Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

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Sadhana Chheda, Susan E. Keeney, and Armond S. Goldman

Immunology of Human Milk and Host Immunity

Paul Ehrlich reported in 1891 the first evidence that immunity could be transmitted through breast-feeding in experimental animals.[1,2] Few organized studies of that possibility in humans were reported, however, until the 1920s, when Woodbury[3] and Grulee and colleagues[4,5] in separate studies found that the incidence and severity of diarrheal diseases were much lower in breast-fed than cow's milk–fed infants. Those observations were confirmed repeatedly in developing and industrialized countries.[6–14] Furthermore, it was found that the specificity of the protection provided by breast-feeding encompassed bacterial and viral enteric infections due to pathogens such as *Shigella* species,[8–10] *Salmonella* species,[9] *Escherichia coli*,[9] *Vibrio cholerae*,[11] rotavirus,[12–14] and poliovirus.[15]

The following explanations for the protection provided by breast-feeding were advanced:

1. Because human milk was less contaminated with pathogenic microorganisms than formula feedings, fewer infections would be transmitted to the breast-fed infant.
2. Because of the increased spacing of births in lactating women due to contraceptive effects of lactation, the density of children susceptible to common contagious agents would be lower in families where breast-feeding was practiced.[16]
3. In addition, infants who were breast-fed would be less likely to be in group-care facilities and thus would be less exposed to children harboring microbial pathogens.

These propositions were reasonable, but they did not completely explain the protection provided by breast-feeding. In that respect, Wyatt and Mata from Guatemala found that manifestations of infection in breast-fed infants were low even when bacterial enteropathogens such as *Shigella* were recovered from the nipples and areola of the breast of the mother.[6] Furthermore, some evidence emerged that breast-fed infants may be more resistant to certain common respiratory infections.[17–20]

Despite those earlier studies, the concept, characteristics, and many of the components of the immune system in human milk were not revealed until the last half of the 20th century.[21] By 1973, the following general features of the antimicrobial agents of the immune system in human milk were evident:[22]

1. They are common to mucosal sites.
2. They are adapted to persist in the hostile environment of the gastrointestinal tract.
3. They inhibit or kill certain microbial pathogens synergistically.
4. They are often pluripotent.
5. They protect without triggering inflammatory reactions.
6. The daily production of many factors is inversely related to the ability of the recipient infant to produce those agents at mucosal sites.

The last feature of antimicrobial agents in human milk strongly suggested a relationship between the evolution of the development of the immune system of the infant and the evolution of

the abilities of the mother to produce and secrete immune factors from the lactating mammary gland.[23] Since then, several other somewhat overlapping evolutionary outcomes concerning the relationships between the immune system produced by the mammary gland and the developmental status of the immune system of the infant have been identified.[24] The seven known evolutionary outcomes are as follows:

1. Certain postnatal developmental delays in the immune system are replaced by those same agents in human milk.
2. Other postnatal delays in the immune system are offset by dissimilar agents in human milk.
3. Agents in human milk initiate or augment functions that are otherwise poorly expressed in the infant.
4. Agents in human milk alter the physiologic and biochemical states of the alimentary tract from one suited for fetal life to one that is appropriate for extrauterine life.
5. Defense agents in human milk protect without provoking inflammation, and some agents in human milk inhibit inflammation.
6. Defense agents in human milk have an enhanced survival in the gastrointestinal tract of the recipient infant.
7. Growth factors in human milk augment the proliferation of a commensal enteric bacterial flora.

The realization of many of those evolutionary outcomes came about as a consequence of the discovery of an expanded immune system in human milk that consisted of not only antimicrobial agents but also of anti-inflammatory[25,26] and immunomodulating agents.[26] The nature and functions of these agents are described in following sections of this chapter.

ANTIMICROBIAL FACTORS

The physical features, functions, and quantities of antimicrobial agents in human milk are summarized in Table 163–1 and are discussed in the following sections.

Proteins

The principal proteins in human milk that are antimicrobial are secretory immunoglobulin A (IgA) antibodies, other immunoglobulins, lactoferrin, lysozyme, mucins, and lactadhedrin. These proteins, except for immunoglobulins other than secretory IgA, are better represented in human milk than other mammalian milks used in human infant nutrition.

Antibodies

The concentrations of IgM are much lower in human milk than in serum.[27] IgM molecules in blood and milk are pentamers. However, unlike serum IgM, some human milk IgM is complexed to secretory component, and the antibody specificities of human milk IgM may be similar to those of secretory IgA in human milk (see later discussion). IgG is also present in human milk, albeit in modest amounts.[27] All IgG subclasses are represented in human milk,[28] but the relative proportion of IgG4 is higher in human milk than serum.[28] Very little IgD is present in human milk.[29] IgE, the immunoglobulin responsible for immediate hypersensitivity reactions, is essentially absent in human milk.[30]

Secretory IgA comprises more than 95% of the immunoglobulins in human milk.[27] This type of IgA consists of two identical IgA monomers united by a 15-kD polypeptide called the joining chain and complexed to a 75-kD glycopeptide, the secretory component.[31,32] 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 receptors on the basolateral surface of epithelial cells.[33]

Investigations of the unusual specificities of antibodies in human milk were spurred by epidemiologic evidence that human milk protects against common enteric and respiratory infectious pathogens and the discovery of secretory IgA in human milk by Lars Å Hanson.[34,35] This led to studies of the origins of B cells that are responsible for the production of the immunoglobulin part of those antibodies and mechanism of the assembly of the final molecule, secretory IgA. The specificities of many antibodies were found to be due to immunogen-triggered events in the intestinal tract.[36] It was later ascertained that antigen-stimulated B cells from Peyer patches of the lower small intestinal tract migrated to the mammary gland and that the process was under hormonal control.[37,38] In addition, a B-cell pathway between lymphoid tissues in the bronchi and the mammary gland was discovered.[39]

This process may be controlled by a mucosal adhesion-cell adhesion system (e.g., mucosal addressin cell adhesion molecule, or MAdCAM[40]), and its counterstructure, $\alpha 4\beta 7$ integrin,[41] and certain cytokines. During mucosal antigenic stimulation, cytokines released from mononuclear cells in Peyer patches induce local B cells to switch from IgM^+ to IgA^+.[42–45] These isotype-switched B cells then migrate sequentially into local intestinal lymphatic channels and lymph nodes, the thoracic duct, and the vascular circulation. Because of lactogenic hormones and other influences that are poorly understood, the cells move from the vascular compartment to the lactating mammary gland. These IgA^+ B cells differentiate to IgA producing-secreting plasma cells that remain in the lamina propria of the mammary gland. In keeping with other mucosal lymphoid tissues, IgA dimers produced by plasma cells in the mammary gland principally contain λ-light chains, whereas κ-light chains predominate in immunoglobulins in human sera.[46]

IgA dimers produced by those plasma cells bind to polymeric immunoglobulin receptors on the basolateral external membranes of mammary gland epithelial cells.[31,32,47,48] The resultant receptor–dimeric IgA complex is transported to the apical side of

TABLE 163–1

Primary Functions of Antimicrobial Agents in Human Milk

Agents	Primary Antimicrobial Functions
Proteins	
Lactoferrin	Bacteriostasis produced by Fe^{3+} chelation Bacterial killing due to lactoferricin
Lysozyme	Lyses bacterial cell walls by degrading peptidoglycans
Secretory IgA	Binds bacterial adherence sites, toxins, and virulence factors
MUCI	Inhibits the binding of S-fimbriated *Escherichia coli* to epithelial cells
Lactadhedrin	Binds rotavirus and thus prevents its contact with epithelium
Oligosaccharides and glycoconjugates	Receptor analogues inhibit binding of enteric/respiratory pathogens and their toxins to epithelial cells.
Monoglycerides and fatty acids from lipid digestion	Disrupt enveloped viruses, inactivate certain bacteria, defend against infection from *Giardia lamblia* and *Entameoba histolytica*

the cell where the original intracytoplasmic portion of the receptor is cleaved away. The remaining molecule, secretory IgA, is secreted into milk. Thus, enteromammary and bronchomammary pathways protect the immunologically immature infant against the pathogens in the environment of the dyad (Table 163–2). This is important given that secretory IgA antibodies and the antigen-binding repertoire of immunoglobulin molecules are not optimally produced during early infancy.[49] Furthermore, some secretory IgA molecules in human milk are antiidiotypic antibodies and therefore may operate as immunizing agents.[50]

The quantity of secretory IgA declines as lactation proceeds, but a considerable amount of secretory IgA is transmitted to the recipient infant throughout breast-feeding.[51–54] The concentrations of secretory IgA in human milk are highest in colostrum[51] and then gradually decline to a plateau of about 1 mg/mL.[52] The approximate mean intake of secretory IgA per day in healthy full-term breast-fed infants is approximately 125 mg/kg per day at 1 month and approximately 75 mg/kg per day by 4 months.[54]

Secretory IgA is resistant to intestinal proteases such as pancreatic trypsin.[55] Although the first IgA subclass, IgA1, is susceptible to bacterial proteases that attack the hinge region of the molecule,[56] the second subclass, IgA2, is resistant to those proteases and is disproportionally increased in human milk.[27] Furthermore, secretory IgA antibodies against these bacterial IgA proteases are found in human milk.[56] In keeping with those observations, the amount of secretory IgA excreted in the stools of low birth weight infants fed human milk was about 30 times that in infants fed a cow's milk formula.[57] In addition, the urinary excretion of secretory IgA antibodies in the recipients increased as a result of human milk feedings.[58, 59] The origin of secretory IgA antibodies in the urine of infants fed human milk is undetermined. It is improbable that they are from human milk because there is no known mechanism for the transport of the entire molecule from the gastrointestinal tract to the blood or from blood to urine.

Lactoferrin

Lactoferrin is a single-chain glycoprotein with two globular lobes, each of which displays a site that binds ferric iron.[60] In over 90% of lactoferrin in human milk,[61] iron-binding sites are available to compete with siderophilic bacteria and fungal enterochelin for ferric iron.[62–65] The chelation of iron disrupts the proliferation of those microbial pathogens. In addition, the chelation is enhanced by bicarbonate, the principal buffer in human milk.

Lactoferrin also kills some bacteria[66] and fungi,[67] and the responsible part of the molecule (lactoferricin)[67, 68] acts by damaging outer membranes of pathogens.[68] The action is dependent on Ca^{2+}, Mg^{2+}, or Fe^{3+} but not on the ability to chelate Fe^{3+}.[68]

TABLE 163–2

Secretory IgA Antibodies in Human Milk Against Microbial Pathogens

Bacteria-Toxins Virulence Factors	Viruses	Fungi and Parasites
Escherichia coli	Adenovirus	*Giardia lamblia*
Campylobacter sp.	Cytomegalovirus	*Candida* sp.
Clostridium botulinum	Enteroviruses (polio)	
Clostridium difficile	HIV	
Haemophilus influenzae	Influenza virus	
Helicobacter pylori	Respiratory syncytial virus	
Klebsiella pneumoniae	Rotavirus	
Streptococcus pneumoniae		
Vibrio cholerae		
Salmonella sp.		
Shigella sp.		

There is also evidence that lactoferrin inhibits certain viruses in a manner that is independent of iron chelation.[69–72]

The mean concentration of lactoferrin in human colostrum is between 5 and 6 mg/mL.[51] As the volume of milk production increases, the concentration falls to about 1 mg/ml at 2 to 3 months of lactation.[52] The mean intake of milk lactoferrin in healthy breast-fed full-term infants is about 260 mg/kg per day at 1 month and 125 mg/kg per day by 4 months.[54]

Because of resistance of lactoferrin to proteolysis,[73] the excretion of lactoferrin in the stools is higher in infants fed human milk than in those fed a cow's milk formula.[57, 74, 75] The quantity of lactoferrin excreted in stools of low birth weight infants fed a human milk preparation is approximately 185 times that excreted by infants fed a cow's milk formula.[57] That estimate, however, may be too high because of the presence of immunoreactive fragments of lactoferrin in the stools of human milk-fed infants.[76] There is also a significant increment in the urinary excretion of intact and fragmented lactoferrin as a result of human milk feedings.[57, 76] Stable isotope studies suggest that those increments in urinary lactoferrin and its fragments originate from ingested human milk lactoferrin.[77]

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.[78] High concentrations of lysozyme are present in human milk during all stages of lactation,[51–54] but longitudinal changes in quantities of lysozyme during lactation are unlike most other immune factors in human milk. The mean concentration of lysozyme is about 70 μg/ml in colostrum,[51] 20 μg/ml at 1 month, and 250 μg/ml by 6 months of lactation.[52] The approximate mean daily intake of milk lysozyme in healthy full-term, completely breast-fed infants is 3 to 4 mg/kg per day at 1 month and 6 mg/kg per day by 4 months of age.[54] The high content of lysozyme in human milk and its *in vitro* resistance to proteolysis are in keeping with an eightfold increase in the amount of lysozyme excreted in the stools of low birth weight infants fed human milk compared with findings in infants fed a cow's milk formula.[57] However, in contrast to secretory IgA and lactoferrin, the urinary excretion of this protein is not increased in infants fed human milk.[59]

The lysozyme C gene gave rise some 300 to 400 million years ago to a gene that codes for α-lactalbumin, a protein expressed only in the lactating mammary gland. The protein is a component of lactose synthetase. It is of interest that three domains of this evolutionary descendant of lysozyme are antibacterial.[79] Furthermore, multimeric α-lactalbumin may be antineoplastic.[80]

Fibronectin

Fibronectin, a high molecular weight protein that facilitates the uptake of many types of particulates by mononuclear phagocytes, is present in human milk (mean concentration in colostrum, 13 μg/ml).[81] The *in vivo* effects of this broad-spectrum opsonin in human milk are not known.

Complement Components

All components of the classical and alternative pathways of complement are in human milk, but the concentrations of these components, except for C3, are low.[82,83]

Human Milk Mucin

Milk mucins are high molecular weight proteins that are greatly glycosylated.[84] About two-thirds of the mucin in human milk is membrane bound. The concentration of mucin in human milk is between 50 and 90 mg/ml. A number of milk mucins have been identified. The most prominent one is MUC1. MUC1 has molecular weights between 250 and 450 kDa and is primarily bound to membranes of milk fat globules. In that respect, human milk fat

globules and mucin from their membranes inhibit the binding of S-fimbriated *E. coli* to human epithelial cells.[85]

The *in vivo* fate of ingested MUC1 has been investigated. It has been found to be resistant to intragastric digestion in preterm infants.[86] Major fragments of MUC1 are detected in feces of breast-fed infants.[87] Furthermore, mucins from such feces are more able to inhibit bacterial adhesion than feces from formula-fed infants.[88]

Lactadhedrin

It was originally reported that human milk mucin defended against rotavirus, the most common cause of infectious enteritis in human infants, in an experimental murine model.[89] Rotavirus bound not only to the milk-mucin complex, but also to a 49-kDa component of the complex. The active component was later found to be a separate glycoprotein that was designated as lactadherin.[90] Like human MUC1, lactadhedrin is resistant to intragastric digestion.[91]

Oligosaccharides and Glycoconjugates

Oligosaccharides in human milk are produced by glycosyltransferases in the mammary gland. Some of these abundant compounds are receptor analogues that inhibit the binding of certain enteric or respiratory bacterial pathogens and their toxins to epithelial cells.[92–95] Many types of oligosaccharides have been identified in human milk, and new types are still being recognized.[96,97]

Oligosaccharides in human milk are different than those found in commercial milk formulas. Although the quantities of total gangliosides in human and bovine milk are similar, the relative frequencies of each type of ganglioside in milk from these two species are distinct. For example, much more monosialoganglioside 3 and GM_1 are found in human than bovine milk.[97–99]

The chemistry of these compounds dictates the specificity of their binding to the adherence structures of bacterial pathogens. For example, GM_1 gangliosides are receptor analogues for toxins produced by *V cholerae* and *E. coli*,[93] whereas the globotriaosylceramide Gb3 binds to the β subunits of Shigatoxin.[100] A fucosyloligosaccharide inhibits the stable toxin of *E. coli*,[94] whereas a different one inhibits *Campylobacter jejuni*.[101] Oligosaccharides in human milk also interfere with the attachment of *Haemophilus influenzae* and *Streptococcus pneumoniae*.[95] In that regard, G1cNAc(β1-3) Gal-disaccharide subunits block the attachment of *S. pneumoniae* to respiratory epithelium.

In vivo animal experiments also suggest that oligosaccharides and glycoconjugates in human milk protect against certain enteric bacterial infections.[102] In that regard, certain human milk oligosaccharides survive passage through the alimentary tract[103] and some of the absorbed carbohydrate is then excreted into the urinary tract.[104] Sugars that are present in several glycoconjugates including mucins, lactadherin, and secretory IgA also interfere with the binding of bacterial pathogens to epithelial cells.[105]

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*.[106, 107] For example, the growth-promoter activity associated with caseins may reside in the oligosaccharide moiety of those complex molecules.[107]

These factors are responsible to a great extent for the predominance of *Lactobacilli* and *Bifidobacilli* in the bacterial flora of the large intestine of breast-fed infants found in most studies. The bacteria produce large amounts of acetic acid, which aids in suppressing multiplication of enteropathogens. It has also been reported that *Lactobacilli* strain GG aids in the recovery from acute rotavirus infections[108] and may enhance the formation of specific IgG, IgA, and IgM antibodies.[109] In addition, enteric commensal bacteria may stimulate the production of low molecular weight, antibacterial peptides, such as defensins.[101] These types of defense mechanisms may contribute to the comparative paucity in stools of breast-fed infants of bacterial pathogens most often found in urinary tract infections (P-fimbriated *E. coli*).[111]

Lipids

Fatty acids and monoglycerides generated by the enzymatic digestion of lipid substrates in human milk disrupt enveloped viruses.[112–114] These antiviral lipids may aid to prevent coronavirus infections of the intestinal tract[115] and defend against intestinal parasites such as *Giardia lamblia* and *Entameoba histolytica*.[116,117] Monoglycerides from milk lipid hydrolysis also inactivate certain gram-positive and gram-negative bacteria.[118]

The *in vivo* hydrolysis of ingested milk lipids in early infancy occurs because of two enzymatic mechanisms.The first is due to the action of lingual lipase and the second is due to the activation of human milk bile–salt stimulated lipase in the duodenum. Thus, it is likely that the products of lipid digestion contribute to the defense of the breast-fed infant against enteric infections.

LEUKOCYTES IN HUMAN MILK

Living leukocytes are found in human milk.[119] 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 the milk secretions. The highest concentrations of leukocytes in human milk occur in the first few days of lactation ($1–3 \times 10^6$/ml).[120] The several types of leukocytes and their major features follow.

Lymphocytes

The relative frequencies of T cells and B cells among lymphocytes in early human milk secretions are 83% and 6%, respectively.[121] The small number of natural killer (NK) cells in human milk[121] is in keeping with the low cytotoxic activity of human milk leukocytes.[122] The small number of B cells is a reflection that 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,[121, 123] but compared with human blood T cells, the proportion of cytotoxic/suppressor T cells ($CD8^+$) in human milk is increased.[121] Virtually all $CD4^+$ and $CD8^+$ T cells in human milk bear the CD45 isoform, CD45RO, that is indicative of cellular activation.[121,124] In addition, an increased proportion of the T cells displays other phenotypic markers of activation.[121,124]

T cells in human milk produce certain cytokines such as interferon-γ,[124] macrophage migration inhibitory factor,[120] and a monocyte chemotactic factor.[120] The production of interferon-γ is consistent with the CD45RO phenotype of T cells in human milk[121, 123] and the finding that $CD45RO^+$ T cells are the major source of that cytokine.[121] Additional cytokines are produced by human milk leukocytes,[124] but the extents of their production and secretion have not been determined.

Neutrophils and Macrophages

Neutrophils and macrophages in human milk are laden with milk fat globules and perhaps with other membranes that have been phagocytized. Because of these intracytoplasmic bodies, the cells are difficult to identify by common staining methods.They can be identified however by their content of myeloperoxidase (in the case of neutrophils),[120] nonspecific esterase (in the case of macrophages),[120] or by the surface expression of CD14 (in the case of macrophages).[125] Both types of cells in human milk are phagocytic.There is some evidence that the respiratory burst occurs in milk macrophages after stimulation,[126] but their intracellular killing activities appear to be reduced.The macrophages have also been found to process and present antigens to T cells.[127]

After exposure to chemoattractants, human milk neutrophils (compared with blood neutrophils) do not increase their adherence, polarity, directed migration,[128] or deformability.[129] Some of those features appear to be due to agents in human milk. For example, the decreased calcium influx by human milk neutrophils has been duplicated by incubating blood neutrophils in human milk.[130] Unlike human milk neutrophils, the motility of macrophages in human milk is increased compared with their counterparts in blood.[131] These features of neutrophils and macrophages in human milk appear to be due to cellular activation, because these cells display phenotypic markers of activation including an increased expression of CD11b/CD18 and a decreased expression of CD62L (L-selectin).[125]

Potential *in Vivo* Effects

The *in vivo* fate and role of human milk leukocytes in defense of the infant are not well understood. The area about the upper alimentary and respiratory tracts seems to provide potential sites for human milk leukocytes to enter. It is of considerable interest that small numbers of memory T cells are detected in blood in infancy.[132] Thus, it may be possible that maternal memory T cells in milk compensate for the developmental delay in their production in the infant. There is evidence from experimental animal studies that milk lymphocytes enter tissues of the neonate,[120] but that has not been demonstrated in humans. There are also reports of transfer of cellular immunity by breast-feeding.[133] It will be important to ascertain whether those reports will be verified by testing for cellular immunity against many different antigens in young infants who have or have not been breast-fed.

ANTI-INFLAMMATORY AGENTS

Inflammatory agents and systems that give rise to them are poorly represented in human milk.[25] These include (1) the coagulation system, (2) the kallikrein-kininogen system, (3) major components of the complement system, (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 inflammatory processes in the recipient.

In contrast to the paucity of inflammatory agents, human milk contains a host of anti-inflammatory agents.[25] They include (1) factors that promote the growth of epithelium and thus strengthen mucosal barriers, (2) antioxidants, (3) agents such as lactoferrin that interfere with certain complement components,[25,134] (4) enzymes that degrade mediators of inflammation, (5) protease inhibitors,[135] (6) agents that bind to substrates such as lysozyme to elastin,[136] (7) cytoprotective agents such as prostaglandins E_1, E_2, and $F_{2\alpha}$,[137,138] and (8) agents that inhibit the functions of inflammatory leukocytes (Table 163–3).[25] Like the antimicrobial factors, many of these factors are adapted to operate in the hostile environment of the alimentary tract.

The main antioxidants in human milk include an ascorbate-like compound,[139] uric acid,[139] α-tocopherol[140,141] and β-carotene.[140,141] In fact, blood levels of α-tocopherol and β-carotene are higher in breast-fed than formula-fed infants not supplemented with those agents.[141]

Mucosal growth factors in human milk include epithelial growth factor,[142] lactoferrin,[143] cortisol,[144] and polyamines.[145,146] Other hormones and growth factors in human milk[147] may also affect the growth, differentiation, and turnover of epithelial cells. These agents may therefore limit the penetration of free antigens and pathogenic microorganisms and affect other barrier functions of the intestinal tract. In keeping with that notion, there are significant differences between the biophysical and biochemical organization and functions of mucosal barriers in adults and neonates.[148,149] Furthermore, maturation of those functions may be accelerated by human milk.[150,151]

Enzymes in human milk degrade inflammatory mediators that may damage the gastrointestinal tract. In that respect, platelet-activating factor (PAF) plays a role in an intestinal injury in rats induced by endotoxin and hypoxia.[152] Furthermore, an acetylhydrolase that degrades PAF is present in human milk,[153] and the production of human PAF-acetylhydrolase is developmentally delayed.[154] Published results of investigations also indicate that human milk feedings lessen intestinal permeability in young infants.[155–157]

IMMUNOMODULATING AGENTS

Three sets of observations provide the basis of the concept of immunomodulating agents in human milk:

1. Epidemiologic investigations suggest that older children who were breast-fed during infancy may be at less risk for developing certain chronic diseases that are mediated by immunologic, inflammatory, or oncogenic mechanisms. The diseases in question are type 1 diabetes mellitus,[158] lymphomas,[159] acute lymphocytic leukemia,[160] and Crohn's disease.[161] Although preventing or lessening infections by antimicrobial agents or by anti-inflammatory agents in human milk may have long-term consequences, agents that influence the development of systemic or mucosal defenses of the infant may also be responsible for those possible long-term effects.
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 infections.[162] 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[163] and secretory[163,164] antibody responses and of secretory IgA in external secretions[57–59] including urine,[58,59] which is far removed from the route of ingestion. Therefore, those increments are not due to absorption of those same factors from human milk.
3. The third line of evidence is the discovery that all leukocytes in human milk are activated (see previous section on leukocytes). Investigations revealed that human milk enhances the movement of blood monocytes *in vitro*. In addition, much of that motility was abrogated by antibodies to tumor necrosis factor-α (TNF-α).[165] Subsequently, TNF-α in human milk was detected immunochemically.[166]

TABLE 163–3

Anti-Inflammatory Factors in Human Milk.

Categories	Examples
Cytoprotectives	Prostaglandins E2,F2α
Epithelial growth factors	Epidermal growth factor, lactoferrin, polyamines
Maturational factors	Cortisol
Enzymes that degrade mediators	PAF-AH
Binders of enzymes	α1-antichymotrypsin
Binders of substrates of enzymes	Lysozyme to elastin
Modulators of leukocytes	Interleukin-10
Antioxidants	Uric acid, α-tocopherol, β-carotene, ascorbate

PAF-AH = Platelet activating factor–acetylhydrolase

Many other cytokines have been found in human milk. They include Th1 cytokines such as interferon-γ,[167] interleukin (IL)-12,[168] and IL-18[169]; proinflammatory cytokines including IL-1β)[170] and IL-6[171, 172]; chemotaxins including IL-8,[173] regulated on activation, normal T expressed and secreted (RANTES),[174] and eotaxin[174]; antiinflammatory agents such as transforming growth factor-β (TGF-β)[173, 175] and IL-10[176]; and the cellular growth factors EGF,[142] granulocyte colony-stimulating factor (G-CSF),[177] macrophage-CSF,[178] hepatic growth factor,[179] and erythropoietin[180] (Table 163–4). There are controversies concerning the quantities of some of these agents in human milk. The discrepancies between the results of some of the studies may depend on differences in storage conditions of the specimens and the types of immunoassays. The sites and extents of their effects on the recipient infant are not determined.

Several other immunomodulating agents are in human milk including β-casomorphins,[181] prolactin,[182, 183] antiidiotypic antibodies,[50] α-tocopherol[140, 141] and a host of nucleotides that enhance NK-cell, macrophage, and Th1-cell activities.[184–186]

RELATIONSHIPS BETWEEN THE IMMUNE SYSTEMS IN HUMAN MILK AND THE RECIPIENT

As previously mentioned, seven somewhat overlapping evolutionary outcomes concerning the relationships between the immune status of infants and defense agents in human milk have been recognized.[50, 51] In respect to the first evolutionary outcome, many aspects of the human immune system are incompletely developed at birth, and the immaturity is most marked in very low birth weight infants. These developmental delays include (1) the mobilization and function of neutrophils,[187] (2) the production of lysozyme[188] and secretory IgA[189,190] at mucosal sites, (3) memory T cells that bear CD45RO,[135] (4) the complete expression of the antibody repertoire,[191] and (5) the production of certain cytokines including TNF-α,[192, 193] IL-4,[194] interferon-γ,[194,195] IL-6,[192] IL-10,[193] G-CSF,[196] GM-CSF,[197] and IL-3.[196]

Many of those developmentally delayed defense factors are well represented in human milk (Table 163–5). For example, secretory IgA antibodies in human milk compensate for the low production of secretory IgA at mucosal sites during early infancy. It is also important that the antibody response achieved through this pathway is polyclonal and is directed against not only protein, but also polysaccharide antigens, because infants display a more restricted clonality[198] and do not mount an IgG antibody response to polysaccharide antigens.[199] The problem has been modified by the introduction of conjugate vaccines. Even so, the antibody response to conjugate vaccines is higher in breast-fed than cow's milk-fed infants.[200]

An additional example is the interrelationship between the amount of lysozyme produced by the infant and the quantity secreted into milk. Indeed, the necessity of high lysozyme levels in human milk is coupled to the low production of the protein by mucosal cells during infancy.[188] It is likely that the attainment of normal intraluminal concentrations of lysozyme in infancy is dependent on breast-feeding. This is in keeping with the finding of higher lysozyme activities in stools of breast-fed than in non-breast-fed infants.[57]

The potential *in vivo* effects of immune factors in human milk in the recipient infant depend on the survival of those agents. Although it may be argued that defense agents in human milk would be destroyed by the digestive processes in the gastrointestinal tract, many of these agents may be bioactive in the alimentary and respiratory tracts for the following reasons:

1. Protein components may affect the epithelium, leukocytes, or other cells of proximal parts of the alimentary or respiratory tracts where proteolytic enzymes are not produced.
2. Ingested proteins may escape intragastric-intraduodenal digestion because of developmental delays in the production of gastric HCl and pancreatic proteases.[201] This resistance to digestion may be augmented by the protection provided by the buffering capacity of human milk that shields some acid-labile components of milk, antiproteases in human milk,[135] inherent resistance of many defense agents in human milk to digestive processes, and the protection against digestion of some defense agents in human milk because they are compartmentalized.[166, 172] In that respect, much of the TNF-α in human milk is bound to soluble receptors.[202]

This thesis is borne out as previously discussed by an increased survival of certain human milk defense agents in the alimentary tract of the recipient infant.

PROTECTION OF PREMATURE INFANTS BY HUMAN MILK

Maturational delays of the immune system are generally more profound in premature infants. Furthermore, the potential immunologic problems are compounded by the shortened duration of placental transfer of IgG to the fetus.[203] That predisposes premature infants to certain opportunistic infections. Moreover, major medical problems during the newborn period including pulmonary diseases,[204] nutritional imbalances, and invasive clinical procedures increase the risks of premature infants to infections.

TABLE 163–4

Potential Functions of Certain Cytokines in Human Milk

Cytokines	Possible Functions
Interferon-γ	T-helper 1 cytokine-macrophage activator
Interleukin-1β	Activates T cells and macrophages
Interleukin-6	Enhances IgA production
Interleukin-8	Chemotaxin for neutrophils and $CD8^+$ T cells
Interleukin-10	Th2 cytokine
	Inhibits production of many pro inflammatory cytokines
Interleukin-12	Th1 cytokine
	Enhances production of interferon-γ
TNF-α	Enhances production of polymeric Ig receptors
TGF-β	Enhances isotype switching to IgA^+ B cells
G-CSF	Increases granulocyte (neutrophil) production
M-CSF	Increases monocyte production

G-CSF = granulocyte colony stimulating factor; M-CSF = monocyte colony stimulating factor; TGF-β = transforming growth factor-β; TNF-α = tumor necrosis factor-α.

TABLE 163–5

Representative Immune Factors in Human Milk the Production of Which Is Delayed in the Recipient Infant

Agents	Time of Maturation
Secretory IgA	~4–12 mo
Full antibody repertoire	~2 yr
Memory T cells	~2 yr
Lysozyme	~1–2 yr
Lactoferrin	?
Interferon-γ	?
Interleukin-6	?
Interleukin-8	?
Interleukin-10	?
TNF-α	?
PAF-acetylhydrolase	?

PAF-AH = platelet activating factor-acetylhydrolase; TNF-α = tumor necrosis factor-α

Milk from women who have delivered prematurely contains many of the same antimicrobial factors that are found in milk from women who have delivered after a full-term pregnancy.[205] These include secretory IgA, lactoferrin, and lysozyme. The concentrations of those defense agents are higher in preterm than term milk. Those higher concentrations may be in large part due to a lower volume of milk produced by women who have delivered prematurely. That may not be the total explanation for the higher concentrations in that the patterns of the concentrations of some of the antimicrobial factors in preterm and term milk are not exactly the same.[205] Moreover, the concentrations of most anti-inflammatory and immunomodulating factors in preterm milk have not been established.

In addition to the protection against enteric infections and respiratory infections such as otitis media, there are several indications that human milk feedings protect premature infants against systemic infections that are more prone to occur in immature infants. Winberg and his colleagues in Sweden[206] reported that the risk of bacterial sepsis was less in premature newborn infants who were fed human milk. These observations were confirmed by Yu and co-workers in Australia[207] and Nayaryanan and her associates in India,[208] who found that supplemental feedings of expressed human milk were associated with a reduced frequency of infections in low birth weight infants.

Human milk also protects against many cases of necrotizing enterocolitis (NEC).[209] The factors in human milk that are responsible for this protection remain to be elucidated, but evidence from human and experimental animal studies suggests that IgA,[210] erythropoietin,[211,212] PAF-acetylhydrolase,[153] and IL-10[213] are likely possibilities. In each case, there is a developmental delay in the production of the suspected factor, and the agent in question is well-represented in human milk.

Two contrasting experimental animal models of cytokine gene deficiency suggest that anti-inflammatory cytokines in human milk may prevent disorders due to inflammatory processes. Mice homozygous for the TGF-β1 null gene display spontaneous, infiltrations of macrophages and T cells in many organ sites; the lungs, heart, and salivary glands are most prominently involved.[214–216] Furthermore, there is experimental evidence that the effects of the TGF-β1 deficiency are mitigated by the ingestion of that cytokine in murine milk.[216]

In the second animal model, a targeted IL-10 gene deletion was engineered in mice. In those IL-10–deficient animals, a fatal enterocolitis began directly after weaning, and it was dependent on establishment of an enteric bacterial flora.[213] The enterocolitis had some features of Crohn's disease and NEC. Much of the enterocolitis in those animals was prevented by intraperitoneal injections of IL-10 given at the start of weaning.[217]

Although it has not been established whether human milk feedings protect against the pulmonary and vascular effects of hyperoxia, some experimental evidence suggests that one of the anti-inflammatory components of human milk, α_1-antitrypsin, prevents many of those features in hyperoxic neonatal rats including elevations in pulmonary elastolytic activity.[218]

The possible effects of human milk upon the development of atopic diseases have been investigated by many groups, but there is no consensus whether breast-feeding protects against those disorders,[219] except for atopic dermatitis[220] or when food allergens are avoided by complete breast-feeding. Much of the disagreement is probably due to confounding variables including variations in the genetic predisposition to atopic disorders, the sufficiency of breast-feeding, dietary exposures not appreciated by the parents, and exposures to inhalant allergens or irritants that might lead to lung damage. Furthermore, there is evidence that increased exposures to infectious diseases facilitate Th1 responses that lead to the development of cellular immunity, whereas much lower exposures engender Th2 responses that lead to antibody formation and hence to possible IgE-mediated hypersensitivity. Thus, the effect of breast-feeding on the risk of atopic diseases may well depend on a multiplicity of factors that are not equally represented in all investigated populations.

Moreover, the question is complicated by the transmission of foreign food antigens in human milk[221] and the triggering of allergic reactions by those antigens in some recipient infants.[222] Why only a subpopulation of breast-fed infants develops atopic diseases is unknown. To establish whether a breast-fed infant is reacting to a foreign food antigen in human milk, it is necessary to conduct trials of dietary elimination and oral challenge with the food in question in the mother while she is breast-feeding.[223] If those trials suggest that the infant is reacting to a foreign food antigen in human milk, then the problem may be avoided by eliminating the food allergen from the maternal diet. If the food allergen is a basic food such as cow's milk, the woman must have a diet that supplies the correct types and quantities of nutrients to meet the needs of lactation.[20] If long-term elimination is impractical, then breast-feeding may be stopped and the infant tried on a hypoallergenic formula. In addition, the development of allergic disease in breast-fed infants may be due to alterations in the types of fatty acids found in milks produced by mothers of the allergic infants.[224,225]

The influence of human milk feedings upon the rate of rehospitalizations of premature infants was examined in the 1988 National Maternal and Infant Health Survey conducted by The National Institutes of Child Health and Human Development.[226] Although a cause-effect relationship could not be definitively established, the feeding of human milk was an independent predictor of decreased risk for rehospitalization. Thus, human milk feeding may have beneficial effects on the premature infant that extend beyond the initial hospitalization.

CODA

Human milk contains an array of host resistance factors that are antimicrobial, anti-inflammatory, or immunomodulating. This immune system is adapted to function at mucosal sites and to protect the recipient against a host of infectious and inflammatory processes that are common in the developing infant. In addition, there may be long-term health benefits to the recipient by human milk feedings that apparently are due to alterations in the immune system.

The precise ways in which the immunologic agents in human milk protect the child and how those agents interact with the developing immune system of the recipient are not well understood. These research issues will require the coordinated efforts of neonatologists, immunologists, molecular biologists, and other clinical and basic scientists.

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164 Neonatal Pulmonary Host Defense Mechanisms

Andrew Metinko

The lungs are unique internal organs, situated within the body yet interposed between the host and its environment. The development of pulmonary host defense mechanisms capable of restricting the growth of environmental pathogens was therefore an essential step in the evolution of air-breathing animals. To effect gas exchange with the environment, the lungs must be able to buffer the potentially injurious effects to airways and alveoli of multiple substances, including pathogenic organisms, which may be present in the air stream. In a 3.5-kg neonate, with a typical minute ventilation ranging from 100 to 150 ml/(kg • min), this requires the lungs to filter approximately 30 L of inhaled air hourly; a problematic task in that the alveolar surface area requiring protection is 20 times the average neonatal body surface area.[1] Mechanisms must also exist to prevent, or contain, effects of potential pathogens delivered by aspiration of oropharyngeal secretions. Concomitantly, the lungs as reticuloendothelial structures are also responsible for filtering all blood returning to the left atrium via the pulmonary circulation. Thus, the extensive alveolar-capillary membrane, composed of both immune and nonimmune cells, may encounter pathogens by hematogenous routes, as well as by inhalation. Beyond this significant environmental exposure, host defense of the lung presents other unique challenges.[2] Individual alveoli are exposed to the environment in parallel, and thus must be somewhat self-sufficient in initial antigen response. Furthermore, the alveolar-capillary interface, teleologically evolved for gas exchange, offers little barrier to pathogen movement in either direction. Finally, even mild inflammation in this critical location can significantly impair gas exchange, and threaten host survival. Because airways in the lower respiratory tract normally contain few colonies of essentially commensal organisms, evolved pulmonary mechanisms of pathogen containment and clearance, both immunologic and nonimmunologic, are clearly effective.

Available pulmonary host defenses can be broadly categorized as either mechanical or immunologic. Examples of mechanical defenses include the larynx and epiglottis (which are anatomically situated to minimize aspiration of oropharyngeal material) airway angulation, mucus secretion, and mucociliary clearance mechanisms, including the cough reflex. These mechanisms result in progressive filtering of about 99% of inhaled particles as they pass through the conducting airways, so that overall level of antigen exposure at a given site is inversely related to its depth within the respiratory tree. Mechanical barrier components of host defense thus minimize "bulk" exposure to pathogens, and antigens, minimizing the frequency of host immune response activation.

Available immunologic mechanisms are by recent convention broadly categorized as either innate, or adaptive. Innate immune responses are nonspecific, relying on host recognition of "pathogen-associated molecular patterns," such as peptidoglycans, endotoxin, or fungal mannans. Such foreign patterns are recognized, and ligated, either by soluble bioactive substances within the airway (defensins, collectins) or by pattern recognition receptors on macrophages. Subsequent generation of "early response cytokines" (tumor necrosis factor, interleukin [IL]-1) and chemotaxins (leukotrienes, chemokines, split components of complement) leads to recruitment of additional cellular elements of innate immunity, granulocytes and natural killer cells. By means of concurrent cytokine networking with other cells in the alveolar milieu (including epithelia and fibroblasts), alveolar macrophages activate antigen-presenting cells, and lymphocytes move into the alveolar compartment; recruitment of the specialized lymphocytes, T cells and B cells, heralds the onset of the adaptive immune response. These cells manifest specific receptors somatically generated in response to specific antigens, facilitating immunologic "memory" and long-term cell-mediated and humoral immunity. Adaptive immunity thus initiates a targeted response aimed at containment and clearance of a specific antigen, allowing titration of nonspecific, and potentially host injurious, alveolar inflammation. Because of the anatomic location of elements of pulmonary innate immunity, these responses typically precede those of adaptive immunity. However, complex