Review

Breastfed at Tiffany's

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The importance of breast milk for the growing infant is undisputed; breast-feeding decreases infantile mortality by tenfold and decreases the incidence of infectious diseases. Despite its recognized benefits, the structural richness of breast milk has also impeded the characterization of the multiple effects of milk components on infant physiology. However, the important roles of some components of breast milk are beginning to be dissected. For instance, molecules such as immunoglobulin A (IgA) and milk oligosaccharides protect from gastrointestinal infections and influence the development of the gut microbiota. Deciphering the complex composition of breast milk brings to light multifaceted contributions that combine to make breast milk the ultimate personalized medicine.

Breastfeeding Protects Child and Mother

Breast milk is often described as the gold standard of infant nutrition, because breast milk provides all the macronutrients and vitamins required for the optimal development of the suckling infant. Recent meta-analyses underline the beneficial effects of breastfeeding on short-term child health by decreasing infant mortality and morbidity [1], and on long-term development by reducing the risk for obesity [2]. Health benefits also extend to the nursing mother, because breastfeeding protects against breast cancer [3]. Given the strong impact of breast milk on child health, the WHO recommends breastfeeding exclusively for the first 6 months of life and then using breast milk as a complement to solid food for at least an additional year [4].

More than just a gold standard, breast milk has been shaped by millions of years of evolution that has resulted in a perfect multifunctional fluid. In fact, beyond the supply of nutrients and vitamins, breast milk provides bioactive factors, including immunoglobulins, cytokines, antimicrobial proteins, hormones, and oligosaccharides, which work in concert to fortify mucosal immunity, shape the gut microbiota, stimulate body growth, and even regulate birth spacing in mothers. Breast milk is a rich fluid that fulfills multiple tasks, as discussed in this review.

Breast Milk is a Meal

Let us begin by crunching some numbers. At the beginning of lactation, each human breast produces, on average, 450 g of milk daily. After 15 months, the daily output can still reach up to 200 g of milk, although the amount largely depends on the intensity of breastfeeding [5]. To accommodate this increased energy expenditure, the nursing mother has to increase her daily caloric intake of around 2000 kcal [6] by an additional 500 kcal. This supplement nearly compensates for the 625 kcal required for the daily production of 700–900 g of breast milk. The process itself is efficient, given that the conversion of dietary energy to milk energy has been estimated to reach 80% [7]. Altogether, the energy expenditure bound to milk production is considerable and is comparable to the daily caloric uptake of the brain [8].

The true structural and functional richness of breast milk emanates from the many components included in its fat, protein, and carbohydrate fractions. The composition of breast milk differs largely among mammals. For example, marine mammals have a milk that is rich in fat, fast-growing mammals have a milk that is rich in proteins, and marsupials and primates have a milk

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that is rich in carbohydrates [9]. In humans, the ethnicity and age of nursing mothers have little impact on overall milk composition [10], but the stage of lactation has the largest effect on the individual classes of macronutrient. In general, **colostrum** (see Glossary) has high concentrations of bioactive proteins and oligosaccharides, whereas mature milk has proportionally high levels of lipids and caseins. The maternal diet has little effect on most macronutrient classes, although dietary lipids definitively influence the fatty acid composition of breast milk [11]. Lipids are the largest source of calories, yielding 40–50% of the total dietary energy of breast milk [12]. In addition to triglycerides and cholesterol, the lipid fraction of early milk includes several lipid mediators, such as anti-inflammatory lipoxins and resolvins [13]. Milk proteins are often subdivided into insoluble caseins that build micelles, and soluble whey proteins, which include bioactive proteins, such as secretory IgA (sIgA), lactoferrin, lysozyme, and α-lactalbumin. The carbohydrate fraction comprises lactose (50–70 g/l) and complex oligosaccharides (7–12 g/l). Despite its structural simplicity and the universal occurrence of glucose (Glc) and galactose (Gal) in living organisms, the disaccharide lactose that combines Glc and Gal is only found in mammals.

Breast Milk is a Clock

Lactose is synthesized in the secretory epithelium of the mammary gland by coupling Gal in a β1-4 linkage to Glc. Lactose synthase (EC 2.4.1.22) is a dimer comprising the β1-4 galactosyltransferase B4GALT1 [14] found in the Golgi apparatus of all cells, and α-lactalbumin [15], which is specifically expressed in the mammary gland. In the absence of α-lactalbumin, B4GALT1 has a low affinity for Glc as acceptor substrate and preferentially transfers Gal to N-acetylglucosamine (GlcNAc). While associated with α-lactalbumin, the affinity of B4GALT1 for Glc increases 1000-fold [16], thereby enabling the formation of lactose. During pregnancy, the expression of α-lactalbumin is inhibited by high levels of circulating progesterone [17], which counteract the stimulatory effect of the pituitary hormone prolactin, the levels of which increase strongly during the second half of gestation. At parturition, progesterone drops while sustained prolactin secretion induces α-lactalbumin expression, hence stimulating milk production. In addition to its role as a nutrient, lactose is also used as an acceptor substrate for the synthesis of a multitude of oligosaccharides, which are addressed in the next section.

After ingestion of breast milk, lactose must be cleaved back to Gal and Glc to be absorbed and used as a source of energy by the suckling infant. The enzyme responsible for lactose cleavage is the β-galactosidase lactase [18], which is expressed at the brush border membrane of the small intestine. Lactase expression is tightly regulated and is progressively turned off in most children around 2–3 years of age (Figure 1). Decreased lactase activity leads to the passage of lactose to the large intestine, where it is metabolized by microbes, thereby releasing hydrogen, methane, carbon dioxide, and lactate [19]. These fermentation products cause bloating, abdominal cramps, and nausea, which are the typical symptoms of lactose intolerance. The emergence of such symptoms will lead the nursed child to reject breast milk and eventually to natural weaning.

Ovarian follicle maturation is suppressed during lactation because of the elevated prolactin and low gonadotropin levels in nursing mothers [20]. This phenomenon prevents a new pregnancy when a mother dedicates a major fraction of her energy expenditure to breastfeeding. Accordingly, lactase repression and the transition to a state of lactose intolerance can be seen as a natural clock regulating weaning and thereby the return to fertility for the mother (Figure 1). Therefore, the lactose–lactase system has been suggested to act as a biological timer controlling birth spacing in humans [21].

Whereas most humans lose lactase expression during early childhood, approximately 40% of the human population shows lifelong lactase persistence. The geographical distribution of

Glossary

Colostrum: 'first milk', which is produced at the end of pregnancy and is secreted during the first 4 days postpartum. Colostrum is rich in sIgA and milk oligosaccharides, thereby providing a first line of immune defense to the newborn. Bovine colostrum was used as a source of antimicrobial immunoglobulins against infections before the emergence of antibiotic-based therapies.
**Defensin:** short cationic antimicrobial peptides that bind to bacterial and fungal cell walls and kill microbes by destabilizing their membrane integrity. Defensins are mainly produced by leukocytes and by Paneth cells in the crypts of the small intestine. Colonization of the gut by microbiota stimulates the production of defensins.
Endocrine disruptor: chemicals that share structural features with hormones and interfere with endocrine pathways. Animals are exposed to endocrine disruptors through different modes, ranging from skin contact to oral ingestion. Some endocrine disruptors are environmental pollutants, such as dioxin, while others are additives to food and materials, such as bisphenol A, which is found in plastics.
Enterobacteriaceae: family of Gram-negative, facultative anaerobic rod-shaped bacteria including *Escherichia coli*, *Shigella*, *Klebsiella*, *Salmonella*, and *Yersinia*. Most Enterobacteriaceae reside in the intestine of animals. The Enterobacteriaceae includes commensals, pathobionts, and pathogens. Enterobacteriaceae cannot process oligosaccharides and large polysaccharides.
Mucins: a family of highly O-glycosylated hydrophilic proteins that are the main constituents of the mucus that protects epithelial layers on mucosal surfaces. Mucins are also found in bodily fluids, such as saliva and phlegm. Mucins are large proteins that are either anchored to cells through a transmembrane domain, or secreted as massive gel-like aggregates. The mucin MUC2, secreted by goblet cells, is the main constituent of the thick mucus layer lining the gastrointestinal tract.
Pathobiont: organism that normally lives in symbiosis with a host, but
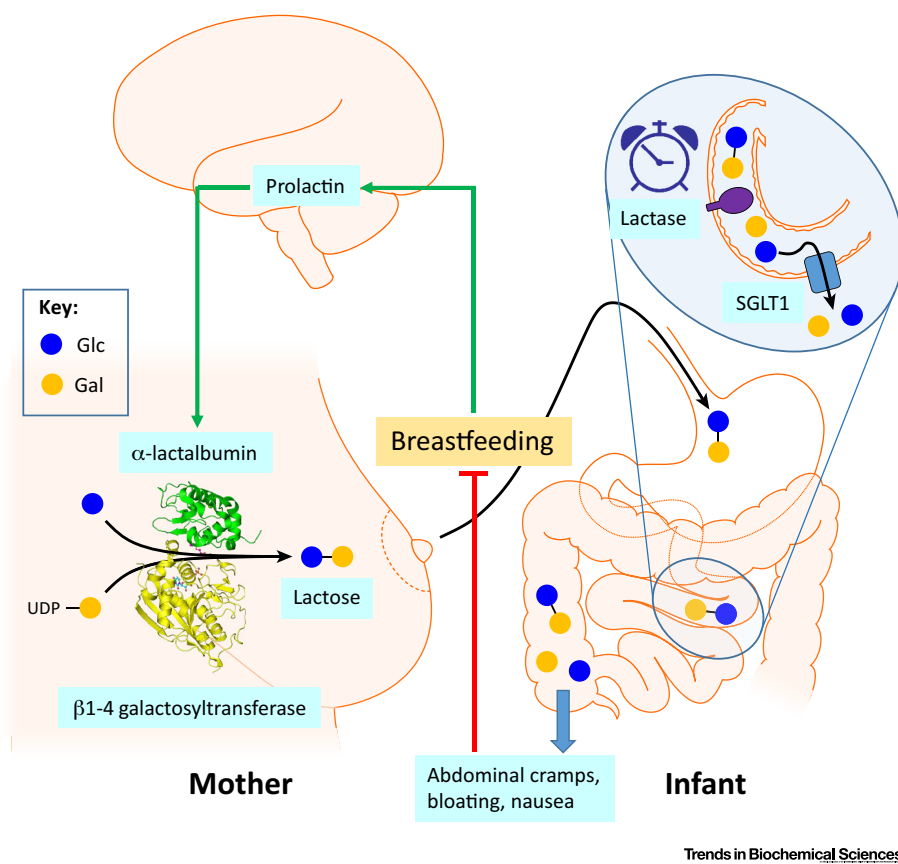
Figure 1. Lactose Biosynthesis and Degradation. Lactose synthase {Protein Data Bank (PDB): 1nhe [16]} is a heterodimer comprising the β1-4 galactosyltransferase B4GALT1 and α-lactalbumin. The pituitary hormone prolactin stimulates the expression of α-lactalbumin in the lactating mammary gland. In the small intestine of the suckling infant, lactose is cleaved by lactase, whose expression is age dependent. The resulting monosaccharides glucose (Glc) and galactose (Gal) are absorbed by the sodium glucose-linked transporter SGLT1. In the absence of lactase, lactose reaches the colon, where it is degraded by intestinal microbes. The increase in bacterial fermentation products causes abdominal cramps, bloating, and nausea, which leads to the cessation of breastfeeding.

can become pathogenic under specific conditions, such as when becoming a dominant taxon in a complex environment. Typical pathobionts among gastrointestinal bacteria are *Helicobacter pylori*, *Clostridium difficile*, and *Escherichia coli*.

Sialic acid (Sia): family of nine-carbon carboxylated carbohydrates found in vertebrates and some bacteria. Sia are mainly found as terminal monosaccharides on glycan chains and are part of carbohydrate epitopes used as receptors for viruses, such as influenza viruses, and toxins, such as cholera toxin. The main forms of Sia found in vertebrates are *N*-acetylneuraminic acid (NeuAc) and *N*-glycolylneuraminic acid (NeuGc). Humans have lost the ability to synthesize NeuGc because of inactivating mutations in the *CMAH* gene encoding the cytidine monophosphate-*N*-acetylneuraminic acid hydroxylase enzyme.

Xenobiotic: a chemical compound detected in an organism that does not synthesize it. Xenobiotics can mediate pharmacological and endocrine effects that can range from toxic to harmless. Xenobiotics include not only drugs, such as antibiotics and their metabolites, but also environmental pollutants that accumulate through the food chain.

lactase persistence is striking because it is mainly localized to Europe, West Africa, the Middle East, and Pakistan/West India. Lactase persistence is in fact a recent trait in human evolution, because the dominant mutation conferring persisting lactase expression appeared only approximately 7500 years ago in Eastern Europe [22]. The high frequency of this lactase haplotype in the European population indicates a strong selection pressure, which coincided with the emergence of dairy cultures across Europe. Distinct mutations in the promoter region of the lactase gene have been reported in West Africa and Asia, indicating that lactase persistence spread across the globe through convergent evolution. The rise of lactase persistence contributed to the expansion of dairy farming and milk consumption in lactose-tolerant populations. The increasing availability of cow's milk introduced alternatives to breast milk for young children and thereby lowered the age of weaning. The resulting shortening of the nursing period also yielded a faster return to fertility in women and, thus, increased the birth rate in Neolithic farming societies.

Breast Milk is a Fertilizer
Breast milk is the first fluid ingested by the newborn; it is not only the first food for the infant, but also a strong conditioner for the gut microbiota, which develop swiftly in the days following birth. As documented by numerous recent studies, the gut microbiota is emerging as a critical organ involved not only in intestinal physiology, but also in influencing general metabolism and affecting

the severity of diseases, such as diabetes [23] and atherosclerosis [24]. Breast milk, as the product of million years of evolution, provides the optimal seeding ground for the development of a healthy gut microbiota. A better understanding of the coordinated action of breast milk constituents in shaping the gut microbiota will lead to a definition of treatments aimed at restoring a healthy gut microbiota in diseases.

In addition to lactose, human breast milk comprises a large number of complex oligosaccharide structures, consisting of three or more monosaccharides, which are also produced in the lactating mammary epithelium. In contrast to lactose, which functions as an energy source for the infant, milk oligosaccharides cannot be digested by the suckling infant. Human colostrum, the milk produced during the first few days after the birth of the baby, contains approximately 22 g/l of oligosaccharides and mature milk still approximately 10 g/l [25]. Human milk comprises close to 200 distinct oligosaccharides [26], a number that is the highest among mammalian milks. This diversity is achieved by combining the four carbohydrates Gal, GlcNAc, fucose (Fuc), and **sialic acid** (Sia) on a lactose core (Figure 2). The term Sia covers a large family of acidic carbohydrates; N-acetylneuraminic acid (Neu5Ac) is the only Sia found in humans, whereas Neu5Ac and N-glycolylneuraminic acid (Neu5Gc) are found in most mammals. The glycosyltransferases involved in the assembly of milk oligosaccharides are the same enzymes that build the glycans decorating glycoproteins and glycolipids. The production of milk oligosaccharides is solely regulated by glycosyltransferase expression in the mammary epithelium. Given that the program of glycosyltransferase gene expression varies among mothers, the amounts of some milk oligosaccharides show a large degree of interindividual variability [27].

Mammals lack the glycosidase machinery required to cleave milk oligosaccharides in the gastrointestinal tract. Therefore, unaltered milk oligosaccharides reach the large intestine, where they are consumed by selected bacterial taxa. The assimilation of oligosaccharides requires glycosidase enzymes, such as fucosidases and sialidases, to break down the oligosaccharides into monosaccharides, and carbohydrate transporters to use the released monosaccharides as a carbon source. Some intestinal bacteria, including *Bifidobacterium* spp. and *Bacteroides* spp., are well equipped to degrade and utilize milk oligosaccharides [28]. Given that bacteria can have preferences for specific milk oligosaccharides, differences in the composition of milk oligosaccharides impact the colonization of the gut by individual bacterial groups. For example, 'non-secretor' mothers, who lack the fucosyltransferase FUT2, produce milk oligosaccharides devoid of α1,2-linked Fuc. Infants of such non-secretor mothers show delayed intestinal colonization with bifidobacteria [29], which include Fuc consumers, such as *Bifidobacterium longum* subsp. *infantis* and *Bifidobacterium bifidum*. Compositional shifts in the gut microbiota induced by different milk oligosaccharide mixtures may have long-term effects on the course of inflammatory diseases. For instance, elevated amounts of the milk oligosaccharide Sia(α2-3)lactose promote the formation of a niche for **Enterobacteriaceae** during lactation, which extensively expands during disease and exacerbates intestinal inflammation in colitis [30]. Surprisingly, Enterobacteriaceae cannot feed on Sia(α2-3)lactose because they lack the sialidase enzymes able to cleave the capping Sia units. In fact, Enterobacteriaceae rely on sialidases released by other intestinal bacteria for that task, such as members of the *Bacteroides* genus [31]. Given that milk oligosaccharides are structurally similar to intestinal **mucin** O-glycans, bacterial glycosidases also digest carbohydrates from the protective mucin layer lining the intestine. The release of carbohydrates from milk oligosaccharides and intestinal mucins mediated by bacterial glycosidases eventually supports the 'cross-feeding' of **pathobionts**, such as Enterobacteriaceae. Recently, milk oligosaccharides and intestinal glycans have been recognized as key players influencing the composition of the gut microbiota under healthy conditions and during disease [32].

Maternal sIgA is another component of breast milk controlling the bacterial colonization of the gut. Bacterial antigens are among the epitopes recognized by maternal sIgA and these
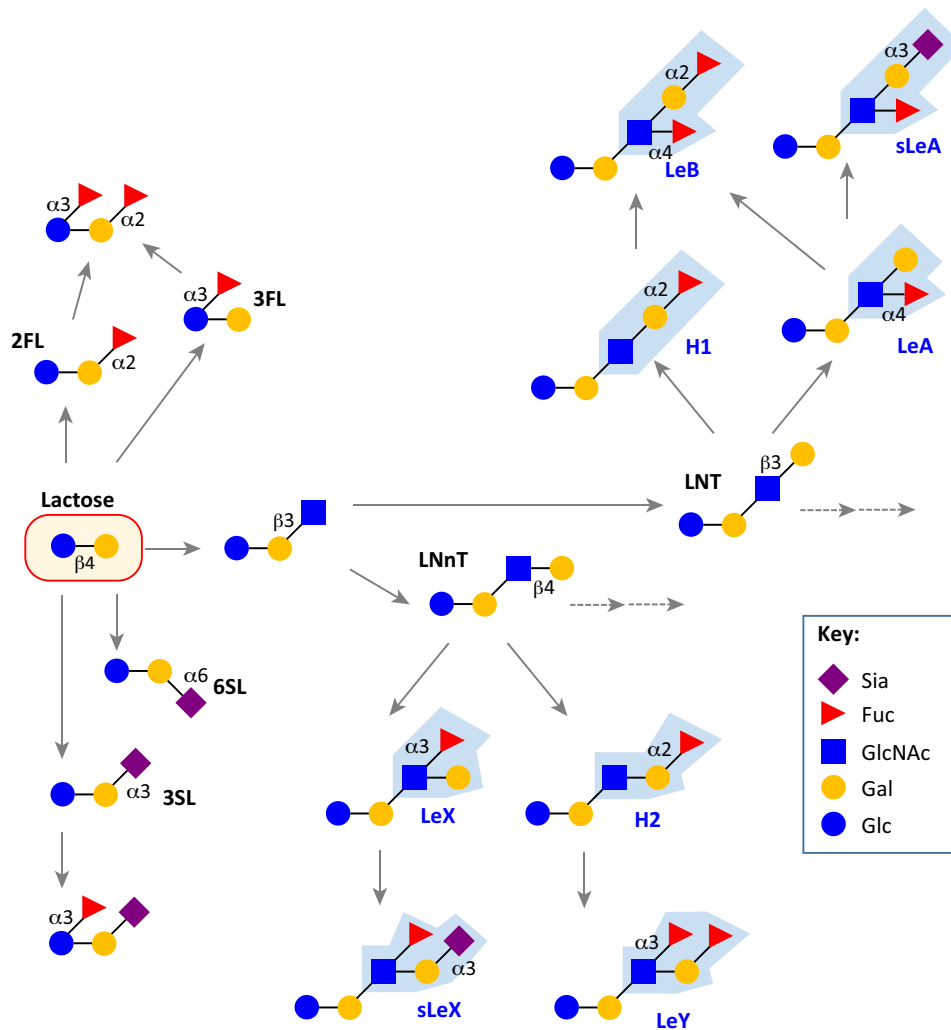
Figure 2. Biosynthetic Pathway of Milk Oligosaccharides. The lactose core (boxed structure) is modified by the addition of fucose (Fuc), sialic acid (Sia), N-acetylglucosamine (GlcNAc), and galactose (Gal). The most common breast milk trisaccharides are fucose(α1-2)lactose (2FL), fucose(α1-3)lactose (3FL), sialyl(α2-3)lactose (3SL), and sialyl(α2-6)lactose (6SL). In human milk, the most common tetrasaccharide is lacto-N-tetraose (LNT), whereas lacto-N-neotreaose (LNnT) dominates in other mammalian milks. In human milk, Lewis antigens (LeA, LeB, LeX, LeY, sLeA, and sLeX) are epitopes (blue-shaded structures) frequently found on milk oligosaccharides. A and B blood group antigens are absent in human milk oligosaccharides, but O antigen type I (H1) and type II (H2) are common. The LNT and LNnT cores can be further elongated (broken arrows) to yield oligosaccharides comprising more than 20 monosaccharides.

antibodies bind to intestinal bacteria once they reach the infant gut. Some bacterial taxa, such as Enterobacteriaceae, are more widely coated than others, such as *Prevotella* and *Bacteroides* [33]. Coating of bacteria with sIgA hampers their proliferation in the gut, thereby preventing the expansion of colitogenic bacteria [34]. The importance of maternal sIgA in shaping the gut microbiota has also been demonstrated in newborn mice nursed by mothers unable to transfer sIgA into their milk because of a polymeric Ig receptor defect. Mice fed with antibody-deficient milk presented long-lasting and detrimental changes in their gut microbiota, as exemplified by increasing Pasteurellaceae and Lachnospiraceae levels, and increased susceptibility towards colitis induced by dextran sulfate sodium [35]. The milk proteins lysozyme and lactoferrin also influence the gut microbiota by cleaving cell wall polysaccharides and by chelating iron,

respectively. Colostrum is especially rich in lactoferrin [36], which binds with high affinity to iron, thereby restricting its availability for the growth of pathobionts, such as Enterobacteriaceae [37].

Breast Milk is an Umbrella

Breast milk contains physiologically relevant amounts of bioactive proteins, including immunoglobulins, cytokines, **defensins**, and lactoferrin, that contribute to the immune protection of the infant [38] (Figure 3). Some of these immunomodulatory factors, such as macrophage
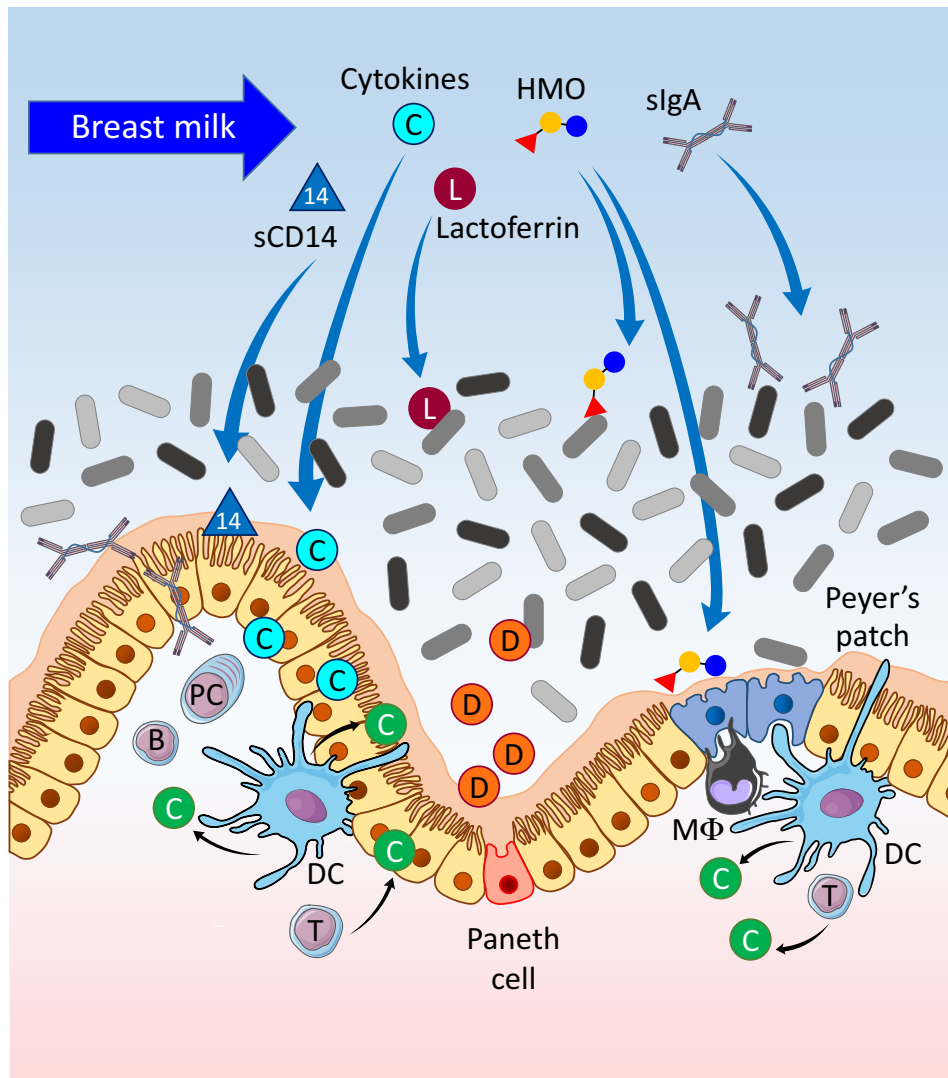
Trends in Biochemical Sciences

Figure 3. Immune-Active Compounds of Breast Milk. Human breast milk delivers cytokines (cyan circles), such as transforming growth factor (TGF)-β, interleukin (IL)-10, and macrophage colony-stimulating factor (M-CSF), soluble CD14 (sCD14, blue triangles), lactoferrin (red circle), human milk oligosaccharides (HMO), and secretory immunoglobulin A (sIgA), to the infant gut. Upon resorption by the intestinal mucosa, sCD14 contributes to innate immune protection by recognizing microbe-associated molecular patterns. Milk lactoferrin, sIgA, and Paneth cell-derived defensins (orange circles) prevent bacterial (gray rods) overgrowth. HMO act as receptor decoys, inhibiting bacterial adhesion to mucosal surfaces. HMO can be also taken up by M-cells (blue cells) in Peyer's patches, which may contribute to the induction of tolerogenic responses towards structurally related mucosal glycans. In the intestinal mucosa, dendritic cells (DC), macrophages (MΦ), T lymphocytes (T), B lymphocytes (B), and plasma cells (PC) orchestrate mucosal immunity through the secretion of cytokines (green circles).

colony-stimulating factor [39], are produced by epithelial cells in mammary ducts, whereas others, such as transforming growth factor β (TGFβ) [40], are produced by leukocytes present in the breast milk. Importantly, these bioactive proteins remain active after passage through the stomach because of a higher gastric pH in infants of approximately 3–5 compared with a gastric pH of 1–2 in adults. The stability of milk proteins is further maintained by ∝1-antitrypsin in early milk, which protects other proteins from gastric proteolysis [41].

The first bioactive proteins identified in the breast milk were immunoglobulins. Transfer of immunity from mother to child was described in 1903 and this effect was linked to antibodies contained in the milk. Most immunoglobulins in breast milk belong to the IgA class. Levels of sIgA reaching 12 g/l are commonly detected in the colostrum, while mature milk contains approximately 1 g/l [42]. Since the intestinal immune system at birth is immature with a low production of sIgA during the first weeks of life, the high levels of sIgA in the colostrum significantly contribute to the immune protection of an infant. Thus, the transfer of adaptive secretory immunity from mother to an infant in the form of sIgA provides direct protection against a variety of pathogens until the infant immune system takes over by producing sufficient sIgA levels around a month after birth [43]. Lactoferrin, which reaches concentrations of 1–3 g/l in breast milk, is another immune protective factor. Lactoferrin efficiently chelates iron, at the same time reducing the growth of certain bacteria relying on iron and increasing absorption of iron by the infant through binding to the intestinal lactoferrin receptor ITLN1 [44]. Importantly, the cleavage of lactoferrin by pepsin in the stomach yields lactoferricin, which acts as an antimicrobial peptide by disrupting the membrane of Gram-negative bacteria [45]. In addition, lactoferrin induces macrophage phagocytosis, thereby promoting the elimination of certain bacteria [46]. The major milk protein ∝-lactalbumin also shares antimicrobial properties when partially unfolded and associated with oleic acid [47]. The resulting complex furthermore induces apoptosis in tumor cells and, therefore, has been called HAMLET ('human ∝-lactalbumin made lethal to tumor cells'). The antitumor activity of the HAMLET complex demonstrates that milk proteins have therapeutic potential; for example, in treating colon cancer [48].

During the first weeks of life, the two anti-inflammatory cytokines interleukin-10 (IL-10) and TGFβ, which are transferred through breast milk, contribute to the maturation of mucosal immunity [38]. Indeed, the milk levels of TGFβ correlate with sIgA production in breastfed infants [49], and with a decreased risk for child diseases, including allergy [50]. Further studies performed in mice showed that milk TGFβ promotes immune tolerance to oral antigens during mucosal maturation [51]. Similarly, targeted deletion of IL-10 in mice leads to spontaneous enterocolitis under conventional housing conditions, which can be prevented by parenteral administration of either IL-10 [52] or TGFβ [53].

In addition to cytokines, breast milk provides passive immune-protective factors, such as lysozymes, defensins [54], and soluble CD14 (sCD14) [55], which assist the infant innate immune system in coping with infections (Figure 3). The concentration of sCD14 in breast milk is 20-fold higher than in the serum of mothers. Milk ∝-lactalbumin binds sCD14 and thereby protects it from degradation when passing through the stomach. The elevated epithelial permeability in the neonate intestine enables the efficient absorption of sCD14, which sensitizes the innate immune system towards Gram-negative bacteria, thus contributing to the maintenance of microbial homeostasis in the neonatal intestine.

In addition to their prebiotic action discussed above, milk oligosaccharides also exert antimicrobial functions by acting as soluble receptors for pathogens. For example, H2 type oligosaccharides (Figure 2) inhibit the adhesion of *Campylobacter jejuni* to the intestinal epithelium [56], and fucosylated milk oligosaccharides from secretor mothers inhibit norovirus infection [57].

Oligosaccharides carrying Lewis X antigens are recognized by the DC-SIGN lectin on intestinal dendritic cells. Such oligosaccharides prevent the binding of HIV through DC-SIGN, thereby decreasing the presentation of the virus to CD4+ T cells [58]. Some milk oligosaccharides have been shown to directly regulate immune cells. For example, the oligosaccharide lacto-*N*-fucopentaose III induces the production of IL-10 in spleen cells [59]. Also, oral supplementation of mice with Sia(α2-3)lactose increases activation of intestinal CD11c+ dendritic cells [60]. The activating properties of specific milk oligosaccharides may be related to their structural similarity with carbohydrate epitopes found on pathogens. Indeed, α2,3-linked Sia is present on the surface of various pathogenic bacteria, such as group B *Streptococcus* [61], *Campylobacter jejuni* [62], *Haemophilus influenzae*, and *Neisseria meningitidis* [63].

Breast Milk is a Remote Control

As well as contributing to the development of the gut microbiota and the maturation of the mucosal immune system, breast milk also affects metabolic pathways and supports the growth of the suckling infant. Several hormones occurring in breast milk likely mediate the same functions as they do as endocrine factors. Accordingly, leptin in breast milk [64] is probably involved in controlling satiety and fat storage; insulin-like growth factor 1 (IGF1) [65] is probably involved in stimulating body growth; and adiponectin [66] is probably involved in regulating blood glucose levels and fatty acid oxidation. Although these hormones certainly have a role in the early growth and development of breastfed infants, the true significance of milk-borne hormones is elusive because clear experimental support is difficult to obtain.

Assessing the biological contribution of breast milk hormones is a challenging task when trying to differentiate their effects from those mediated by the same hormones produced endogenously. Also, the pleiotropic actions of several hormones render the identification of specific effects difficult and can lead to ambiguous conclusions. For example, some studies have attributed behavioral functions to breast milk cortisol by associating cortisol levels in maternal milk with human infant temperament [67], and with reduced anxiety as investigated in rats [68]. Far from discrediting such studies, it must be remembered that cortisol, as the main glucocorticoid hormone, exerts multiple actions and that behavioral changes may be indirect and consecutive to numerous metabolic and immunologic effects. In fact, cortisol is an important factor controlling intestinal immunity [69]. Accordingly, cortisol delivered through breast milk probably contributes to the maintenance of anti-inflammatory conditions during the early phase of intestinal microbial colonization in infants.

Breast Milk is a Waste Basket

Breast milk not only provides various protective compounds as discussed above, but also conveys lipophilic **xenobiotics** that accumulate in the maternal breast tissue. The list of environmental contaminants is long, featuring heavy metals, pesticides, synthetic additives, and **endocrine disruptors**, among others. Thanks to vigilant scientists, such as Rachel Carson [70], who raised awareness in the general community of the health risks of such contaminants, several xenobiotics have been banned over the past 20 years. For example, dichlorodiphenyltrichloroethane (DDT) was broadly used as pesticide in agriculture before the warning call of Rachel Carson, which eventually led to the ban of DDT in 49 countries by 1995. DDT and its metabolites affect bird reproduction and are highly toxic to fish. In humans, exposure to DDT has been associated with preterm birth [71] and increased risk for breast cancer [72]. The USA banned the use of DDT in 1972 and several European countries had restricted its use by 1970. The levels of DDT measured in the breast milk of Swedish mothers peaked in 1970 at 3 μg/g lipids and had steadily declined to zero by the end of the 20th century [73]. Additional studies addressing the accumulation of DDT in the human body revealed a half-life for DDT in human fatty tissues, such as breast tissue, of approximately 4 years.

Whereas several xenobiotics have been blacklisted, others are still widely used. For example, phthalates are noncovalent additives found in plastics, textiles, personal-care products, and so on. Phthalates are released in the environment and accumulate in fat tissues. They are found not only in dietary products, such as butter, but also in breast milk. Phthalates have been claimed to act as endocrine disruptors [74]. Positive correlations have been described between specific phthalates in breast milk and altered levels of sexual hormones in suckling infant boys at 3 months of age. Especially noteworthy was the detection in such infants of a higher ratio of luteinizing hormone to testosterone than is normal, which is indicative of the antiandrogenic action of some phthalates [75].

In addition to xenobiotics, breast milk is also involved in the transmission of pathogens, such as HIV and cytomegalovirus (CMV), to the suckling infant. Newborns from CMV-positive mothers are protected prenatally by the transfer of anti-virus IgG through the placenta [76]. The situation is not as positive in the case of HIV, because transmission of the virus has been documented in 10–40% of mother–infant pairs [77]. Consequently, the Center for Disease Control (CDC) recommends avoiding breast-feeding for HIV-positive mothers [78]. In general, only a few maternal viruses are transmitted through breast milk, which underlines the general safety of breastfeeding.

Concluding Remarks

Beyond the biological functions of breast milk addressed in this review, the act of breastfeeding itself is the topic of emotional discussions related to the philosophical question of motherhood. Should society encourage breastfeeding simply because it is 'natural'? Is a woman who stops nursing her baby after 3 months a bad mother? Does breastfeeding depreciate the economic and social status of women [79]? Similar provocative questions keep the general debate on breastfeeding alive and remind us that the discussion on breast milk transcends biology (see Outstanding Questions). Breast milk is ultimately why Carolus Linnaeus, as the father of seven children, chose the term *Mammalia* to define our own class of animals in the tree of life.

Outstanding questions

What is the nature of the selection pressure that led to the rapid propagation of lactase persistence in human populations?

Why does human breast milk contain close to 200 oligosaccharide structures, whereas the milk of other mammals has on average only 10–50 oligosaccharide structures?

Do milk oligosaccharides contribute to the development of an immune tolerance towards carbohydrate epitopes, thereby decreasing the long-term risk for autoimmune diseases and allergies?

Do glucocorticoids, β-endorphin, and oxytocin delivered in breast milk shape infant behavior and maternal bond?

Do breast milk constituents, such as free Sia, contribute to ganglioside biosynthesis and brain development, thus promoting intellectual abilities in breastfed children?

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