Chapter 9
The Immune System of Breast Milk: Antimicrobial and Anti-inflammatory Properties

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Introduction

Human breast milk is recognized as the optimal infant feeding. Human milk contains the nutrients necessary to support the infant's development [1–3]. Breast milk also contains components that protect young children against various infectious diseases and, as more recently described, constituents necessary to support the development of the infant's immune system (ontogeny) [1–3]. This includes various antimicrobial substances, constituents that promote tolerance and priming of the infant immune system, as well as anti-inflammatory components. It has recently become clearer that protection provided through breast milk against some infections extends well beyond weaning [4].

The immune system of the neonate differs considerably from that of an adult [5]. At birth, cells of the innate immune system (dendritic cells, macrophages, and neutrophils) and IgM- and IgG-producing cells are present in the infant's intestinal mucosae, but IgA secreting cells are extremely rare [6]. Because at least 90% of microorganisms infecting human beings use the mucosae as portal of entry [7, 8], young infants are exposed to a large number of microorganisms and are at increased risk for infection [9]. However, the intestinal immune system develops rapidly in the early postnatal period. During this critical period of immunological vulnerability, resistance to infection relies both on the protective factors in milk and on the infant developing his own innate and adaptive immunity. Breast milk provides the missing components while also actively stimulating maturation of the infant's own intestinal defense [3].

In addition to eliminating infectious agents and minimizing the damage they cause, the neonatal immune system must develop the ability to acquire tolerance. This means to discriminate between antigens that are harmless and those that are potentially dangerous. Induction of tolerance is believed to occur primarily in the gut and is facilitated by the specialized B and T cells, the production of secretory IgA (SIgA), and the skewed Th2 response [10, 11]. Failure to regulate tolerance and

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active immune responses is hypothesized to contribute to food-related allergy, autoimmunity, and inflammatory bowel disorders. Human milk promotes oral tolerance in the infant [12] and exclusive breast-feeding may even prevent or delay the onset of atopic illness such as allergies.

Immunological Components of Human Milk

Breast Milk Cells

The cellular content of breast milk has certainly not yet revealed all its surprises. Recent studies demonstrated that, particularly in mature milk, epithelial cell adhesion molecule (EPCAM+) cells from the mammary gland are the most represented cell type in breast milk [13]. The exact role of these cells ingested by neonates and breast-feeding infants remains unknown. Stem/progenitor cells have also been identified by their specific surface markers in breast milk [14]. Their physiological role, if any, is equally mysterious.

Excluding epithelial cells, neutrophils comprise roughly 80% of breast milk cells, macrophages 15%, and lymphocytes 4% [15].

Lymphocytes

Various lymphocyte types are coexisting in breast milk: CD3+ T cells (representing roughly 83% of lymphocytes, almost equally distributed in CD4+ and CD8+ lymphocytes), gdT cells (11%), CD16+ NK cells (3–4%), and B cells (2%). Breast milk T and B lymphocytes share three major characteristics that differentiate them from circulating lymphocytes. First, memory T and B lymphocytes are overrepresented in breast milk, the majority of cells failing to express CD45RA receptor characterizing naïve cells [16, 17]. More than 70% of breast milk B cells are IgD-CD27+ memory B cells [18]. Second, many T and B lymphocytes from breast milk are activated, as they frequently express markers such as HLA-DR and CD38 [16–20]. This is paradoxical since human milk per se is remarkably not prone to immune activation. As compared with other media including plasma, blood lymphocytes incubated in the presence of human milk display considerably less frequently activation markers at their surface (E. Tuaillon, personal communication). Most likely, breast milk lymphocytes become activated through the extravasation process and/or their transepithelial migration [18–21]. Breast milk B cells are frequently switched memory cells primed to secrete antibodies with overrepresentation of large-sized B cells, plasmablasts, and plasma cells [18] and are not expressing complement receptor [22]. Third, breast milk T [16] and B cells [18] predominantly harbor mucosal homing markers (CD49f, b7 integrin, CD103, CD44) confirming that most of these cells migrated from the highly compartmentalized mucosal-associated lymphoid tissue (MALT) to the mammary gland as an effector site. At least in milk-derived B cells, migration seems to have occurred preferentially from gut-associated lymphoid tissue (GALT) confirming the predominant entero-mammary axis of the mucosal immune system. Migrating B cells, mainly those primed to secrete IgA antibodies, are attracted to and anchored within the mammary acini by the mucosae-associated epithelial chemokine CCL28 [23], other cells being released in breast milk.

CD8+ cytotoxic T lymphocytes can be found in human milk. These cells result from antigenic exposure of maternal mucosal surfaces and are thought to be functional [24–27].

These characteristics of breast milk lymphocytes reinforce the idea that human milk provides neonates and infants with a supplemental, highly immunoactive system primed to recognize the maternal environment and to protect from potential pathogens the mother–infant dyad may encounter.

Table 9.1 Distribution of immunoglobulins and other soluble substances in the colostrum and milk delivered to the breast-fed infant during a 24-h period

	Concentration in mg/day at postpartum			
Soluble product	<1 week	1–2 weeks	3–4 weeks	>4 weeks
IgG	50	25	25	10
IgA	5,000	1,000	1,000	1,000
IgM	70	30	15	10
Lysozyme	50	60	60	100
Lactoferrin	1,500	2,000	2,000	1,200

Adapted from ref. 34

Macrophages

Macrophages have long been considered to represent the major cell type in colostrum and breast milk consisting of 40% of breast milk leukocytes in early lactation and up to 85% in mature milk [19, 28, 29]. However, in these studies, macrophages were defined by morphologic characteristics and by using forward and scatter flow cytometry analyses. More recent studies strongly suggest that the number of breast milk macrophages has been overestimated since only a small proportion of breast milk cells harbor surface markers of macrophages such as CD14 [13–15]. Indeed, macrophages may represent only 15% of breast milk leukocytes [15].

Also, breast milk macrophages are distinct in terms of phenotype and functions from blood monocytes/macrophages conferring them a higher phagocytic capacity and a more efficacious defense against pathogens [30]. Breast milk macrophages are frequently activated [31] and their motility is much enhanced as compared with blood monocytes/macrophages [32]. Breast milk macrophages are morphologically distinct from blood macrophages and spontaneously secrete granulocyte–macrophage colony-stimulating factor (GMCSF), an important cell growth factor that enhances effector functions and cell signaling pathways [30]. They express DC-SIGN gene and protein (a DC-specific lectin that mediates interaction with HIV-1), and differentiate into CD1+ dendritic cells after incubation with IL-4 [30]. In addition, breast milk macrophages contain secretory IgA that can be released during the phagocytosis process [33] and are profuse secretors of soluble factors such as lactoferrin and complement factors C3 and C4 [29].

Immunoglobulins

The mammary gland produces SIgA in high quantity with lesser amounts of SIgM and SIgG (Table 9.1). They are one of the predominant proteins in breast milk and found at highest level in colostrum (5 mg/mL in colostrum, about 1 mg/mL in mature milk, [35]). IgA is synthesized in human milk by resident B-cells anchored in the mammary gland tissue through CCL28. These cells have migrated from the mother's intestine via the enteromammary axis [36]. IgA are synthesized as a dimer and linked to a secretory component (SC). SIgA is relatively resistant to proteolytic enzymes from the infant's gastrointestinal tract, allowing to provide a supply of IgA antibodies to the infant's gut [2]. Human milk is not only a rich source of SIgA that could be involved in situ in immune exclusion, but the specificity of the SIgA is directed against microbes common to both the mother and her infant [1].

SIgA provides antimicrobial defense in three ways (Fig. 9.1): preventing bacteria and viruses from attaching to mucosal surfaces by immune exclusion and mucosal painting, as well as neutralizing microbial toxins. Through these mechanisms, SIgA would prevent the establishment of bacterial colonies in the intestine and/or translocation across the mucosal barrier, thereby preventing an inflammatory response that would be damaging to the infant. This provides a potential mechanistic

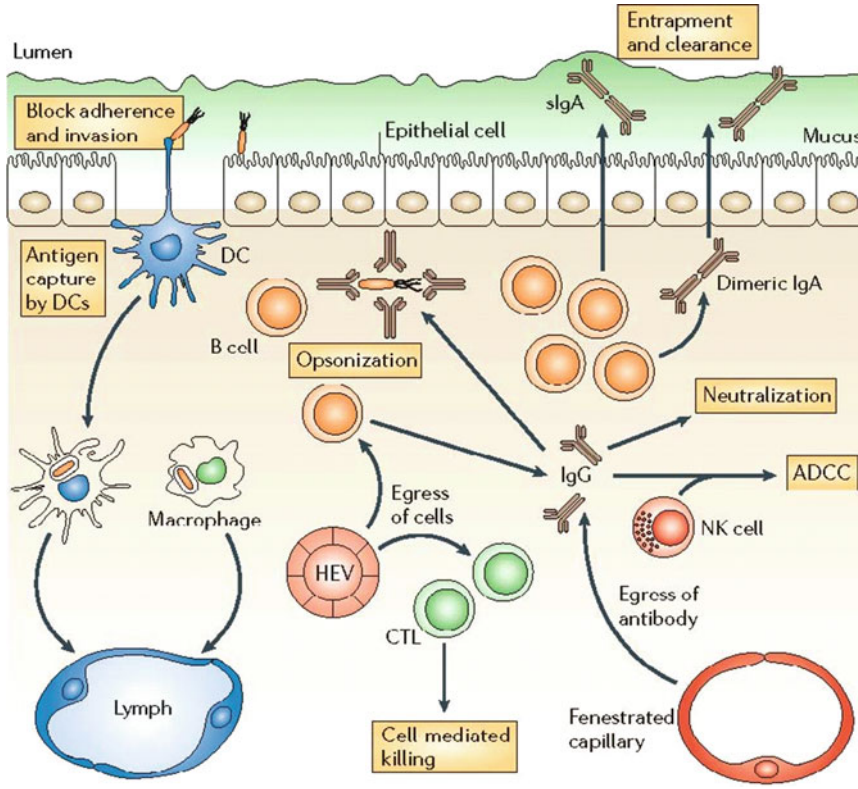

Fig. 9.1 Mechanisms of immune protection of human mucosae. From ref. 37, with permission. Abbreviations: *ADCC* antibody-dependent cellular cytotoxicity, *NK cell* natural killer cell, *CTL* cytotoxic lymphocyte responses, *DC* dendritic cells, *sIgA* secretory immunoglobulin A, *HEV* high endothelial venules

explanation for the reduced neonatal septicemia associated with breast-feeding [1]. SIgA have been identified in breast milk against many bacterial pathogens including *Escherichia coli*, *Vibrio cholerae*, *Campylobacter*, *Shigella*, *Giardia lamblia*, *Haemophilus influenzae*, *Clostridium difficile*, and *Streptococcus pneumoniae* [4], viruses such as Rotavirus, CMV, HIV, Influenza virus, respiratory syncytial virus, and yeast [36]. SIgA against maternal microflora and dietary proteins have also been identified in human milk [38]. Vaccination of mothers in late gestation will produce specific SIgA in her breast milk that may protect her infant against exposure.

Animal experiments strongly suggest that SigA is the crucial protective component of breast milk [39]. Knock-out mice lacking SIgA and SIgM show reduced protection against certain epithelial infections [6]. However, in striking contrast to humans, immunoglobulins from breast milk in many animal species (rodents, bovines, cats, ferrets, etc.) are transported across the intestinal epithelium into the neonatal circulation [40]. This is an evident limitation in translating observations made from animal models into humans.

Secretory Component

The secretory component (SC) is a glycoprotein present in many external secretions that is usually coupled with polymeric immunoglobulins. SC is also abundant in human milk [41]. It may interfere with the mucosal adhesion of enterotoxigenic *E. coli*, salmonellae, and *C. difficile* toxin A, as well as interact with a pneumococcal surface antigen [6]. SC may inhibit certain toxins, such as *C. difficile* toxin A [6].

Lactoferrin

Lactoferrin is a predominant whey protein in mature milk (≈1 g/L), its maximal concentration is found in colostrum (≈7 g/L). The concentration of lactoferrin in breast milk is controlled by the reproductive hormones prolactin and estrogen [1, 3].

Lactoferrin is an iron-binding protein. It is believed that its ability to withhold iron from pathogens explains a great part of its antimicrobial potency [42]. However, this protein has antibacterial activities that are independent of iron chelation. It may also act against some pro-inflammatory cytokines such as IL-1β, TNF-α, and IL-6 [1].

Lactoferrin may inhibit the attachment of bacteria to intestinal cells and has a direct activity against a wide range of Gram-positive and Gram-negative bacteria, fungi, viruses, including HIV-1 [43], and tumor cells [44, 45].

Lysozyme

Lysozyme is also a major whey protein of human milk. Together with SIgA, lactoferrin, and other antimicrobial compounds, this protein plays a major role in the immune defense of the infant. Lactoferrin removes lipopolysaccharide from the outer cell membrane so that lysozyme can enter and degrade the inner proteoglycan matrix of the membrane of invading cells [46].

Oligosaccharides

Nondigestible oligosaccharides are an abundant constituent of human milk. They are found in high concentration in colostrum (≈22 g/L) and are less abundant in mature milk (≈12 g/L). The types of oligosaccharides also change during lactation. Oligosaccharides are produced by an antigen-independent mechanism in epithelial cells in the mammary gland. Oligosaccharides resist hydrolysis by gastro-intestinal enzymes, indicating that they would remain intact in the small intestine.

These compounds function like bacterial receptor analogues by competing with pathogens for binding sites on the epithelial surfaces of the intestine. They can bind pathogens present on the mucosal epithelia, such as *E. coli*, *Campylobacter jejuni*, and *S. pneumoniae* [44].

Other Nonantibody Protein Defense Agents and Other Innate Immune Components

Many microorganisms express surface molecules that are believed to act like oligosaccharides [47]. These include lactadherin, mucins, and antisecretory lectins.

Lactadherin is a mucin-related glycoprotein produced by mammary epithelial cells during lactation. Its concentration in milk peaks immediately postpartum and declines thereafter. It has been reported to bind human rotavirus by preventing viral attachment to host cell receptors [48, 49]. Its concentration has been inversely associated with rotavirus symptoms in infected breast-fed infants [50].

In addition, several hydrolysis products of casein and of α-lactalbumin have antimicrobial activity against *E. coli*, *Klebsiella pneumoniae*, staphylococci, streptococci, and *C. albicans* [51].

The protein haptocorrin has been shown in vitro to resist digestion and to inhibit the growth of enterotoxigenic *E. coli* [51].

The secretory leukocyte protease inhibitor (SLPI), a serine protease inhibitor, is present at potentially active concentrations (1,100 ng/mL) in colostrum and transition milk, and it can inhibit HIV-1 entry into host cells in vitro [52].

Human milk contains active lactoperoxidase. The antibacterial properties of lactoperoxidase are closely connected to the oxidation of thiocyanate, both in vitro and in milk.

Mature milk is reported to contain a soluble form of CD14 (sCD14), a cell receptor expressed on macrophages. sCD14 is a glycoprotein that binds bacterial wall components; it is produced by mammary epithelial cells in concentration 20-fold higher than in serum [1].

Several components (C3 and C4), receptors (CF2, CD21), and activation fragments of complement are found in human milk. They might participate in immune bacteriolysis, neutralization of viruses, immune adherence, cytolysis, and enhanced phagocytosis in the infant's intestine.

Human β-defensin-1 has been demonstrated to have antimicrobial activity against *E. coli* and might also be involved in upregulating the adaptative immune system in the gut [53].

Toll-like receptors, lacking in the neonate's gut, have recently been reported in breast milk. They play a crucial "sensing" role in the early innate immune response to invading pathogens [1].

Fatty Acids

The fat content of breast milk is 3–4 g/L in colostrum, transitional and mature milk, with 93–97% of the lipids in the form of triglycerides. In addition to their nutritive and developmental benefits, milk fats have been demonstrated to provide antimicrobial activity in infant gut [54]. The mechanism for antimicrobial effects of fatty acids and monoglycerides has not been established, but it has been suggested that free fatty acids damage bacteria by disrupting their cell membranes or by changing intracellular pH.

Compounds that Promote "Healthy" Intestinal Microflora

It is well documented that the colon of breast-fed infants contains fewer potentially pathogenic bacteria, such as *E. coli*, *Bacteroides*, *Campylobacter*, and *Streptococcus*, and more beneficial bacteria, such as *Lactobacillus* and *Bifidobacterium*, as compared to formula-fed infants [55]. Activation of SIgA-producing plasma cells in the young infant's gut is dependent of the colonization of the gut by *Bifidobacteria* and *Lactobacilli* stimulated by fermentation of nondigestible oligosaccharides which are found in large amounts in human milk [10]. These are bifidus factors (compounds that enhance the growth of bifidobacteria in the intestine), prebiotics, and compounds with prebiotic activity that stimulate the growth of beneficial bacteria. Bifidogenic peptides from lactoferrin have been isolated from human milk. Lactoferricin, a cationic peptide derived from digested lactoferrin has been found to be structurally related to certain bifidogenic peptides and is hypothesized to participate in the colonization of newborn infants' flora [56]. The SC of IgA may also have some prebiotic properties [56]. It has also been hypothesized that the long-chain polyunsaturated fatty acids (LCPUFAs) in human milk facilitate the adhesion of probiotics to mucosal surface [57].

Effect of Human Milk on the Infant's Immune Development (Table 9.2)

The infant's intestinal immune system develops rapidly in the early postnatal period as it comes into contact with breast milk components as well as dietary and microbial antigens [6]. During the first 4–6 weeks of life, subepithelial plasma cells are unable to secrete a sufficient amount of SIgA to protect the infant mucosae [6]. This has to be compensated by breast milk intake.

Table 9.2 Compounds in human milk with possible influence on the infant's immune development

Maternal mammary epithelial cells
Maternal immune cells
Macrophages and dendritic cells
Neutrophils
Natural Killer cells
T-cells
B-cells and their immunoglobulins
Stem/progenitor cells
Cytokines
Nucleotides
Other immune components
Chemokines
Other soluble factors
Long-chain polyunsaturated fatty acids
Compounds that promote microbiological colonization of the infant's colon
Hormones and bioactive peptides

Adapted from ref. 1

Table 9.3 Cytokines found in human milk

TNF-α	IL-7
TGF-β	IL-8
IFN-γ	IL-10
IL-1β	IL-12
IL-2	IL-13
IL-4	IL-16
IL-6	IL-18

Adapted from ref. 1

Cells

The immune cells present in mother's milk play a pivotal role in bridging the gap between birth and the child's development of a fully functional immune system.

These cells may secrete immunoregulatory factors such as cytokines. The cytokines may stimulate IgA production by peripheral blood lymphocytes [2]. The same cytokines are found in breast milk, and the presence of transforming growth factor-β (TGF-β), IL-6, IL-7, and IL-10 is important for immunologic development and differentiation of IgA-producing cells in the infant [6, 58, 59].

Breast milk neutrophils are also present in activated form, but seem to have limited functional capacity once secreted into milk.

Cytokines

Human milk contains an array of cytokines, some in concentrations that could potentially influence immune function (Table 9.3). The exact source of the cytokines present in the aqueous fraction of breast milk remains to be determined, though it is suspected that the primary source is from cells present in the mammary gland [60]. The extent to which cytokines survive passage through the infant stomach is largely unknown, but recent work suggests that some cytokines may be sequestered and

protected until they reach the intestine [61]. For example, IL-6 may be unable to survive the passage through the gastro-intestinal tract, unless it is released from milk macrophages in the neonatal gut and thereby enhance the development of the mucosal immunity.

In general, the concentration of cytokines varies widely in human milk, changes through lactation, and is influenced by the mother's health, making it difficult to assess their roles in the development of the infant's immune system.

The intake of cytokines through human milk has the potential to influence the maturation and development of immune cells in the infant. Neonates have a limited ability to produce many cytokines found in breast milk [1]. For example, TGF-β is present in breast milk and has been proposed to stimulate appropriate maturation of naïve infant intestinal immune system [4, 62, 63]. IL-6 is hypothesized to enhance the development of the infant's mucosal immunity [6]. Additionally, the cytokines present in milk assist both the transport of maternal leukocytes into milk and across the infant's gut epithelium [64]. There are many factors in breast milk that could either facilitate or inhibit cytokine activities that are not accounted for in studies conducted in vitro.

Hormones

Little is known about the activity of these compounds on the naïve immune system of an infant when delivered orally. However, it can be suspected that they impact immune development in some synergistic manner [1].

Nucleotides

Nucleotides are present in small amounts in human milk. It is likely that they play an important role for the rapid early expansion of the lymphoid system as well as the early rapid growth of the gut of the young infant. Dietary nucleotides are reported to benefit the immune system by promoting lymphocyte proliferation, NK activity, macrophage activation, and by producing a variety of other immunomodulatory factors [65]. It is suggested that nucleotides promote a Th1 response and modulate the differentiation of T and B cells [65].

Long-Chain Polyunsaturated Fatty Acids

Docosahexaenoic acid (DHA) and arachidonic acid (AA) constitute a small fraction of LCPUFAs in human breast milk, but have recently been suggested to participate in immune development [66]. Adding the LCPUFAs DHA and AA to preterm formula resulted in lymphocyte populations and cytokines more similar to human milk-fed infants than to infants who received unsupplemented formula [66].

Immune Tolerance

In addition to eliminating infectious agents and minimizing the damage they cause, the infant's immune system also has to process harmless antigens and has evolved intricate mechanisms to discriminate between antigens with the potential to cause damage and those without (tolerance). Induction of tolerance is believed to occur primarily in the gut by unique features of GALT, including specialized B- and

T-cells, the production of IgA, and the Th2 response [11, 67]. Failure to regulate tolerance and active immune responses can lead to diseases such as food-related allergy, autoimmunity, and inflammatory bowel disorders.

Although it is well established that dietary antigens/potential allergens are present in human milk (reviewed in ref. 68), the consequences on the infant's immune system remain unclear. It is hypothesized that breast milk promotes tolerance to dietary antigens and microflora via immunosuppressive cytokines (IL-10 and TGF-β) and antigens present in breast milk [6].

Compounds in Human Milk that May Be Involved in the Induction of Tolerance

1. TGF-β and IL-10
   Acquisition of tolerance involves the downregulation of the immune system through the secretion of cytokines such as TGF-β and IL-10. Low levels of TGF-β in breast milk have been related to an increased risk of atopic illness in infants, which supports the role of this cytokine in the development of tolerance [67]. Other dietary nutrients may influence the concentration of TGF-β in human milk, as levels of TGF-β were reported to be positively correlated to polyunsaturated fatty acids (PUFA) content [69]. IL-10 present in breast milk is bioactive. Human milk inhibits the proliferation of human blood lymphocytes in vitro and this inhibition is lessened by adding antihuman IL-10 antibodies in the milk [70]. IL-10 plays also a role in the synthesis of IgA [70].
2. $N-6$ and $n-3$ fatty acids
   In human milk, there are roughly ten different long chain (LC)-PUFAs consistently detected, representing both the $n-3$ and the $n-6$ series and including AA and DHA [71]. Maternal intake of PUFA prior to and during lactation is reflected in breast milk PUFA content [72]. Membrane phospholipids fatty acid composition in infants is strongly influenced by maternal diet and can alter the function of the immune cells [73]. Low $n-6/n-3$ ratios (due to higher content of $n-3$) in breast milk have been related to lower risk of atopic illness in the breast-fed population [4]. Animal studies suggest that the essential fatty acid content of the maternal diet significantly affects the development of immunological tolerance to food antigens in the suckling offspring [74]. Immunoregulatory benefits have been attributed to $n-3$ LCPUFA [68]. Dietary intervention during critical early stages of immune development before the establishment of allergic responses is thought to be most preferable [68]. Cohort studies have shown lower levels of $n-3$ PUFA in the milk of mothers whose infants showed symptoms of atopic illness before the age of 18 months [68].

Priming of the Immune System

A balance between tolerance and sensitization (priming) is necessary for the gut immune system to discriminate between harmless antigens and those associated with pathogenic and nonpathogenic microbes [67]. Antigen exposure via mother's milk has been shown to prime the immune response of the suckling pup against that antigen in rats [11]. Clinical trials have shown that breast-fed babies have enhanced specific antibody titer to some, but not all vaccines [4, 5]. The ability to transfer vaccinations from mother to infant via her milk is of great interest because it could eliminate potential problems associated with directly vaccinating the infant [76]. One possible explanation for the ability to immunize infants with their mother's milk has been attributed to the presence of anti-idiotypic antibodies in their breast milk [4]. Anti-idiotypic antibodies are antibodies with specificity against other autologous antibodies [10]. Therefore, anti-idiotypic antibodies, if present in breast milk, could have the capability of priming the infant's antibody response against the antigen the idiotype is directed to [40].

Anti-inflammatory Properties of Breast Milk

Inflammation is a necessary part of the immune response that helps protect the infant from infection. The inflammatory response traps pathogens and signals the arrival of immune cells to destroy the antigen. However, this process results in a great deal of adverse events on healthy tissue if it is not controlled [1].

The immature human intestine overreacts to both indigenous (IL-1β) and exogenous (Lipopolysaccharide-LPS) inflammatory stimuli to produce an excessive inflammatory (i.e., IL-8) response [3]. In preterm infants, this excessive inflammatory response may contribute to the high incidence of necrotizing enterocolitis. Furthermore, the immature human intestinal epithelium cannot distinguish between pathogens and commensal colonizing bacteria, and reacts to both with an inflammatory response [77]. This immature inflammatory response has to be controlled in order to prevent premature newborns from being in a chronic state of inflammation. Breast milk contains many protective anti-inflammatory components that prevent excessive inflammation until the infant can develop its own mature anti-inflammatory mechanisms.

In a human intestinal model, colostral whey incubated with a human small intestinal xenograft not only reduces the epithelial secretion of IL-8 (inflammatory response), but also downregulates the luminal expression of TLR-4 expression, thereby reducing innate inflammation.

These studies indicate that breast milk protection from infection could be due in part to anti-inflammatory components in the human milk that prevent inappropriate immune response of the infant against nondeleterious antigens.

There have been very few experimental studies on the anti-inflammatory properties of human milk. Neutrophils are the main immune cells involved in the inflammatory process and in vitro studies have shown that human milk can limit the oxidative injury produced by them (reduced cytochrome *c* and consumed H_2O_2) [78].

The explanation for the prophylactic nature of human milk is not currently known. However, some components of human milk have potential anti-inflammatory effects; these include cytokines (as well as their receptors and antagonists), antioxidants, antiproteases, and fatty acids [79].

Cytokines

IL-10

IL-10 inhibits the production of pro-inflammatory cytokines, providing the necessary balance to ensure that the inflammatory response is limited to destroying the pathogen and not healthy tissue. In vivo evidence of the necessity of IL-10 as an anti-inflammatory cytokine is provided by genetically altered mice that are not able to produce IL-10. These mice mount an immune response to the normal microflora in their gut, but without the IL-10 to suppress the inflammation, they develop enterocolitis (similar to ulcerative colitis and celiac disease in humans) [80]. This suggests that IL-10 in human milk might help regulate aberrant immune responses in the infant.

TGF-β

TGF-β inhibits the production of inflammatory cytokines and promotes the healing of intestinal cells damaged by injury or infection. A feeding trial examining the effectiveness of a diet with proteins and supplemented with TGF-β in the management of pediatric Crohn's disease provides the most

convincing in vivo evidence of the anti-inflammatory properties of TGF-β [81]. The enteral diet containing high levels of TGF-β resulted in decreased mucosal IL-1 mRNA (pro-inflammatory cytokine) and clinical remission in 79% of the children [81].

IL-1 Receptor Antagonist

The IL-1 receptor antagonist (IL-1ra) is present in human milk. IL-1ra limits inflammation by competing with IL-1 (pro-inflammatory cytokine) for receptor binding. The reduced inflammatory response in rats with colitis fed human milk compared to formula was similar to the inflammatory response in rats fed infant formula supplemented with IL-1ra [82]. These results suggest that the IL-1ra content of human milk contributes to its anti-inflammatory properties.

Antioxidants

Free radicals, or reactive oxygen species, are produced during the normal metabolic activity of cells. These free radicals can damage cells by lipid peroxidation and alteration of protein and/or nucleic acid structures leading to oxidative stress [83]. Antioxidants in both milk and formula prevent significant lipid oxidation, breast milk suppresses oxidative DNA damage better than formula does. We have yet to identify all of the compounds in human milk that have antioxidant properties; however, there are several antioxidants in human milk that can scavenge free radicals and thereby limit the damage caused by oxidative stress. These compounds include α-tocopherol, β-carotene, cysteine, ascorbic acid, catalase, and glutathione peroxidase. The ability of human milk to resist oxidative stress is greater than formula [84]. In vitro studies have shown that human milk degrades the naturally occurring hydrogen peroxide as well as that produced by neutrophils. This is possibly due to the catalase content of human milk [79].

Lactoferrin has been shown to inhibit the production of proinflammatory cytokines (IL-6 and TNF-α) as well as inflammatory mediators (nitric oxide, GMCSF [4]). The anti-inflammatory activity of lactoferrin is generally attributed to its ability to search out free iron, which is a potent oxidizer.

Antiproteases

Inflammatory cells produce proteases, which allow the cells to enter the affected area. Some pathogens also produce proteases in order to enter the body. Human milk contains active protease inhibitors (e.g., α-1-antitrypsin, α-1-antichymotrypsin, and elastase inhibitor) that can limit the ability of pathogens to gain entry into the body and limit the inflammation caused by the inflammatory response.

Long-Chain Polyunsaturated Fatty Acids

It is hypothesized that the effects of LCPUFA $n-3$ fatty acids on immune function are mediated by their ability to compete with the metabolism of the $n-6$ fatty AA [85]. AA can be metabolized into the pro-inflammatory prostaglandin-E2 (PGE2) or leukotriene-B4 (LTB4) [86].

Prostaglandin E2 is one of the most important prostaglandins formed as it initiates the typical symptoms associated with inflammation: pain, fever, and swelling [87]. The metabolism of AA to

yield PGE2 and LTB4 can be inhibited by DHA, thereby decreasing the capacity of immune cells to synthesize eicosanoids from AA. DHA will then give rise to PGE3 and LTB5, which are considered less biologically potent than the eicosanoids derived from AA. However, their activities have not yet been fully investigated.

Others

Other substances in breast milk can also produce anti-inflammatory effects in the newborn intestine. For example, lactoferrin can reduce the production of inflammatory cytokines in monocytes by inhibiting nuclear factor kappa light-chain enhancer of activated B cell (NFκB) activation.

Protection Against Infection

The adoption of exclusive breast-feeding for the first 6 months of life has been estimated to be the single most effective preventive strategy for saving lives of young children in low-income settings, with a potential estimated reduction of 13% in infant mortality rate [82]. A large part of this protective effect can contribute to a dramatic reduction in infectious diseases incidence, as it has been observed for decades [88–92].

Protection by breast-feeding against death, hospitalization, diarrheal diseases, and acute lower respiratory tract infections (ALRI) is undisputed in children living in conditions of poor hygiene, where mucosal pathogens are major killers in young children.

In developing nations, it has been demonstrated for many years that the risk of dying from diarrhea is dramatically reduced in breast-fed children [7, 93–95]. For example, Scrimshaw and Taylor [96], in the years 1955–1959, demonstrated that the infant mortality rate in villages from Punjab, India, was 950 per 1,000 live births in formula-fed babies compared with 120 in the breast-fed ones. In Rwanda, case-fatality rates for diarrhea, pneumonia, and measles were significantly lower in breastfed, than in completely weaned, hospitalized children, for all three diseases [92].

Additional data from developing countries have confirmed and extended these early observations [97–106]. In communities with a high prevalence of malnutrition, such as rural Bangladesh, breast-feeding may substantially enhance child survival up to 3 years of age [98]. Lack of breast-feeding is an important risk factor for pneumonia and ALRI mortality in developing countries [101, 103–105]. In a population-based study of infant mortality in two urban areas of southern Brazil, the type of milk in an infant's diet was found to be an important risk factor for deaths from diarrheal and respiratory infections [102]. Compared with infants who were breast-fed with no milk supplements, and after adjusting for confounding variables, those completely weaned had 3.6 times the risk of death from respiratory infections [102]. In a prospective observational study conducted in slum areas of Dhaka, Bangladesh [103], partial or no breast-feeding was associated with a 2.23-fold higher risk of infant deaths resulting from all causes and 2.40- and 3.94-fold higher risk of deaths attributable to ALRI and diarrhea, respectively, when compared with exclusive breast-feeding. Betrán et al. [104] have strongly suggested that 5% of infant deaths from diarrheal disease and acute respiratory infections in Latin America are preventable by exclusive breast-feeding among infants aged 0–3 months and partial breast-feeding throughout the remainder of infancy. A strong association between delayed initiation of breast-feeding and increased neonatal mortality has been shown in a large observational study in rural Ghana [99, 100].

A beneficial effect of breast-feeding has also been demonstrated in the industrialized world. Numerous careful studies in children from wealthy nations have now confirmed that breast-feeding

protects against several common diseases such as diarrhea, acute otitis media (AOM), respiratory tract infections, invasive *Haemophilus influenzae* type B infections, neonatal septicaemia, and necrotising enterocolitis [6, 8, 107–109]. Chen and Rogan [110] have assessed the effect of breast-feeding on postneonatal mortality in the USA using recent (1988) National Maternal and Infant Health Survey data. Breast-feeding was associated with a statistically significant reduction in the risk for postneonatal death [104]. In a study of the epidemiology of middle ear disease in Massachusetts children, absence of breast-feeding was a risk factor for AOM and/or recurrent AOM (together with male gender, a sibling history of recurrent AOM, and early onset of otitis) [111].

In industrialized countries, breast-feeding has been shown to reduce the severity of lower respiratory infection in the first 6 months of life and an inverse relation between the duration of ALRI symptoms and the length of exclusive breast-feeding has been demonstrated [112]. In these countries, an effect is seen principally among infants living in crowded conditions in lower socioeconomic strata [112].

In the Republic of Belarus, an experimental intervention (modeled on the Baby-Friendly Hospital Initiative of WHO and UNICEF and emphasizing health care worker assistance with initiating and maintaining breast-feeding and lactation and postnatal breast-feeding support) was shown to decrease the risk of gastrointestinal tract infection in the first year of life [113]. In the UK, breastfeeding, particularly when exclusive and prolonged, protected against severe morbidity from diarrhea and ALRI in a large cohort of healthy, singleton, term infants born from 2000 to 2002 [114]. In Alicante, Spain, full breast-feeding was shown to lower the risk for hospital admission as a result of infections among infants younger than 1 year [115].

Conclusion

Human milk is extraordinary complex in composition. Notably, its content evolves over time. In early lactation phases, SIgA, SIgM, antiinflammatory factors and, most probably immunologically active cells provided by breast milk are substituting for the relatively immature neonatal immune system. Thereafter, breast milk continues to adapt remarkably to the infant ontogeny, to its immune protection needs and to its nutritional requirements. In particular, breast-milk antibodies are targeted against potentially deleterious infectious agents and antigens that are present in the maternal environment, which are those likely to be encountered by the infant. In that sense, it is legitimate to consider the maternal and the infant's immune systems as a continuum, with the placenta and the mammary glands/breast milk as interfaces, now known as the mother–offspring immune dyad [116].

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