Human Milk: Immunologic-Nutritional Relationships

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INTRODUCTION

The incidence and severity of certain bacterial and viral infections of the intestinal tract and respiratory system are significantly lower in breast-fed compared to non-breast-fed infants¹⁻⁴ (TABLE 1). The protection provided by breast feeding includes a contraceptive effect and a lesser exposure of breast-fed infants to infectious agents (TABLE 2). Even when the breast-fed infant ingests pathogenic bacteria during nursing, often no enteric symptoms develop.³⁻⁵ These observations lead to the prediction that the protection afforded by human milk was due to antimicrobial agents in human milk. That prediction proved to be correct.⁶ It is now recognized that the spectrum of protective agents in human milk includes not only preformed direct acting antimicrobial agents but also 1) agents created through limited digestion of substrates in milk, 2) antiinflammatory factors, and 3) immunostimulants (TABLE 2).

Direct Acting Antimicrobial Agents in Human Milk

The antimicrobial system in human milk consists of a complex group of biochemical agents. Although they differ widely in their structures, they commonly occur at other mucosal sites (TABLE 3), persist throughout lactation,⁷⁻¹⁰ resist digestion in the alimentary tract, and protect by noninflammatory mechanisms.

Lactoferrin

Lactoferrin, a member of the transferrin family of iron-binding glycoproteins, has a Mr of about 80 kDa and two lobes, each of which contains a ferric iron binding site.¹¹ Lactoferrin is one of the most prominent whey proteins in human milk.⁷⁻¹⁰ Although most of the protein is intact, a 20-kDa fragment corresponding to half of the N lobe of the protein has recently been found in human milk.¹² About 80% of the iron-binding sites on human milk lactoferrin are unsaturated. The apoprotein competes with siderophilic bacteria for ferric iron and thus interferes with the multiplication of those microorganisms.¹³ A great deal of the apoprotein is ingested by breast-fed mature infants during the first several months of life¹⁰ (TABLE 4). A number of fecal excretion studies suggest that some

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TABLE 1. Infectious Disorders That Are Prevented or Lessened by Breastfeeding

1. Respiratory infections
a. Respiratory syncytial virus
b. Haemophilus influenzae
c. Streptococcus pneumoniae
d. Enteroviruses
2. Enteric infections
a. Poliovirus
b. Other enteroviruses
c. Escherichiae coli
d. Shigella
e. Salmonella
f. Rotavirus
g. Cryptosporidium

of the ingested apoprotein persists throughout the alimentary tract,¹⁴⁻¹⁶ although an increase in the production of lactoferrin at the mucosal site of the infant is an alternative possibility.¹⁵ In addition to the antimicrobial effects, lactoferrin may positively influence cell growth¹⁷ and negatively effect inflammation.^{18,19} Comparatively little is known, however, about the fate and functions of this factor in the recipient.

Lysozyme (N-Acetylmuramide Glyconohydralose)

Lysozyme, a 12-kDa single chain protein, is found in relatively high concentrations in external secretions including human milk (TABLE 3). Lysozyme lyses susceptible bacteria by hydrolyzing β -1,4 linkages between N-acetylmuramic acid and 2-acetyl-amino-2-deoxy-D-glucose residues in cell walls.²⁰ Large amounts of this enzyme are provided to the infant by breast feeding.⁷⁻¹⁰ The agent is relatively resistant to digestion by trypsin²¹ or denaturation due to acid.²² It was thus anticipated that this enzyme in human milk would persist in the gastrointestinal tract of the recipient infant. The amount of lysozyme that is excreted in the stools of human-milk-fed, low birth weight infants is in fact about eight times that in cow's-milk-fed infants.¹⁵ Otherwise, the *in vivo* fate and functions of the agent remain to be determined.

Secretory IgA

Secretory IgA comprises over 90% of the immunoglobulin in human milk.⁷⁻¹⁰ This immunoglobulin is formed through two intricate processes. The first is the entero-bron-

TABLE 2. Types of Protection Due to Breastfeeding

1. Poorer conveyance of pathogens by human milk.
2. Contraceptive effect of breast feeding.
3. Direct acting host defense agents in human milk.
4. Host defense agents created in the recipient's alimentary tract by limited digestion of substrates in ingested human milk.
5. Promotors of protective microflora.
6. Antiinflammatory factors in human milk.
7. Immunostimulants in human milk.

TABLE 3. Characteristics of Host Protective Agents in Human Milk

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1. Diverse group of compounds common to mucosal sites.
 2. Agents adapted to survive in the alimentary tract.
 3. Agents interact with each other and with defense factors in the infant.
 4. Factors protect by noninflammatory mechanisms.
 5. Agents decrease attack rate and/or lessen the injurious effects of infections.
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cho-mammary pathway for the migration of B lymphocytes that mature into polymeric IgA-producing plasma cells that populate the subepithelial zones.²³⁻²⁶ The second is the binding of polymeric IgA to polymeric immunoglobulin receptors (membrane bound secretory component) located on the basolateral plasma membranes of epithelial cells of the mammary gland and the subsequent transport of polymeric IgA complexed with a major fragment of its receptor through the epithelial cell and secretion of the assembled complex into milk.^{27,28} These processes may be regulated by hormones produced late in pregnancy or during lactation²³ and by cytokines.^{29,30} The specificities of secretory IgA antibodies in human milk are a reflection of the enteric and respiratory antigens that trigger the migration of B lymphocytes into the entero- and broncho-mammary pathways. For example, a bacterial antigen taken up by M cells overlying Peyer's patches is recognized by B cells that bear specific antibodies against the antigen. Under the influence of interleukin 5, the isotype of the cell surface antibodies switches from IgM to IgA. These IgA-bearing B cells then enter afferent lymphatics and migrate sequentially to mesenteric lymph nodes, the cisterna chyli, thoracic duct, and systemic circulation and then home to the mammary gland where they undergo terminal maturation to plasma cells that produce polymeric IgA antibodies against the original eliciting antigen. Therefore, secretory IgA antibodies are directed against many important epitopes of enteric and respiratory pathogens.³¹ Considerable amounts of these antibodies are ingested by the nursing infant particularly during the first months of complete breast feeding⁷⁻¹⁰ (FIG. 1). The amount of IgA which is excreted in the stools of low birth weight infants fed human milk is about 25-fold over that found in infants fed cow's milk preparations.¹⁵ Furthermore, evidence is mounting that protection by breast feeding against enteric pathogens such as enteropathogenic *Escherichiae coli* or *Vibrio cholerae* is in large part due to specific secretory IgA antibodies in human milk.^{32,33}

Fibronectin

Fibronectin, a high molecular weight protein that facilitates the uptake of many types of particulates by phagocytic cells, is present in human milk (mean concentrations in colostrum, 13.4 mg/L).³⁴

TABLE 4. Major Antiinflammatory Agents in Human Milk

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1. Agents that double as direct protective agents.
 2. Antioxidants.
 3. Enzymes that degrade inflammatory mediators.
 4. Anti-enzymes.
 5. Cytoprotective agents.
 6. Modulators of leukocyte activation.
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Oligosaccharides-Glycoconjugates

Human milk is rich in carbohydrate moieties, some of which protect against bacterial pathogens.³⁵⁻³⁹ They include monosialogangliosides that are receptor analogues for the heat labile toxins from *E. coli* and *V. cholerae*, fucose containing oligosaccharides that inhibit the binding of the classical strain of *V. cholerae*, and mannose containing high molecular weight glycoproteins block the binding of the El Tor Strain of *V. cholerae*. Glycoproteins as well as glycolipids interfere with the binding of CFA/II fimbriae on enterotoxigenic *E. coli* onto epithelial cells. Moreover, human milk oligosaccharides interfere with the adherence of *Streptococcus pneumoniae* and *Haemophilus influenzae*. GlcNac(β1-3) Gal-disaccharide units may aid in blocking the attachment of *Streptococcus pneumoniae*. The nonnutritive functions of other carbohydrate compounds in human milk is still undetermined.

Leukocytes

Living white blood cells are present in human milk. The concentrations of these leukocytes are highest in the earliest phase of lactation and gradually decline during the

FIGURE 1. The quantities of lactoferrin, secretory IgA, and lysozyme ingested by mature breast-fed infants. The quantity of each ingested protein is expressed as the mean (bar) and the standard deviation (T).

next 2-3 months of milk production.⁷ Neutrophils and macrophages are the most prominent cells in human milk. Although human milk neutrophils are phagocytic, they are unable to adhere to common substrata, move as rapidly as neutrophils from venous blood,⁴⁰⁻⁴³ or respond to chemotactic agents.⁴² In contrast, macrophages in milk are far more motile than their counterparts, the blood monocytes.⁴³ Human milk macrophages have also been found to produce toxic oxygen radicals⁴⁴ and the protein products of the class I and II genes in the major histocompatibility complex.⁴⁵

Lymphocytes are also found consistently in human milk. They are principally T cells and the relative frequencies of the major subsets of T cells (CD3⁺4⁺ and CD3⁺8⁺ cells) in human milk are similar to those in human blood.⁴⁶ The cytotoxic responses of these cells are poor,⁴⁷ but the cells are capable of generating certain lymphokines including interferon-γ and monocyte chemotactic factor.^{48,49} The *in vivo* role of these cells remains to be explored.

Protective Factors Produced by Limited Enzymatic Digestion of Substrates from Human Milk

Human milk may also protect by supplying substrates that are partially digested in the recipient's alimentary tract to produce defense factors. Fatty acids and monoglycerides

produced from milk fats by lipases in milk or from the recipient are able to disrupt enveloped viruses.^{50,51} These antiviral lipids may aid in preventing coronavirus infections of the intestinal tract.⁵² They may also defend against intestinal parasites such as *Giardia lamblia*.⁵³ Similar antimicrobial lipids are generated from fat found in cow's milk. The second example is the production of β -casomorphins from ingested human κ -casein.⁵⁴ These peptide fragments have opioid effects,⁵⁵ but the agent has also been found to be an immunostimulant.⁵⁶

Fragments of human lactoferrin have also been demonstrated in the stools of human milk-fed infants.¹⁵ A 20-kDa fragment of lactoferrin has been described in human milk,¹² but the multiplicity of fragments in the stools suggests that apolactoferrin from human milk feedings or produced in the alimentary tract is partially cleaved in the gastrointestinal tract of the recipient. Similar fragments of lactoferrin have been demonstrated in the urine of those infants⁵⁷ suggesting that they originated from those produced in the gastrointestinal tract. The role of these fragments in those excreta is undetermined.

Finally, it is also possible that other immunologic factors are compartmentalized in human milk and are released during intestinal digestion. This is likely given the complex physical structure of human milk.

Promotion of Protective Microflora

It has been known for many years that human milk contains growth promoters of the dominant resident bacteria in the lower intestinal tract of breast-fed infants.⁵⁸ Certain oligosaccharides promote the growth of lactobacilli in the lower intestinal tract of the infant. Acetic acid produced by those bacteria in turn inhibits the multiplication of bacterial pathogens such as *E. coli*, salmonella, or shigella.

Antiinflammatory Effects of Human Milk

One of the extraordinary features of the protection afforded by human milk is the virtual absence of clinical signs of inflammation during the process. This may be due in part to the more rapid elimination or neutralization of microbial pathogens by defense agents from human milk, but other features of human milk suggest that this is not the sole explanation. Phlogistic agents and the biochemical systems that give rise to them are poorly represented in human milk.^{59,60} Moreover, human milk contains a host of anti-inflammatory agents^{59,60} (TABLE 4). Some of these antiinflammatory agents, such as lactoferrin, secretory IgA, and lysozyme, double as antimicrobial factors. Like the antimicrobial factors, they are well adapted to operate in the hostile environment of the alimentary tract. In addition, two of the agents, α -tocopherol⁶¹ and β -carotene,⁶² are absorbed into the circulation where they may have systemic effects.

Inducers of Immunity in Human Milk

A number of experimental observations suggest that human milk actively as well as passively protects the recipient infant. Several reports claim that the production of IgA at mucosal sites is enhanced by human milk.^{15,57,63-66} Although in most of those studies it has been difficult to exclude the effect of passively transferred secretory IgA, in two investigations the excretion of IgA was increased in the urinary tract,^{57,66} a system far removed from direct contact with human milk. Furthermore, in one report the urinary excretion of secretory component was also remarkably increased in infants fed human

milk.⁵⁷ Since the Mr of these proteins far exceeds the size of molecules filtered by glomeruli, and since neither secretory component nor secretory IgA are specifically transported from the systemic circulation into epithelial cells, it is most likely that human milk feedings stimulated the synthesis of secretory component by epithelial cells in the urinary tract and that this in turn enhanced the transport of secretory IgA into the urine of the infants. The components in human milk that may be responsible for such an enhancement are undetermined at this time.

The second piece of evidence is the response of breast-fed infants to respiratory syncytial virus (RSV).⁶⁷ As compared to infants who were not breast fed, serum levels of interferon- α were strikingly increased in breast fed infants in the first 2–4 weeks after RSV infection. Since there is little interferon- α in human milk, it seems likely that human milk is able to prime leukocytes in the host to produce that cytokine. In addition, there is some evidence that the plasma concentrations of fibronectin appear to be higher in breast-fed than non-breast-fed infants³⁴ (237 and 17/mg/L, respectively). Since the amount of ingested fibronectin is not sufficient to account for the increment in plasma fibronectin that has been observed, it seems likely that human milk induces the synthesis of that opsonic protein in the infant.

If human milk stimulates certain defense systems in the infant, one might predict that the effects might lead to long lasting resistance. Supporting epidemiologic evidence for that premise has been mounting for the past several years. The incidence of juvenile diabetes mellitus appears to be less among children who have been breast fed during infancy.⁶⁸ In addition, a recent retrospective analysis suggests that breast feeding lessens the risk from lymphomas.⁶⁹ In each of these retrospective studies, considerable reliance has been placed upon the ability of mothers to recall the type and duration of feeding given to their offspring; yet recall of events that transpired many years beforehand may be suspect. Undoubtedly, prospective studies of the possible long-term protective role of human milk will be required to further explore the possible long-term immunologic benefits of human milk.

The last piece of evidence comes from the discovery of immunostimulators in human milk. It was previously noted that human milk contains a high concentration of α -tocopherol. In addition to its antioxidant effects, the vitamin is known to stimulate the development of immunity.^{70–73} More recently it was reported that agents in the human milk activate the random motility of human monocytes. The discovery emanated from observations that macrophages from human milk are more motile than monocytes from human blood.⁴³ It was therefore predicted that human milk would contain chemokinetic agents for human monocytes. In subsequent investigations, it was found that the rate of movement of blood monocytes was increased by incubating them in human milk.⁷⁴ The activators of monocytes were heat-sensitive, acid-resistant proteins. Three peaks of chemokinetic activity were found by column chromatography to correspond to 50–55, 20–25, and 15–20 kDa. Furthermore, the chemokinetic activities were significantly decreased by antibodies to one of the major monokines, tumor necrosis factor- α . The identity of these chemokinetic agents is still uncertain, but it is possible that one or more of the activators may be tumor necrosis factor- α , immunologically similar agents, or agents that stimulate the production of that cytokine.

Coda

Human milk emerges as a complex system of nutrients, some of which double as defense agents; preformed host defense agents that are comparatively resistant to digestion; defense agents produced by partial digestion of substrates from human milk; anti-inflammatory factors; growth factors for protective microflora; and agents that speed the

maturation of defense systems. The results of metabolic balance studies in breast-fed infants provide an insight into the ultimate fate of the members of the defense systems in human milk. It appears that the bulk of these lipids, carbohydrates, and proteins must be utilized to meet the nutritional requirements of the breast-fed infant.⁷⁵ Given the resistance of many of the components to digestive processes, it appears that the agents are not completely degraded until they reach the more distal regions of the alimentary tract, or after they are absorbed for systemic use. The precise ways in which these efficient defense systems in human milk operate in the recipient to directly fend off infections, minimize phlogistic reactions, or speed the maturation of the infant's defense systems should be the subject of intense study during the next decade. These investigations may well include the binding of defense agents to epithelial receptors, the consequences of receptor binding, the systemic absorption of certain components and their distribution and fate in the body, the site of and rate of digestion of the components, the specificity of the *in vivo* actions of the components directly upon microbial pathogens, the *in vivo* role of antiinflammatory agents, and the nature of and short- and long-term effects of immunologic stimulators upon the recipient.

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