk leukocytes in the infant are poorly understood. Only a small numbers of memory T cells are detected in infancy[149]; thus maternal memory T cells in milk may compensate for that developmental delay in the infant. There is evidence from experimental animal studies that milk lymphocytes enter tissues of the neonate,[141] but that has not been demonstrated in humans. In addition, cellular immunity may be transferred by breast-feeding.[150]

ANTI-INFLAMMATORY PROPERTIES

Inflammatory agents and systems that give rise to them are poorly represented in human milk.[25,26] These include (1) the coagulation system; (2) the kallikrein-kininogen system; (3) most complement components; (4) IgE; (5) basophils, mast cells, eosinophils; and (6) cytotoxic lymphocytes. Certain proinflammatory cytokines (see subsequent discussion) are found in human milk, but there is no clinical evidence that they generate inflammation in the recipient.

Human milk also contains many antiinflammatory agents[25,26] including (1) factors that promote epithelial growth and thus strengthen mucosal barriers; (2) antioxidants; (3) agents such as lactoferrin that interfere with some complement components[26,151]; (4) enzymes that degrade mediators of inflammation; (5) protease inhibitors[152]; (6) agents that bind to substrates such as lysozyme to elastin[153] and lactoferrin to the toxic moiety of lipopolysaccharide, lipid A[154]; (7) cytoprotective agents such as prostaglandins E_1, E_2, and $F_2\alpha$,[155,156]; and (8) agents that inhibit the functions of inflammatory leukocytes[26] such as binding of LPS to CD14 by lactoferrin[154] and the down-regulation by lactoferrin of LPS-induced cytokine production by mononuclear phagocytes via NFκB[157] (Table 158-3). Furthermore, many antiinflammatory agents in human milk are adapted to survive in the alimentary tract.

The main antioxidants in human milk include an ascorbate-like compound,[158] uric acid,[158] α-tocopherol,[159,160] and β-carotene.[159,160] Blood levels of α-tocopherol and β-carotene are higher in breast-fed than formula-fed infants not supplemented with those agents.[160] The in vivo action of those agents in human milk is unknown.

Mucosal growth factors in human milk include epithelial growth factor,[161] lactoferrin,[162] cortisol,[163] polyamines,[164,165] and peptides produced from α-lactalbumin and lysozyme.[166] Other hormones and growth factors in human milk[167] also affect the growth and differentiation of epithelium and thus limit the penetration of antigens and pathogens into the intestines. In that respect, the biophysical and biochemical organization and functions of mucosal barriers in adults and neonates are different.[168,169] Furthermore, their maturation may be accelerated by human milk.[170,171]

TABLE 158-3

Antiinflammatory Agents in Human Milk

Categories	Examples
Cytoprotectives	Prostaglandins E2, F2α
Epithelial growth factors	EGF, lactoferrin, polyamines
Maturational factors	Cortisol
Enzymes that degrade inflammatory mediators	PAF-acetylhydrolase
Binders of enzymes	α1-Antichymotrypsin
Binders of substrates of enzymes	Lysozyme to elastin
Binders of toxins	Lactoferrin to lipid A of LPS
Modulators of inflammatory leukocytes	IL-10, TGF-β1
Antioxidants	Uric acid, β-carotene, ascorbate

Enzymes in human milk degrade inflammatory mediators. Platelet-activating factor (PAF) plays a role in an intestinal injury in rats induced by endotoxin and hypoxia.[172] Human milk, however, contains an acetylhydrolase that degrades PAF,[173] whereas the production of human PAF-acetylhydrolase is developmentally delayed.[174] As a result of these agents in human milk, intestinal permeability is lessened in breast-fed infants.[175-177]

IMMUNOMODULATING AGENTS

Observations suggest that immunomodulating agents in human milk are important:

1. Epidemiologic investigations suggest that older children who are breast-fed during infancy may be at less risk for developing certain chronic diseases mediated by immunologic, inflammatory, or oncogenic mechanisms. The diseases are type 1 diabetes mellitus,[178-180] type 2 diabetes mellitus,[181] lymphomas,[182,183] acute lymphocytic leukemia,[183,184] and Crohn's disease.[185,186]
2. Increased levels of certain immune factors in breast-fed infants cannot be accounted for by passive transfer of those substances from human milk. Breast-feeding primes the recipient to produce higher blood levels of interferon-α in response to respiratory syncytial virus (RSV) infections.[187] In addition, increments in blood levels of fibronectin achieved by breast-feeding cannot be accounted for by the amounts of that protein in human milk. Moreover, breast-feeding leads to a more rapid development of systemic[188] and secretory[188,189] antibody responses and of secretory IgA in external secretions[60-62] including urine,[61,62] which are far removed from the route of ingestion. There is no evidence that those increments are caused by absorption of those same factors from human milk.
3. Thymic growth,[190] T-cell emigration from the thymus possibly caused by increased IL-7 in human milk,[191] T-cell maturation, and IL-10 production[192] are increased in breast-fed infants compared with infants who are not breast-fed.
4. All leukocytes in human milk are activated.

Cytokines

After it was ascertained that human milk leukocytes were activated, activating agents in human milk were sought. It was found that human milk enhances the movement of blood monocytes in vitro and that much of that motility was abrogated by antibodies to tumor necrosis factor-α (TNF-α).[193] Subsequently, TNF-α

TABLE 158-4

Cytokines in Human Milk

Types	Examples
T-cell production augmentation	IL-7
Cellular immunity enhancement	Interferon-γ, TNF-α, IL-12, and IL-18
Humoral immunity enhancement	TGF-β2, IL-4, IL-10
Macrophage stimulation	IL-1β, IL-6, IL-6, MIF
Chemokine activities	IL-8, RANTES, MIP-1, CCL28
Interferon-inducible proteins	IP-10 and MIG
Antiinflammatory actions	TGF-β1, IL-10
Growth stimulation	EGF, M-CSF, G-CSF, erythropoietin

in human milk was detected immunochemically.[194] Since then, many cytokines have since been found in human milk (Table 158-4). They include the following:

1. IL-7, which promotes intrathymic development of T cells and maintenance of mature T cells in the peripheral lymphoid system.[192]
2. IL-2 with IL-7, which promotes the proliferation of recent thymic immigrants.[195]
3. Th1 (interferon-γ,[196] IL-12,[197] and IL-18[198]) and Th2 (IL-10[199,200] and IL-4[201]) cytokines.
4. Macrophage-stimulating cytokines including IL-1β,[202] IL-6,[203] and MIF.[86]
5. Chemotaxins including IL-8,[204] RANTES,[201] CCL28,[85] and eotaxin.[201]
6. Interferon-inducible proteins IP-10 and MIG.[205]
7. Antiinflammatory agents such as Il-4,[201] TGF-β1,[206] TGF -β2[207] and IL-10[199,200]
8. Growth factors EGF,[161,167] granulocyte colony-stimulating factor,[208] macrophage-CSF,[209] hepatic growth factor,[210] Il-4[196] and erythropoietin.[211]

It should be pointed out that the in vivo fate, action, and interactions of these cytokines in human milk are complex and largely unexplored. For example, IL-2 greatly decreases the expression of IL-7 receptor α-chains (IL-7Rα).[212] Because IL-7Rα is a component of receptors for IL-7 and thymic stromal lymphopoietin, IL-2 may negatively regulate signals by each of these cytokines. It is unclear whether the in vitro actions pertain to the in vivo effects upon the infant.

Other Modulators

Other immunomodulating agents in human milk include β-casomorphins,[213] prolactin,[167,214] antiidiotypic antibodies,[48] α-tocopherol,[159,160] nucleotides that enhance NK-cell, macrophage, and Th1 activities,[215] cell adhesion molecules ICAM-1, VCAM-1, E- and L-selectin,[216] mannan-binding lectin—which activates complement by the lectin pathway after recognizing surface saccharide motifs on microorganisms[217] and soluble CD14, a B-cell mitogen.[218]

Toll-like receptors (TLR) in human milk are being investigated. Soluble TLR-2 is present in human milk.[219] Furthermore, a protein of approximately 80 kD in human milk enhances the response of TLR4 and TLR5 receptors on umbilical cord blood mononuclear leukocytes.[220]

In addition to the antimicrobial and antiinflammatory functions of lactoferrin, this single-chain glycoprotein also promotes the differentiation of dendritic cells from monocytes.[221] It will be important to establish whether lactoferrin in human milk has a similar in vivo effect in the recipient infant.

In addition to their antimicrobial properties, some oligosaccharides are immunomodulatory. Lacto-N-fucopentaose III and lacto-N-neotetraose increase the production of murine IL-10.[222] Furthermore, human milk acidic oligosaccharides increase the number of interferon-producing $CD4^+$ and $CD8^+$ T cells and IL-13 production by $CD8^+$ T cells.[223] In addition, more CD25 is expressed on $CD4^+$ T cells after exposure to those oligosaccharides.[223]

TABLE 158-5

Immune Factors in Human Milk Whose Production Is Delayed in the Recipient

Immune Functions	Representative Agents
Antimicrobial	SIgA
	Lactoferrin
	Lysozyme
Antiinflammatory	IL-10
	PAF-acetylhydrolase
	Lactoferrin
	Lysozyme
Immunomodulatory	Memory T cells
	IL-4
	IL-10
	IL-12
	G-CSF
	TNF-α
	Interferon-γ
	RANTES

IMMUNE SYSTEM IN HUMAN MILK AND THE RECIPIENT INFANT

Several evolutionary outcomes concerning the relationships between the immune status of infants and defense agents in human milk have been recognized. In respect to one of the main evolutionary outcomes, many aspects of the human immune system are incompletely developed at birth, and the immaturity is most marked in very-low-birth-weight infants. They include (1) the mobilization and function of neutrophils,[224-226] (2) the maturation of dendritic cells,[227,228] (3) the recognition by monocytes and macrophages to bacterial agents by toll-like receptors TLR2 and TLR4,[229] (4) the production of lysozyme[230] and secretory IgA,[231] (5) memory T cells that bear CD45RO,[149] (6) the full expression of the antibody repertoire,[232] and (7) the production of certain cytokines including TNF-α,[233-235] IL-4,[236,237] interferon-γ,[237-239] IL-6,[234,240] IL-10,[233,241] IL-12,[242,243] IL-18,[243] G-CSF,[244] GM-CSF,[245] IL-3,[244] and RANTES.[246]

Many developmentally delayed defense agents are present in human milk (Table 158-5). For instance, secretory IgA antibodies in milk compensate for low production of secretory IgA during early infancy.[248] Human milk antibodies are polyclonal and directed against protein and polysaccharide antigens. This is important because infants display a more restricted antibody clonality[248] and do not produce IgG antibodies to polysaccharides.[249] Conjugate vaccines have been introduced, but antibody responses to them are higher in breast-fed than cow's milk-fed infants.[250]

An additional example is the interrelationship between the production of lysozyme by the infant and the mammary gland. High lysozyme levels in human milk[54-56] are coupled to low production of the protein by tracheo-bronchial mucosal cells during infancy.[230] Indeed, normal intraluminal concentrations of lysozyme in infancy may depend on breast-feeding. This is in keeping with higher lysozyme activities in stools of breast-fed infants than in infants who are not breast-fed.[60]

The functions of immune factors in human milk in the recipient infant depend on their survival in the infant. The following are germane: (1) Proteins may affect the epithelium, leukocytes, or other cells of the proximal GI or respiratory tracts where proteolytic enzymes are not produced. (2) Some proteins are inherently resistant to proteolysis. (3) Ingested proteins may escape

digestion because of developmental delays in production of gastric HCl and pancreatic proteases.[251]

This resistance to digestion may be augmented by (1) the buffering capacity of human milk that shields some acid-labile components of milk, (2) antiproteases in human milk,[152] (3) the inherent resistance of many defense agents in human milk to digestive processes, and (4) the compartmentalization of some defense agents in human milk.[86,94,95] In that respect, much of the TNF-α in human milk is bound to soluble receptors.[252]

PROTECTION OF PREMATURE INFANTS BY HUMAN MILK

Maturational delays of the immune system are generally more profound in premature infants. Furthermore, the problem is compounded by the shortened duration of placental transfer of IgG to the fetus,[253] medical problems during the newborn period,[254] nutritional imbalances, and invasive clinical procedures that increase the risks to infections.

Milk from women who deliver prematurely contains many of the same antimicrobial factors that are found in milk from women who have delivered after a full-term pregnancy. These include secretory Ig, lactoferrin, and lysozyme.[255] The concentrations of some defense agents are higher in preterm than term milk. Those higher concentrations may be in large part due to a lower production of milk by women who deliver prematurely. However, that may not be the total explanation for the higher concentrations in that patterns of the concentrations of some antimicrobial agents in preterm and term milk are not the same.[255]

Bacterial Sepsis

In addition to the protection against enteric infections and respiratory infections, there are several indications that human milk feedings protect premature infants against systemic infections that are prone to occur in such infants.[256-258]

Necrotizing Enterocolitis

Human milk protects against necrotizing enterocolitis (NEC).[259] Human and experimental animal studies suggest that IgA,[260] erythropoietin,[261,262] PAF-acetylhydrolase,[172-174] and IL-10[199,200,263,264] protect against NEC. These possibilities are in keeping with two important findings: 1) each of these defense agents is well represented in human milk and not in artificial feedings, and 2) the production of each agent in human infants is developmentally delayed.

One animal model suggests that IL-10 in human milk may prevent intestinal inflammation. Mice homozygous for IL-10 null genes develop a fatal enterocolitis that begins soon after weaning and is dependent on an enteric bacterial flora.[263,264] The enterocolitis had some features of Crohn's disease and NEC. Much of the enterocolitis in those animals is prevented by intraperitoneal injections of IL-10 given at the start of weaning.[263]

One study suggests that variations in the concentrations of IL-10 in human milk may be responsible for some of the risk of NEC in premature infants.[200] Two distinct populations of women were found in respect to the concentrations of IL-10 in their milk—72% were high producers, and 28% were very low producers.[200] In women whose infants developed NEC while receiving their own mother's milk, IL-10 was barely detected or undetected in milk from more than 90% of them.[200] The study awaits verification.

Lung Disease

Although it is unknown whether human milk protects against pulmonary and vascular effects of hyperoxia, α_1-antitrypsin prevents many of the features observed in hyperoxic neonatal rats including elevated pulmonary elastolytic activity.[265] Furthermore, a murine model suggests that TGF-β1 in human milk protects against certain pulmonary inflammatory diseases. Mice homozygous for the TGF-β1 null gene display infiltrations of macrophages and T cells in many organ sites, particularly the lungs, heart, and salivary glands.[266-268] Further, the effects of TGF-β1 deficiency are mitigated by ingestion of TGF-β1 in murine milk.[268] Furthermore, one study suggests that greater exposure to human milk TGF-β1 lessens the risk of asthma in the first year of life.[206]

ATOPIC DISEASE

Some studies suggest that human milk protects against atopic dermatitis[269] and asthma[206,270] and that some of the protection against asthma is mediated by TGF-β1[206] and soluble CD14 in human milk.[270] However, there is no consensus whether as to whether breast-feeding protects against atopic diseases.[271] Much of the disagreement may be due to confounding variables including variations in genetic predisposition to atopic disorders, the sufficiency of breast-feeding, unappreciated dietary exposures, and exposures to inhaled allergens or irritants. Further, increased exposures to infectious diseases facilitate Th1 responses that lead to cellular immunity, whereas lower exposures engender Th2 responses that lead to antibody formation and hence to possible IgE-mediated hypersensitivity. Thus, the effect of breast-feeding on atopic diseases may depend on factors that are not equally represented in all investigated populations.

Moreover, the question is complicated by foreign food antigens in human milk[272] and the triggering of allergic reactions by those antigens in some infants.[273] To test whether a breast-fed infant reacts to a foreign food antigen in human milk, dietary elimination and oral challenge with the food in question in the breast-feeding mother are needed.[273] If the infant reacts to a foreign food antigen in human milk, then the food should be eliminated from the maternal diet. If the allergen is a basic food, the elimination diet should contain the correct types and quantities of nutrients to meet the needs of the mother.[20] If dietary elimination is impractical, breast-feeding may be stopped and a hypoallergenic formula instituted. In addition, allergic disease in breast-fed infants may be due to alterations in fatty acids in human milk.[273-275]

IMMUNOLOGIC TOLERANCE

Evidence for induction of immunologic tolerance by breast-feeding comes from studies of alloreactivity. Maternal renal allografts are better tolerated in children from women who breast-feed transplant recipients than those who do not.[276,277] The difference in alloreactivity is also shown with blood lymphocytes from mothers and their children. Less alloreactivity occurs when lymphocytes from the mother (stimulators) are co-cultured with her breast-fed child's cells (reactors).[278] The tolerance may be induced by HLA-DR antigens on fat globules[279,280] and macrophages[137,141,144] in human milk.

CONCLUSION

Although much has been learned, there is much to be discovered. This includes: 1) other defense agents present in human milk, 2) regulation of production of the agents during lactation, 3) the precise molecular form of each defense agent in human milk, 4) where compartmentalized and soluble-receptor bound agents in human milk are released, 5) whether other defense agents are created in the infant's gastrointestinal tract by partial digestion of substrates in human milk, 6) mechanisms responsible for activating leukocytes in human milk, 7) in vivo fate and action of the

defense agents in human milk, 8) effects of commensal bacterial flora induced by breast-feeding upon the immune system of the recipient, 9) tolerogenic effects of human milk, and 10) long-term effects of antiinflammatory, immunomodulating, and antineoplastic agents in human milk.

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Neonatal Pulmonary Host Defense

Like the skin and gastrointestinal (GI) tract, the lungs are a mucosal organ with a large surface area exposed to the external environment. Unlike the skin and GI tract, the lung is considered to be largely sterile below the glottis whereas the skin and GI tract are colonized with bacteria termed "commensal flora." Despite the lower airway being sterile, the upper airway becomes rapidly colonized with bacteria that can be aspirated into the lower airway, thus the lung has evolved an array of host defense mechanisms to prevent development of infection in the air space. This robust development of pulmonary host defense mechanisms was an essential step in the evolution of air-breathing animals. The major physiologic aspect of the lung is to perform gas exchange, namely the exchange of oxygen and carbon dioxide across the alveolar capillary membrane. To maintain this function, the lungs must have buffering capacity in the airway and alveolar space to neutralize potentially injurious agents including pathogens. In a 3.5-kg neonate with a typical minute ventilation ranging from 100 to 150 mL/(kg•min), the lungs are required to filter approximately 30 L of inhaled air hourly. This is a problematic task in that the alveolar surface area requiring protection is 20 times the average neonatal body surface area.[1] In addition to normal tidal breathing or gas exchange the lung must be able to handle larger insults because of what may occur upon aspiration of oropharyngeal or gastric contents.

Available pulmonary host defenses can be broadly categorized as either structural or immunologic. Examples of structural defenses include the larynx and epiglottis (which are