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Immunological Components of Milk: Formation and Function

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I. INTRODUCTION

Among the external secretions, milk is unique in its complexity and high concentrations of mucosal immune products. This chapter explores the nature of that uniqueness and attempts to explain its basis. Throughout the chapter, we emphasize human milk since it differs significantly from that of other nonprimate species. However, because bovine milk commonly is consumed by humans, particularly infants and children, we contrast human and bovine milk when major differences are known. The immunological outcomes of breast-feeding in the infant are discussed in Chapter 52.

The high degree of complexity of human milk, relative to the other external secretions, apparently has evolved to provide key factors for the nutritional, metabolic, and immunological needs of the infant. If the lactating woman is nourished adequately, her milk will contain essentially all the nutrients required by the infant for at least the first 6 months of life. In addition, many immunological factors are present in human milk, presumably to protect the lactating mammary gland and the nursing infant from pathogenic microorganisms. The immunological constituents of milk must enhance the probability that maternally derived nutrients will be transferred to the infant without contamination or degradation, so they can be used optimally for the growth and development of the infant.

Another source of complexity of human milk is the dynamic change in composition that occurs during lactation. In this sense, milk is really a series of secretions that is produced as lactation progresses and the infant matures. An interesting speculation is that the changes in milk composition are timed to respond to changing needs of the infant, whose own mucosal immune system is developing rapidly.

In trying to understand the function of the immunological factors in milk, we must recognize that the *in vivo* fate and functions have been demonstrated to date for a limited number of the factors. In particular, limited information exists concerning the distribution, survival, and function of immunological factors within the infant.

Finally, evidence suggests that the immunological impact on the infant of the feeding of human milk may not be totally passive in nature. Some milk factors may interact with factors produced by the infant, whereas others may act as stimuli for the development of the infant's own mucosal immune system. Thus, milk may be part of a dynamic mother–infant interaction that supports the newborn infant's growth and immunological development to a level that enhances the chances of survival in the extrauterine environment.

II. ANATOMY, CELL BIOLOGY, AND PHYSIOLOGY OF MILK PRODUCTION

A. General Features

The human mammary gland is a compound tubuloalveolar organ. The glandular secretions empty into ducts that coalesce as they course through the stroma of the breast toward the nipple. During pregnancy, the number and size of the alveolar clusters increase until, at parturition, 70% of the gland is composed of parenchymal tissue. The process of lactation is modulated by hormones including prolactin, insulin, and growth hormone. The release of some of these hormones, particularly prolactin, is triggered by a neurosensory–endocrine pathway that is initiated by nursing. The autocrine and paracrine events that control lactation remain poorly defined.

B. Secretory Pathways

Four pathways exists for the secretion of milk components. The first one is responsible for secretion of many of the aqueous components of milk including casein, α-lactalbumin, and lactose. Caseins, after translation, are transferred to the Golgi apparatus, where they are phosphorylated or glycosylated. As these proteins pass from the terminal cisternae of the Golgi compartment into secretory vesicles, their concentration increases to the millimolar range, causing aggregation of the casein molecules into micelles (Morr *et al.*, 1971). The secretory vesicles migrate to the apical membrane, fuse with it, and release their contents by exocytosis. One prominent milk protein, α-lactalbumin, forms a complex with the enzyme galactosyltransferase in the Golgi, where the complex catalyze the synthesis of lactose. The osmotic activity of this disaccharide draws water and presumably ions into the Golgi, providing the driving force for fluid secretion.

The second or apocrine pathway is responsible for secre-

tion of milk fat. The substrate for milk fat synthesis derives from two sources. Fatty acids are synthesized from glucose by mammary alveolar cells that contain fatty acid synthetase. These fatty acids have somewhat shorter chain lengths (10–16 carbons) than those synthesized in adipose tissue because of the presence of a mammary gland-specific enzyme called thioesterase II. In addition, plasma triacylglycerols are cleaved within the mammary gland by the enzyme lipoprotein lipase. The resulting fatty acids are transported into the mammary alveolar cells, where they are reesterified with glycol to make the neutral fat molecules that coalesce to form milk fat globules. Alveolar cells have a columnar shape with copious endoplasmic reticulum surrounding the nucleus in the basal region. During milk secretion, the Golgi apparatus and secretory vesicles become more numerous toward the apical pole. Abundant cytoplasmic lipid droplets enlarge as they move toward the luminal end of the cell. As they press into the apical plasma membrane, the droplets are released into the milk as membrane-bound milk fat globules (Moyer-Mileur and Chan, 1989). Other globules that are formed during this process contain fewer lipids but larger amounts of other cytoplasmic constituents (Patton and Huston, 1985).

The third pathway transports certain small molecules including sodium, potassium, chloride, and glucose across the apical membrane of the cell. The secretion of the monovalent ions is effected by the electrical gradient across this membrane.

The fourth pathway transfers proteins and possible other substances from the interstitial space to the lumen using receptors and intracellular vesicles. This mechanism will be considered in the description of the formation of secretory IgA (SIgA) in milk via polymeric immunoglobulin receptors.

Finally, in addition to soluble components, certain cells and cellular membranes enter human colostrum and milk. Epithelial cells or their membranes are shed directly into milk. Most B cells that home to the mammary gland remain sessile, whereas many T cells, macrophages, and neutrophils that have entered the lamina propria pass through the intercellular junctions of alveolar cells into the milk (Brandtzaeg, 1983). The mechanisms that attract the leukocytes to the mammary gland and trigger the migration of those cells into the milk are considered in a subsequent section.

C. Milk Ejection

Milk secretions are stored in the alveoli and small ducts until they are ejected during nursing. Epithelial cells of the alveoli and ductules are surrounded by contractile basket-

like epithelial cells. The ejection of milk from the breast is mediated by neuroendocrine events that culminate in the contraction of those myoepithelial cells. As a result of stimulation of sensory nerves at the nipple and areola during nursing, oxytocin is released from the hypothalamus into the posterior lobe of the pituitary gland and then into the peripheral circulation. Oxytocin triggers myoepithelial cells to contract, forcing milk into the larger ducts and finally through the orifice of the nipple.

III. COMPOSITION OF MILK

A. Cellular Elements in Milk

Human milk from early in lactation differs from most other external secretions because it contains viable leukocytes. The concentration of leukocytes is highest in colostrum and declines rapidly during the first months of lactation. Table I displays estimates of the numbers of neutrophils, macrophages, and lymphocytes in human milk during the first months of lactation. These numbers are based on morphological characterisitics. However, distinguishing neutrophils from macrophages in human milk is difficult because the morphology of both cells is dominated by the large amount of lipid-containing vesicles in the cytoplasm (Smith and Goldman, 1968). The mechanism by which maternal leukocytes enter the milk is poorly understood. However, one potentially important clue to this process is the finding that essentially all these cells have surface markers or physiological features of activated cells. Since most of the surface markers of activation on milk leukocytes are also present on leukocytes found in sites of inflammation, and are known to be important in homing and egress of leukocytes from the vascular compartment, the mechanism of migration of leukocytes into the milk may be similar to that involved in inflammation. Although the array and mechanisms of production of inflammatory mediators in the lactating mammary gland are not well understood, several cytokines that may be involved in leukocyte migration have been detected in human milk, as described in later sections of this chapter. The following sections discuss our current knowledge of the morphology and in vitro function of the milk leukocytes.

1. Macrophages

Macrophages may have been first photographed in human milk by the French microscopist Alfred Donné (1844). How-

Table I Estimated Mean (std) Concentration ($\times 10^9$/ml) of Leukocytes in Human Milk by Phase of Lactation[a]

	2–3 days	4 wk	24 wk	52 wk
Macrophages/neutrophils	3.6 (2.7)	0.06 (0.12)	0.04 (0.09)	<0.01
Lymphocytes	0.2 (0.1)	0.02 (0.03)	0.01 (0.02)	<0.01

[a] Adapted with permission from Goldman et al. (1982).

ever, little attention was paid to the cells in milk until Smith and Goldman (1968) described milk cells with the morphology and phagocytic activity consistent with activated macrophages. The concentration of macrophages in early milk is usually greater than that of their counterparts in peripheral blood, the monocytes.

More recent studies have demonstrated that the milk macrophages are more motile than their counterparts in blood (Özkaragöz et al., 1988; Mushtaha et al., 1989) and display a pattern of surface markers associated with activation (Keeney et al., 1993). These cells also actively produce toxic oxygen radicals (Tsuda et al., 1984). Attributing other activities to the milk macrophages is difficult since most studies of milk leukocytes have used unseparated cells. The role of the milk macrophage in vivo has not been established.

2. Polymorphonuclear Leukocytes

Early in lactation, the concentration of neutrophils in milk approaches that in peripheral blood (Table I). Neutrophils in human milk have been demonstrated to be phagocytic (Smith and Goldman, 1968). However, the adherence, response to chemotactic factors, and motility of these cells are less than those of neutrophils from peripheral blood (Thorpe et al., 1986; Özkaragöz et al., 1988). Studies of surface markers suggest that some of these functional features may be the result of prior activation of the neutrophils (Keeney et al., 1993). Activation of neutrophils may occur during the process of egression from the vascular space, may relate to the process by which large numbers of milk globules are engulfed by neutrophils in milk, or may be the result of exposure to cytokines demonstrated to exist in milk (see subsequent text).

3. Lymphocytes

Although the concentration of lymphocytes in human milk is small relative to that in peripheral blood, these cells are consistently present in milk obtained during the first few months of lactation. Approximately 80% of milk lymphocytes are T cells (Wirt et al., 1992). The precise distribution of T-cell subpopulations in milk lymphocytes is controversial, since different investigators have reported numbers of CD4$^+$ and CD8$^+$ cells similar to those in peripheral blood (Keller et al., 1986), a CD8$^+$-cell predominance (Richie et al., 1982), or moderate increase in the proportion of CD8$^+$ cells relative to peripheral blood (Wirt et al., 1992). These differences may be the result of selection of certain subsets during the fractionation of milk or of the analytic limitations of direct fluorescent microscopy. These problems were avoided in the last study by using flow cytometry of unfractionated milk (Wirt et al., 1992). As in the case for other milk leukocytes, the increased display of certain surface phenotypic markers, including CD45RO, CD25 (IL-2R), and HLA-DR, suggests that the T lymphocytes are activated memory cells (Wirt et al., 1992).

Milk lymphocytes can be activated to proliferate using mitogens, but their responses are weaker than those of peripheral blood T cells (Parmely et al., 1976; Goldblum et al., 1981). The spectrum of antigen-specific responses, as measured by proliferation, is thought to differ from that of

peripheral blood lymphocytes from the same donor (Parmely et al., 1976), but the T-cell receptor repertoire of milk T lymphocytes has not been investigated. Although evidence exists for the production of interferon (Emodi and Just, 1974; Keller et al., 1981; Bertotto et al., 1990) and monocyte chemotactic factor (Keller et al., 1981), the full array of cytokines produced by milk cells has not been determined.

B. Particulate Structure of Milk

In addition to viable cells, several different types of particles are suspended in human milk, including casein micelles, globules packed with lipid, and globular structures containing less lipid but more cytoplasmic structures (Patton and Huston, 1985). With low speed centrifugation, some of these particles sediment with the cells whereas many of the membrane-bound lipid-filled particles rise to the surface and coalesce. Understanding this particulate structure of milk may be important, since several studies suggest that some host defense factors are compartmentalized within these structures. For instance, centrifugation of human milk causes the concentration of lysozyme to increase approximately fivefold over the value obtained by sampling milk prior to centrifugation (Goldblum et al., 1975). Crago et al. (1979) demonstrated that some of the SIgA antibodies in human milk are contained in lipid-filled particles. Little is known about the direct effects on host defenses of the particles suspended in human milk, although some of them may be neutrophil activators (Keeney et al., 1992) or may interfere with the attachment of enterobacteria to epithelial cells (Schroten et al., 1992).

C. Soluble Factors in Milk

1. Proteins and Peptides

a. Immunoglobulins and Ig transport fragments. The major class of immunoglobulins in human milk is IgA. In contrast, mature cow's milk contains predominantly IgG. The structure and function of SIgA, which makes up at least 80% of the milk IgA, is discussed in Chapters 7 and 11, respectively. Chapter 21 considers the distribution and characteristics of the cells that produce immunoglobulins within the mammary gland. The demonstration of a very high density of IgA1- and IgA2-producing cells in the lactating mammary gland (Brandtzaeg, 1983) helps explain why human colostrum and milk contain the highest concentrations of SIgA of any secretions. The high proportion of SIgA in human milk of the IgA2 isotype (~40%) relative to plasma (10%) also must be related to the isotype distribution of these cells in the mammary gland. These findings also provide evidence that most of the IgA secreted into the milk is produced locally within the breast, rather than transported from the plasma.

The mechanism of antigenic sensitization and migration of IgA-committed B cells into the mammary gland is considered in several earlier chapters. Briefly, current evidence indicates that many of the IgA antibody responses detected in human milk originate from antigenic stimulation at specialized mucosal sites in the intestinal and respiratory tracts

(Goldblum *et al.*, 1975; Roux *et al.*, 1977). IgA-committed B cells emerging from mucosal sites, such as Peyer's patches in the small intestine, migrate preferentially to other mucosal sites, including the lactating mammary gland. Migration to the mammary gland is under hormonal regulation (Weisz-Carrington *et al.*, 1978). The wide array of specific antibodies found in human milk suggests that some of these cells are derived from memory B cells rather than from recent antigenic exposure. In the breast, B cells mature into plasma cells, the predominant product of which is polymeric IgA.

As in other secretory mucosae, transport of immunoglobulins into colostrum and milk is accomplished predominantly by the polymeric immunoglobulin receptor (PIgR). Chapter 10 describes in detail the mechanism by which PIgR mediates specific binding, endocytosis, and transcellular transport of immunoglobulins. Proteolytic cleavage of PIgR at the apical membrane of the mammary alveolar cell releases the polymeric IgA molecule, covalently complexed to a fragment of PIgR termed secretory component (SC). This complex is called SIgA.

Some of the PIgR molecules are transported and cleaved by the epithelial cells without any attached immunoglobulin. The resulting proteolytic fragment, free SC, is also present in high concentrations in human milk. The function of free secretory component has not been established clearly. One study (Wilson and Christi, 1991) suggests that these molecules may be able to inhibit the enzyme phospholipase A_2, a function that could reduce inflammatory reactions along mucosal surfaces as well as, perhaps, the fluid accumulation produced by some intestinal pathogens (Peterson and Ochoa, 1989).

From this brief outline of the immunogenesis of SIgA, we can deduce that this system is well adapted for the production, and secretion into milk, of specific antibodies against pathogenic microorganisms to which the mother's mucosal immune system has been exposed. Since the mother–infant pair normally shares many environmental exposures, this system may be ideal for protecting the infant from potential pathogens entering the environment. As indicated in Chapter 52, several epidemiological studies have shown that the presence in milk of specific SIgA antibodies against enteric pathogens diminishes the incidence or severity of diarrhea caused by bacterial and viral organisms.

Immunoglobulins of the other major isotypes also are found in human milk, although at lower concentrations than in plasma. IgM in milk is in its typical pentameric form, although some of the molecules have noncovalently attached SC, suggesting that IgM is transported into the milk via the PIgR. However, the binding of free SC in milk could generate the same complexes. The antibody specificity of IgM in milk has not been tested extensively, but seems to parallel that of IgA when examined. The presence of IgM antibodies with attached SC should be considered when designing studies of SIgA in human milk, since detection systems based on anti-SC antibodies also may detect secretory IgM (Mellander *et al.*, 1985).

In cow milk, IgG is the major isotype. However, only small amounts of each of the subclasses of IgG have been detected in human milk. The relative proportion of IgG4 is greater in milk than in serum, suggesting that more of this isotype may be produced locally or selectively transported by the interstitium (Keller *et al.*, 1983). This hypothesis has not been borne out in a more recent investigation (Mehta *et al.*, 1991). Nonetheless, the total amount of IgG4 is so low that attributing biological functions to this or the other IgG isotypes will be difficult.

The concentration of IgD in human milk is very low, even relative to serum concentrations (Keller *et al.*, 1984). IgE is essentially absent from human milk (Underdown *et al.*, 1976).

Antibodies against a large number of microbes and specific antigens have been detected in human milk. Table II provides a summary of some of these specificities. This list should not be considered complete or thought to imply functional significance of the presence of antibodies of particular specificity, since it respresents a summary of studies from groups with particular interest in the antigens tested.

The stability of the immunoglobulins in human milk has been the subject of a number of studies, most of which have concentrated on SIgA. The majority of the IgA molecules in postnatal milk seems to be intact, based on Western blots using anti-α chain antibodies as developing reagents (Cleveland *et al.*, 1991). Thus, during active lactation, little degradation must occur within the mammary gland. Following the fate of the SIgA within the infant is difficult. Fecal excretion of SIgA has been examined in low birth weight and full-term infants (Schanler *et al.*, 1986; Prentice *et al.*, 1987; Davidson and Lönnerdal, 1987). The finding that SIgA excretion in the

Table II Antimicrobial SIgA Antibodies Detected in Human Milk

Bacteria and bacterial toxins	Viruses	Parasites/fungi
Enteric		
Clostridium difficile	Polio viruses	*Giardia lamblia*
Escherichia coli	Rotaviruses	*Candida albicans*
Klebsiella pneumoniae		
Salmonella sp.		
Shigella sp.		
Vibrio cholerae		
Respiratory		
Haemophilus influenzae	Respiratory syncytial virus	
Streptococcus pneumoniae	Influenza viruses	

feces was 30 times higher in low birth weight infants receiving human milk than in similar infants fed cow milk formulas strongly suggested that a portion of the ingested milk survived the whole gastrointestinal tract. However, when expressed as a proportion of the SIgA fed, approximately 9% of the SIgA was recovered as SIgA (Schanler et al., 1986).

The function of mucosal immunoglobulins is discussed in detail in Chapter 11. Of potential importance for milk immunoglobulins is the amplification of the effect of other milk defense factors by IgA. For instance, some of the lactoferrin in milk is found complexed with IgA (Arnold et al., 1977). These complexes may have enhanced activity since they can be targeted to surfaces of pathogenic microorganisms where lactoferrin could function to chelate selectively the iron needed by the microorganism for growth. Microorganisms that are resistant to the lytic action of lysozyme may become more susceptible in the presence of SIgA and complement (Adinolfi et al., 1966). Galactosyltransferase enzyme also complexes tightly with IgA, although the functional significance of these complexes is not known (McGuire et al., 1989).

b. Lysozyme. Lysozyme, a 12-kDa single polypeptide, catalyzes the hydrolysis of β1–4 linkages between N-acetylmuramic acid and 2-acetylamino-2-deoxy-D-glucose groups in bacterial cell walls, leading to direct lysis of susceptible bacteria, predominantly those without an extensive cell wall. As indicated earlier, interaction with IgA and complement may expand the antimicrobial range of activity of this secretory enzyme.

The quantity of lysozyme supplied to the infant each day are presented in Table III. Although the concentration varies during lactation the amount delivered appears to remain relatively constant for at least the first 4 months (Butte et al., 1984). These concentrations are among the highest of any secretion (Jolles and Jolles, 1967).

The relative stability of lysozyme against acid denaturation and tryptic digestion makes it well suited to function in the gastrointestinal tract of the recipient infant. However, the fate of the ingested lysozyme is not clear. Low birth weight infants who are fed human milk excrete about eight times more lysozyme in their stool than do cow milk-fed infants (Schanler et al., 1986).

c. Lactoferrin. Lactoferrin is the whey protein with the highest concentration in human milk. The daily amount of lactoferrin ingested by the infant at various stages of lactation are shown in Table III. A gradual decline is seen in the amount transferred to the infant, beginning soon after initiation of lactation.

A single chain glycoprotein of 79 kDa, lactoferrin consists of two globular lobes, each with two domains surrounding a binding cleft for an atom of ferric iron and a bicarbonate ion. The major function of lactoferrin is the chelation of iron. In that respect, apolactoferrin robs the siderophilins of microorganisms of iron that is essential for their growth (Spik et al., 1978). The lactoferrin in human milk is well suited for this role, since 90% of the molecules are devoid of iron (Fransson and Lönnerdal, 1980). Several other functions have been suggested for lactoferrin. The finding of a specific receptor for lactoferrin on the mucosa of the upper bowel suggested that lactoferrin might enhance the uptake of milk iron by the infant (Davidson and Lönnerdal, 1988). The low degree of iron saturation in milk lactoferrin makes this function seem unlikely, unless iron was transferred from other compartments during the digestion process. Some evidence also suggests that lactoferrin has trophic effects on enterocytes (Nichols et al., 1987) and may inhibit complement activity (Kijlstra and Jeurissen, 1982).

Few studies have been done on the disposition of milk lactoferrin in the infant. One study was carried out on infants with enterostomies, which allowed the gut contents to be recovered before entering the colon (Hambreus et al., 1989). The results indicated that 9–32% of the ingested lactoferrin could be recovered from this site. Low birth weight infants fed a human milk preparation excreted almost 200 times more lactoferrin in their stool than similar infants fed a cow milk-based formula (Schanler et al., 1986). However, only 3% of the ingested lactoferrin was recovered in the fecal sample. In addition, a major portion of the excreted lactoferrin was partially digested, resulting in molecules of unknown activity (Goldman et al., 1990). Infants fed human milk also had a larger amount of lactoferrin and lactoferrin fragments in their urine (Goldblum et al., 1989); the molecular sizes of those fragments were similar to those in the stools (Goldman et al., 1990). Another study using stable isotope methods also suggested that some lactoferrin derived from the milk may

Table III Quantity (mg/kg/D) of Immune Factors [mean (Std)] Provided by Human Milk[a]

Factor	Phase of lactation (age of infants in months)			
	1	2	3	4
Lactoferrin	275 (75)	190 (80)	167 (70)	120 (25)
SIgA	130 (50)	105 (40)	88 (3)	77 (35)
Lysozyme	4 (3)	5 (3)	4.9 (1.5)	6 (2)

[a] Data modified from Butte et al. (1984).

be absorbed intact from the intestinal tract and then excreted into the urinary tract (Hutchens *et al.*, 1991). If this occurs, the absorbed lactoferrin must be cleared rapidly since human milk ingestion does not increase the serum concentration of lactoferrin (Schanler *et al.*, 1986).

d. Complement components. Many of the components of the classical and alternative complement pathway have been detected in human milk (Ballow *et al.*, 1974; Nakajima *et al.*, 1977). However, with the exception of C3, the concentrations of these factors are very low. The activity of the complement system in human milk is not likely to be great, although interactions with other milk constituents may allow some function (Adinolfi *et al.*, 1966).

e. Bioactive peptides. Several cytokines have been quantified by immunoassays in human milk, including interleukin-1β (IL-1β; Munoz *et al.*, 1990), tumor necrosis factor α (TNF-α; Mushtaha *et al.*, 1989; Rudloff *et al.*, 1992a) and IL-6 (Saito *et al.*, 1991; Rudloff *et al.*, 1992b). The functions of these factors in the infant remain to be elucidated. However, studies of the leukocytes in the milk suggest that some of these cytokines may be active (Söder, 1987; Mushtaha *et al.*, 1989). Of special interest is the finding that incubation of peripheral blood leukocytes with human milk causes monocytes and neutrophils to become activated. Further, addition of neutralizing antibodies against human TNFα abrogated the activating effects of milk on monocytes (Mushtaha *et al.*, 1989).

Some fragments of casein, the β-casomorphines, which are created during proteolysis of casein in the gastrointestinal tract, are biologically active (Teschenmacher, 1987). β-Casomorphines not only have endorphin effects but may be immunoregulatory as well (Parker *et al.*, 1984).

Several different isoforms of prolactin have been demonstrated in human milk that apparently are produced by post-translational modifications in the mammary gland. Although the function of each of these isoforms is not delineated, the basic protein molecule has been found to influence the development of T cells in animal model systems (Chikanza and Panay, 1991; Gala, 1991; Rovensky *et al.*,1991) and to enhance the formation of specific antibodies in serum and milk (Ijaz *et al.*, 1990).

The array of growth factors in human milk includes epidermal growth factor (EGF), insulin, transforming growth factor β (TGF-β; Hooton *et al.*, 1991), and mammary gland derived growth factor (Kidwell *et al.*, 1987). Some of these factors have been postulated to aid in the postnatal development of the mucosal barriers of the intestinal and respiratory tracts. However, the *in vivo* effects of these factors are not well characterized.

2. Carbohydrates and Glycoconjugates

a. Lactobacillus growth factors. Human milk contains high levels of a growth-promoting activity for *Lactobacillus bifidus* var. *Pennsylvania* (György *et al.*, 1974). This activity, which is essentially absent from cow milk, is generated by oligosaccharides (György *et al.*, 1974), glycopeptides, and proteins (Nichols *et al.*, 1975; Bezkorovainy *et al.*, 1979).

Similar activity associated with caseins also may be the result of oligosaccharide moieties on that protein (Bezkorovainy and Topouzian, 1981). The role of these factors in host defense may be related to the predominance of *Lactobacillus* in the bacterial flora in the colon of infants fed human milk. The large amount of acetic acid produced by these organisms suppresses the growth of enteropathogens.

b. Oligosaccharides and glycoconjugates. Human milk is rich in oligosaccharides that appear to be formed in the mammary epithelium by the same galactosyltransferases that glycosylate proteins and peptides, using lactose as the acceptor molecule. Various biological activities have been attributed to the whole group of oligosaccharides (Holmgren *et al.*, 1981) and, more recently, to individually characterized moieties, including fucosylated oligosaccharides that inhibit the hemagglutinin activity of the classical strain of *Vibrio cholerae* (Holmgren *et al.*, 1983) and protect against the heat-stable toxin of *Escherichia coli* (Newburg *et al.*, 1990). Mannose-containing glycoproteins and glycolipids interfere with the fimbria-mediated binding of *E. coli* (Holmgren *et al.*, 1987; Wold *et al.*, 1990). The attachment of *Haemophilus influenzae* and *Streptococcus pneumoniae* to epithelial cells is inhibited by saccharides containing the disaccharide subunit N-acetylglucosamine (1-3)-β-galactose (Andersson *et al.*, 1986). These units may exist as free oligosaccharide or in glycoproteins or peptides. In any case, molecules with these structures may act as false receptors for the lectin-like adherence structures on the microorganism and thereby protect the infant from colonization or infection with these pathogens. Although an *in vivo* role for these oligosaccharides is suggested by animal models (Otnaess and Svennerholm, 1982; Cleary *et al.*, 1983; Ashkanazi *et al.*, 1992) few human studies have been done that pertain to this question.

3. Lipids

a. Unsaturated fatty acids and monoglycerides. Free fatty acids and monoglycerides are produced by the digestion of milk triglycerides by bile salt-stimulated lipases or lipoprotein lipases in human milk. In addition, the lingual and gastric lipase activities of the recipient infant are active on the milk triglycerides (Hosmosh, 1990). The lipid products have several host defense activities including disruption of enveloped viruses (Stock and Frances, 1940; Welch *et al.*, 1979; Issacs *et al.*, 1986; Thromar *et al.*, 1987), which may prevent coronavirus infection in the intestinal tract (Resta *et al.*, 1985). The fatty acids and monoglycerides also may provide some defense against intestinal parasites such as *Giardia lamblia* (Gillin *et al.*, 1983,1985; Hernell *et al.*, 1987).

b. α-Tocopherol and β-carotene. Two vitamins found in human milk (Chapell *et al.*, 1985) also may have host defense activity. High levels of α-tocopherol in milk may serve as an antioxidant, but additionally this vitamin is known to stimulate the development of immunity (Tengerdy *et al.*, 1981; Bendich *et al.*, 1986). β-Carotene, another potent antioxidant, is present in high concentrations in the mammary gland at parturition. This agent is released from the tissue into milk

during the first few days of lactation (Chapell et al., 1985). As a result of the ingestion of α-tocopherol and β-carotene in human milk, the blood levels of these two agents rise substantially in the recipient infant (Chapell et al., 1985; Ostrea et al., 1986). These and other agents in human milk may regulate inflammatory responses and immune functions of the infant.

IV. OVERVIEW OF THE FUNCTION OF HUMAN MILK IN HOST PROTECTION AND CONCLUSIONS

Despite the identification and quantification in human milk of many factors that have the potential to protect the lactating breast and the recipient infant, little currently is known about how these factors function in vivo. Progress in this area has been limited by the types of studies that can be carried out in human infants and by the large species differences in milk composition and function that make experimental animal studies difficult to apply to humans. However, certain patterns of factors may provide clues to unique in vivo function of human milk.

A. Production of Immune Factors by the Breast Is Regulated

In contrast to many mucosal glands, which function on a continuous basis, the mammary gland secretion of immune factors is restricted largely to periods of lactation. The factors that regulate the onset, quality, and quantity of the human milk are only partially understood. Prolactin and other lactogenic hormones are essential for the onset and maintenance of lactation. An array of growth factors including EGF, insulin, and mammary gland derived growth factors that are concentrated in human milk (Kidwell et al., 1987) also may play a role in these processes.

B. Immune Factors in Milk May Prevent Infection without Causing Inflammation

The same proximity of the mucosal surfaces to the external environment that leads to extensive exposure to microorganisms and other antigens allows the mucosal immune system to defend against infections without the need for the extensive inflammatory and phagocytic responses that are typical of the systemic defenses. Thus, if factors in milk can reduce the adherence, colonization, or growth of microorganisms in the infant's respiratory or intestinal tract, the incidence or severity of infection would decrease correspondingly without producing much physiological abnormality in the infant. We have hypothesized previously that a characteristic of the immune system in human milk is the absence of phlogistic factors and the presence of agents with anti-inflammatory activity (Goldman et al., 1986). Demonstrations that infants who receive mother's milk that contains specific antibodies against an enteric pathogen still have culture-proven infections but less diarrhea than those receiving milk without those

antibodies (Glass et al., 1983; also see Chapter 52) are in keeping with this hypothesis. In that respect, the lower morbidity of breast-fed infants infected with rotavirus was not found to be related to the levels of specific antibodies in the milk (Duffy et al., 1986). This result suggests that other agents, including anti-inflammatory factors, may be responsible for some of the protection against certain pathogens.

C. Long-Term Effects of Breast Feeding

Several studies suggest that breast feeding may have effects that last much longer than the breast-feeding period. For instance, breast-fed infants have a lower incidence of juvenile diabetes mellitus (Mayer et al., 1988; Hamman et al., 1988) and Crohn's disease (Koletzko et al., 1989) than those fed formulas. Retrospective analysis also suggests a diminished risk of lymphomas after breast-feeding (Davis et al., 1988). Whether these long-term effects are the result of mucosal immune factors in human milk is unclear. However, speculating that breast-feeding alters the development of the infant's immune system or protects against certain infections during a critical developmental period, thereby preventing illnesses that become manifest later in life, is interesting.

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