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Bioactive proteins in breast milk and their impact on infant gut development

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ABSTRACT

Background: During early stages of life, breast milk is the mainstay of nutrition. Beyond delivering vital amino acids, certain proteins like lactoferrin, α-lactalbumin, sIgA, and lysozyme bolster the infant's gastrointestinal system and provide protective mucosal factors alongside immunomodulatory functions, helping shape the gut microbiome. The goal of this review is to analyze the scientific literature on the types and composition of proteins in breast milk, their biological functions, and their effects regarding the development and protection of the infant's gastrointestinal tract with special focus on the mechanisms of mucosal immunity, mucosal defense, and microbial colonization. **Methods:** A literature search was conducted through PubMed, and Google Scholar databases with the terms bioactive, breastmilk, gut, infant, and microbiota. Only articles published between 2015 and 2025 were chosen for their relevance to the topic and methodological soundness. **Findings:** Whey proteins, which dominate the early lactation phase, contain bioactive peptides that are easily absorbed and support enterocyte maturation. Lactoferrin and sIgA contribute to maintaining mucosal integrity and preventing pathogen colonization. Growth factors such as epidermal growth factor (EGF) and transforming growth factor-beta (TGF-β) accelerate epithelial maturation and strengthen tight junctions, while other proteins like osteopontin and beta-casein help shape a favorable microbial ecosystem. **Conclusion:** Breast milk proteins contribute multifaceted roles in gastrointestinal and immune system development, underscoring the importance of exclusive breastfeeding as a foundation for neonatal gastrointestinal and immunological health. **Novelty/Originality of this article:** This review provides a comprehensive synthesis of the diverse roles of breast milk proteins in shaping infant gastrointestinal development and mucosal immunity by emphasizing perspectives that integrate evidence focused on nutrition, immunology, and the microbiome in a way that has not been fully addressed in previous literature.

KEYWORDS: bioactive proteins; breast milk; infant gastrointestinal tract; mucosal immunity; neonatal microbiota.

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1. Introduction

Breast milk is widely recognized as the most ideal and complete source of nutrition for infants, particularly during the first six months of life. This biological fluid is uniquely tailored to meet the developmental needs of the human neonate. It provides not only macronutrients such as carbohydrates, proteins, and fats, and essential micronutrients like vitamins and minerals, but also a complex mixture of bioactive components that are critical for supporting the maturation of physiological systems most notably, the immune and gastrointestinal systems (Arenas et al., 2025; Afdhal et al., 2025). Its dynamic and adaptive composition enables it to function both as nourishment and as a form of early-life medicine.

As the sole, species-specific nutritional source designed for human infants, breast milk plays a crucial role in shaping health outcomes during infancy and beyond. It protects against infections, fosters proper organ development, and lays the foundation for immunological competence and metabolic regulation. The World Health Organization and numerous pediatric health bodies strongly recommend exclusive breastfeeding for the first six months, followed by continued breastfeeding alongside complementary feeding, owing to its unique and irreplaceable composition. Notably, the long-term health benefits of breastfeeding. such as reduced risk of allergies, obesity, type 2 diabetes, and inflammatory disorders, are attributed to the multifunctional and long-lasting effects of its bioactive components (Hermawati et al., 2025a; Salsabila, 2025; Zulfa et al., 2025). Breastfeeding also helps prevent stunting by supporting optimal growth in early life (Hermawati et al., 2025b). Among the various components of breast milk, proteins occupy a central role due to their dual function as nutritional substrates and biologically active molecules. These proteins contribute to structural growth, metabolic regulation, and most importantly, the modulation and maturation of the infant's immune system. Several key proteins in breast milk have been shown to exert immunomodulatory, anti-inflammatory, and antimicrobial effects that are essential in the early stages of life when the infant's own immune system is still developing (Aquino-Domínguez et al., 2025). These proteins also assist in maintaining the integrity of the intestinal barrier and in establishing a beneficial gut microbiota, both of which are critical for immune education and protection.

The most extensively studied bioactive proteins in human breast milk include α-lactalbumin, lactoferrin, secretory immunoglobulin A (sIgA), lysozyme, and various growth factors. These molecules act in a synergistic manner to enhance intestinal epithelial cell development, maintain mucosal immunity, and establish a healthy microbial ecosystem in the gastrointestinal tract (Le Bras et al., 2025). Each protein has a distinct but complementary function. For example, lactoferrin exerts broad-spectrum antimicrobial activity by binding iron, thereby restricting its availability to iron-dependent pathogens, while also promoting the growth of beneficial bacteria. It also has anti-inflammatory and immunoregulatory properties that help prevent excessive immune activation.

sIgA, on the other hand, is the dominant immunoglobulin in human milk and plays a pivotal role in immune exclusion. It neutralizes pathogens and toxins in the gut lumen without triggering proinflammatory immune responses, thus protecting the infant's mucosal surfaces while allowing tolerance to dietary antigens and commensal microbes. The high concentration of sIgA in breast milk compensates for the immaturity of the neonatal immune system, which has limited capacity for immunoglobulin production in early life (Le Bras et al., 2025). Lysozyme contributes to bacterial lysis by hydrolyzing peptidoglycan in bacterial cell walls, particularly in Gram-positive organisms, and exhibits synergistic effects when combined with lactoferrin.

The composition of breast milk proteins is not static; rather, it changes in response to the phase of lactation and the needs of the infant. During the initial days postpartum, colostrum is produced. It is characterized by a high concentration of immune-related proteins and protective factors. Proteins such as lactoferrin, sIgA, lysozyme, and Bactericidal/Permeability-Increasing Protein (BPI) are abundant in colostrum and play a vital role in providing passive immunity. BPI, in particular, offers potent activity against Gram-negative bacteria by neutralizing endotoxins and promoting phagocytosis, thereby

helping to prevent systemic and intestinal infections during this vulnerable period (Aquino-Domínguez et al., 2025; Syahniar & Suri, 2020).

As lactation progresses from colostrum to transitional and mature milk, the concentration of immunological proteins gradually decreases, while proteins supporting growth and metabolism increase. The early predominance of whey proteins over casein in breast milk during this phase enhances protein digestibility and bioavailability. Whey proteins include a host of bioactive components that not only facilitate rapid nutrient absorption but also regulate cellular and molecular pathways involved in immunity, inflammation, and barrier function. Importantly, the whey-to-casein ratio in human milk shifts over time, yet remains distinct from that of cow's milk, further underscoring its evolutionary adaptation to human infant physiology.

Moreover, the functional role of breast milk proteins extends beyond direct immune defense. They also modulate the development of the infant's gut-associated lymphoid tissue (GALT), which serves as a central platform for immune learning and tolerance induction. In this context, breast milk proteins contribute to the programming of immune responses, reducing the risk of allergic and autoimmune diseases later in life. In addition to proteins, non-protein bioactive components such as human milk oligosaccharides (HMOs) play a complementary role in shaping the infant's immune and gastrointestinal systems. HMOs are structurally diverse, indigestible carbohydrates that serve as prebiotics, promoting the colonization of beneficial microbiota such as Bifidobacteria. They also act as decoy receptors that inhibit the binding of pathogens to the intestinal epithelium. Furthermore, HMOs interact with epithelial and immune cells, modulating cytokine expression and influencing the maturation of innate and adaptive immune responses (Slater et al., 2025). The concerted action of proteins, oligosaccharides, and live microbial components in breast milk illustrates a highly integrated biological system evolved to support infant survival and development. The synergistic relationships among these elements enable breast milk to function as a bio- communicative fluid, transmitting immunological, metabolic, and microbial signals that influence not only immediate health but also the long-term trajectory of immune competence, cognitive development, and disease resistance.

Given the complexity and significance of breast milk composition, a deeper understanding of its protein profile and functional roles is crucial, not only from a nutritional science perspective but also in the context of preventive and therapeutic strategies in neonatology and pediatric care. The clinical implications extend to the design of improved infant formulas, especially for preterm or non-breastfed infants, and to the potential therapeutic application of isolated or recombinant milk proteins in managing neonatal and childhood illnesses. Therefore, this literature review seeks to comprehensively examine the major classes of proteins in breast milk, elucidate their biological mechanisms in supporting neonatal gastrointestinal and immune development, and evaluate their clinical relevance. By integrating current evidence across disciplines including immunology, nutrition, and developmental biology this review aims to highlight the central role of breast milk proteins in shaping early-life health outcomes and in guiding future innovations in maternal-child health interventions.

2. Methods

This study employed a systematic literature review to synthesize current evidence on bioactive proteins in breast milk and their roles in mucosal immunity and infant gastrointestinal health. A comprehensive literature search was conducted through PubMed and Google Scholar using specific keywords, namely bioactive, breastmilk, gut, infant and microbiota. The search was limited to indexed publications from 2015–2025, written in English or Indonesian.

To ensure the relevance and quality of the included studies, predefined inclusion and exclusion criteria were applied. This selection and evaluation process adhered to the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines,

ensuring transparency, reproducibility, and rigor in reporting (Page et al., 2021) as illustrated in Figure 1.

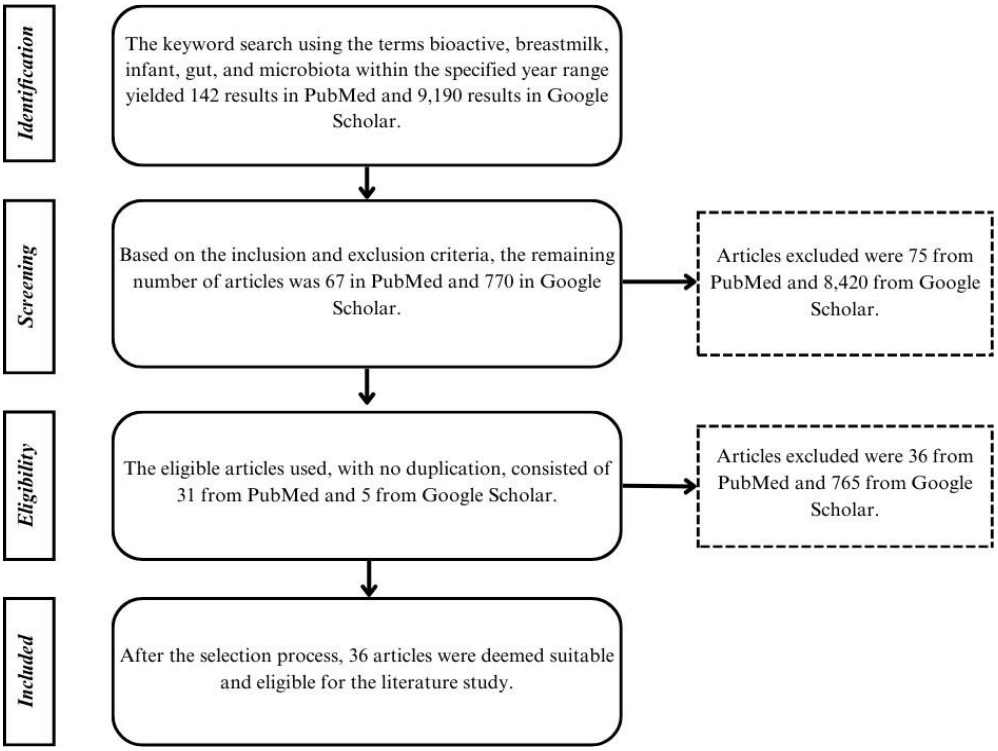

Fig. 1. PRISMA flow diagram of the study selection process

Articles were included if they consisted of original research, review articles, or meta-analyses specifically addressing the types, functions, and mechanisms of bioactive proteins in breast milk, as well as their impact on gastrointestinal physiology, mucosal immunity, or the neonatal microbiota. Conversely, articles were excluded if the full text was unavailable, exhibited methodological weaknesses (e.g., unclear study design or use of unrepresentative samples), were non-scientific publications such as opinion pieces or editorials, or were duplicates of already selected studies. Articles discussing aspects outside the scope of the topic were also excluded from this review, as shown in Table 1.

Table 1. Bioactive proteins in breast milk and their roles in infant gastrointestinal health

No.	Breast Milk Protein	Role in Infant Gastrointestinal Tract	References
1	Lactoferrin	Binds free iron and inhibits pathogen growth; supports mucosal defense and exhibits immunomodulatory activity	Manzoni et al., 2021; Kulesza-Brończyk et al., 2023; Jiang et al., 2024; Mołas et al., 2025; Ceroni et al., 2025
2	Secretory Immunoglobulin A (sIgA)	Prevents pathogen adhesion to intestinal mucosa; promotes immune tolerance to dietary and microbial antigens	Rio-Aige et al., 2021; Mahdally et al., 2023; Donald, 2025
3	Lysozyme	Hydrolyzes Gram-positive bacterial cell walls; contributes to microbial control within the intestinal lumen	Minami et al., 2016; Cheng et al., 2017; Sindi et al., 2025
4	α-lactalbumin	Provides essential amino acids and has antibacterial properties; enhances enterocyte maturation	Layman et al., 2018; Semënova et al., 2022; Menglu et al., 2023; Nagel et al., 2023; Ponchon et al., 2024; Liang et al., 2024; Holgersen et al., 2024

5	Osteopontin	Involved in immune response and regulation of intestinal mucosal integrity	Aksan et al., 2021; Jiang et al., 2023; Sørensen & Christensen, 2023; Ceroni et al., 2025; McClanahan et al., 2025

3. Results and Discussion

3.1 Immunoprotective proteins: Mucosal defense and intestinal immune regulation

Human breast milk contains a variety of bioactive proteins that play a crucial role in supporting the infant's mucosal immune system and regulating intestinal immunity. This function is particularly vital during the early stages of life, when the infant's immune system is still immature and gut microbial colonization is ongoing. Among the key components with immunoprotective activity in breast milk are lactoferrin, sIgA, and lysozyme. These three proteins work synergistically to establish the first line of defense in the infant's gastrointestinal tract while also facilitating immune tolerance to dietary antigens and commensal microbiota (Ceroni et al., 2025; Donald, 2025; Sindi et al., 2025).

Lactoferrin, one of the most abundant iron-binding glycoproteins in breast milk, serves a dual role as both an antimicrobial agent and an immunomodulator. Structurally, lactoferrin has a high affinity for ferric iron (Fe^{3+}), enabling it to inhibit the growth of pathogenic microorganisms that depend on iron for metabolism and proliferation. By sequestering free iron in the intestinal lumen, lactoferrin creates an unfavorable environment for pathogens such as *Escherichia coli, Staphylococcus aureus,* and *Candida albicans*, while simultaneously promoting the growth of beneficial microbes like Lactobacillus and Bifidobacterium (Jiang et al., 2024; Mołas et al., 2025).

Beyond its antimicrobial properties, lactoferrin also enhances both innate and adaptive immune responses. Recent studies demonstrate that lactoferrin can stimulate dendritic cell activation, amplify T-cell signaling, and modulate the production of cytokines such as interleukin-10 and interferon-γ, which are essential for maintaining a balance between immune tolerance and defense against infections (Ceroni et al., 2025). This multifaceted role positions lactoferrin as a key component in the development of the infant's mucosal immune system, which is still under maturation in the first months of life.

sIgA the predominant antibody in breast milk, is primarily found in its dimeric form, bound to a secretory component that protects it from enzymatic degradation in the digestive tract. The main function of sIgA is to provide immunological protection at the mucosal surface of the gut. It prevents the adhesion and invasion of pathogens through a mechanism known as immune exclusion, by binding antigens and microorganisms in the intestinal lumen and facilitating their elimination without triggering inflammatory responses (Mahdally et al., 2023; Rio-Aige et al., 2021). In addition to pathogen protection, sIgA plays a central role in establishing immune tolerance to dietary antigens and commensal microbes. This function is critical for preventing hypersensitivity reactions that may later manifest as food allergies or autoimmune diseases. The underlying mechanism involves the interaction of sIgA with M cells in Peyer's patches and activation of mucosal immune cells, which promote a tolerogenic immune profile, including the upregulation of regulatory T cells that suppress inflammation (Donald, 2025).

Lysozyme, an antibacterial enzyme present in high concentrations in human breast milk, also significantly contributes to intestinal immune defense. This enzyme functions by hydrolyzing the β-1,4-glycosidic bonds in the peptidoglycan layer of bacterial cell walls, primarily targeting Gram-positive bacteria and leading to cell lysis. Although less effective against Gram-negative bacteria, lysozyme's activity is enhanced by lactoferrin, which disrupts the outer membrane of these bacteria, allowing lysozyme better access to their peptidoglycan layers (Minami et al., 2016; Cheng et al., 2017).

Beyond its antimicrobial role, lysozyme helps maintain intestinal microbial balance by preventing the overgrowth of pathogenic organisms. Additionally, it exhibits

immunomodulatory effects by inducing apoptosis in overly active immune cells and suppressing the production of pro-inflammatory cytokines. In the context of intestinal immunity, lysozyme contributes to creating an environment conducive to healthy microbiota colonization and supports immune tolerance (Sindi et al., 2025).

Table 2. Roles of major immunoprotective proteins in human milk on mucosal defense and intestinal immune regulation in infants

Bioactive Protein	Source in Human Milk	Mechanism of Action	Primary Immunological Functions	References
Lactoferrin	Colostrum and mature milk	Binds iron, inhibits microbial growth, modulates cellular immune response	Antimicrobial activity, immune modulation, supports commensal microbiota colonization	Manzoni et al., 2021; Kulesza-Brończyk et al., 2023; Jiang et al., 2024; Mołas et al., 2025; Ceroni et al., 2025
Secretory IgA (sIgA)	Secreted by plasma cells in mammary gland	Blocks pathogen adhesion, neutralizes antigens, promotes immune tolerance	Immune exclusion, mucosal protection, induces oral tolerance to dietary and microbial antigens	Rio-Aige et al., 2021; Mahdally et al., 2023; Donald, 2025
Lysozyme	Present in high concentrations in human milk	Hydrolyzes peptidoglycan in Gram-positive bacteria, synergistic with lactoferrin	Antibacterial activity, microbial homeostasis, mucosal immune modulation	Minami et al., 2016; Cheng et al., 2017; Sindi et al., 2025

These three proteins (Table 2) do not function independently but exhibit synergistic interactions in maintaining immunological homeostasis in the infant's gastrointestinal tract. For instance, the interaction between lactoferrin and lysozyme has been shown to enhance antibacterial effectiveness against various pathogens, while sIgA facilitates the encapsulation of protein-antigen complexes to prevent direct contact with the intestinal mucosa. This combination forms a mucosal immune defense system that is non-aggressive yet highly effective, ideally suited for the vulnerable gut environment of neonates (Ceroni et al., 2025; Donald, 2025; Sindi et al., 2025).

This protective mechanism is particularly crucial during the neonatal period, a time when the adaptive immune system is immature and infants are highly dependent on passive immunity provided through breast milk. Moreover, the interaction between breast milk bioactive proteins and early microbial colonization in the gut significantly influences the long-term development of the immune system, including the risk of chronic inflammatory conditions such as inflammatory bowel disease, asthma, and other autoimmune disorders (Cheng et al., 2017; Jiang et al., 2024; Mahdally et al., 2023).

Research also indicates that exclusive breastfeeding during the first six months of life is associated with a significant reduction in the incidence of gastrointestinal and respiratory infections, as well as increased diversity and stability of the gut microbiota. These findings reinforce the understanding that breast milk serves not only as a source of nutrition but also as an early immunological intervention that programs the infant's immune system for a healthier future (Donald, 2025; Menglu et al., 2023).

Furthermore, recent studies suggest that the levels and activity of these proteins may be influenced by various factors, including the duration of lactation, maternal nutritional

status, and environmental exposures. For example, lactoferrin concentrations are typically higher in colostrum and decrease over time, while sIgA levels remain relatively stable throughout lactation. This understanding is important in the context of public health interventions, particularly in supporting early initiation and continued breastfeeding (Donald, 2025; Mahdally et al., 2023; Rio-Aige et al., 2021). The immunoprotective proteins in human breast milk play an integral role in shaping the foundation of the infant's mucosal immune system. Through a combination of antimicrobial actions, immunomodulatory effects, and promotion of immune tolerance, lactoferrin, sIgA, and lysozyme collectively support both short-term and long-term gastrointestinal and immune health in infants. Enhancing our understanding of these mechanisms provides a crucial basis for promoting breastfeeding practices and developing evidence-based nutritional strategies that emulate the biological advantages of human milk.

In addition, quantitative data provide further clarity on the immunoprotective roles of breast milk proteins. At 42 days postpartum, lactoferrin levels reach approximately 99.4 mg/100 g but significantly decrease to 75.3 mg/100 g by 3 months ($p < 0.01$), consistent with longitudinal analyses showing a progressive decline during the first 6 months of lactation (Kulesza-Brończyk et al., 2023). Similarly, α-lactalbumin decreases from 380.3 mg/100 g to 272.8 mg/100 g, and osteopontin from 24.3 mg/100 g to 18.3 mg/100 g over the same period ($p < 0.01$), in line with recent proteomic profiling of early mature milk (Liang et al., 2024). Lysozyme was not measured in this dataset, but consistent with intervention studies, it is known to increase progressively during lactation and can be modulated by maternal diet (Sindi et al., 2025). Secretory IgA remained relatively stable, ranging from 38.6 mg/100 g at 42 days to 33.0 mg/100 g at 3 months, showing no significant difference ($p = 0.1539$), which supports findings that sIgA levels are maintained throughout lactation to ensure mucosal protection (Rio-Aige et al., 2021). Casein fractions demonstrated a distinct pattern: αs1-casein and β-casein decreased significantly with time, whereas κ-casein and total casein also declined but remained the dominant protein group (>1,100 mg/100 g at 3 months) (Liang et al., 2024). These findings, together with evidence from randomized controlled trials showing that oral lactoferrin supplementation reduces late-onset sepsis and necrotizing enterocolitis (NEC) in preterm infants (Manzoni et al., 2021), underline the translational potential of breast milk proteins as preventive interventions in neonatal care.

Nevertheless, limitations in the current evidence should be acknowledged. Variability in breast milk protein concentrations may arise due to maternal factors such as nutritional status, genetics, and environmental exposures, making it difficult to establish standardized benchmarks. Moreover, most studies remain observational, and further multicenter clinical trials are needed to confirm the long-term effects on immune development and chronic disease prevention. From a clinical and public health perspective, these insights reinforce the importance of exclusive breastfeeding for the first six months as both a nutritional and immunological intervention. At the same time, they provide a scientific basis for the development of fortified infant formulas designed to emulate the protective properties of human milk, thereby bridging gaps in infant nutrition when breastfeeding is not possible.

3.2 Bioactive components of breast milk in mucosal maturation and immune regulation

The neonatal period is a critical window for the development and maturation of the infant's immune and digestive systems. During this stage, the intestinal mucosa is not yet fully developed, rendering it more susceptible to infections, inflammation, and exaggerated immune responses triggered by pathogenic microorganisms and environmental antigens. Therefore, maintaining the integrity of the intestinal mucosa is essential to support systemic homeostasis and protect infants from gastrointestinal and systemic disturbances (Grases-Pintó et al., 2024; Nolan et al., 2019; Torres-Castro et al., 2020).

Breast milk plays a central role in facilitating this process, not only serving as a source of nutrition but also functioning as an active immunological agent. It contains a wide array of bioactive components, including regulatory proteins that have been shown to play key

roles in the maturation and maintenance of intestinal mucosa (Nolan et al., 2019). Among the most extensively studied proteins are Transforming Growth Factor-β (TGF-β), Epidermal Growth Factor (EGF), and osteopontin (OPN). These three proteins exhibit complementary mechanisms of action in regulating epithelial cell differentiation and proliferation, reinforcing tight junction structures between epithelial cells, and directing the development of the mucosal immune system (Torres-Castro et al., 2020; McClanahan et al., 2025).

The gastrointestinal mucosa serves as the first line of defense against pathogens and foreign antigens. In neonates, the maturation of this mucosal barrier is highly dependent on external stimuli, particularly those derived from breast milk. TGF-β is known to suppress inflammation and promote the establishment of oral immune tolerance; EGF facilitates epithelial regeneration and intestinal growth; and OPN exerts immunomodulatory effects and supports intraepithelial lymphoid cell activity (Grases-Pintó et al., 2024; Torres-Castro et al., 2020). Additionally, breast milk contributes to the balance of the gut microbiota, which plays a vital role in the infant's long-term health.

Transforming Growth Factor Beta (TGF-β) is a multifunctional cytokine that plays a pivotal role in the immune system and the development of the infant gastrointestinal tract (Grases-Pintó et al., 2024). Among its various isoforms, TGF-β is the most predominant in colostrum and early-phase breast milk, also the presence of TGF-β at high concentrations in human milk functions as a local immunosuppressant and a systemic immune regulator, which is particularly vital during the early stages of life when the immune system is still undergoing maturation (Torres-Castro et al., 2020).

One of the key functions of TGF-β is to promote the establishment of oral tolerance the immune system's ability to recognize dietary and environmental microbial antigens as non-threatening, thereby preventing excessive immune responses that could damage the mucosal tissue (Grases-Pintó et al., 2024). Studies have demonstrated that exposure to TGF-β through breast milk contributes to the suppression of intestinal mucosal inflammation by reducing the activation of pro-inflammatory immune cells and promoting the differentiation of regulatory T cells (Treg), which are essential for maintaining immune homeostasis. This protective effect is particularly evident in preterm infants who receive breast milk, as they exhibit a significantly lower risk of developing NEC compared to those who do not receive breast milk (Grases-Pintó et al., 2024; Torres-Castro et al., 2020). TGF-β has been shown to play a key role in preventing mucosal injury caused by excessive inflammation, as well as in facilitating the repair of damaged intestinal epithelial tissue (Nolan et al., 2019). Moreover, the administration of donor milk containing TGF-β has also been proven to reduce gastrointestinal inflammation, reinforcing the notion that the bioactivity of human milk is markedly superior to that of infant formula (Hård et al., 2019). EGF is a key bioactive component present in high concentrations in colostrum and early breast milk, particularly during the first few days postpartum. EGF plays a fundamental role in promoting the proliferation, differentiation, and maturation of intestinal epithelial cells, which is crucial for the development of the immature digestive system in neonates (Noel et al., 2021). One of the primary mechanisms by which EGF supports intestinal health is through the upregulation of tight junction proteins, such as occludin and claudin (Noel et al., 2021; Grases-Pintó et al., 2024). These proteins are essential for maintaining the integrity of the intestinal mucosal barrier, preventing the translocation of pathogenic microorganisms and toxins from the intestinal lumen into systemic circulation, thereby protecting infants from infections and inflammation.

In experimental studies, intestinal epithelial cell cultures treated with human breast milk demonstrated increased transepithelial electrical resistance (TER), a key indicator of epithelial barrier integrity and strength (Noel et al., 2021). Additionally, a significant reduction in the production of pro-inflammatory cytokines such as Monocyte Chemoattractant Protein-1 (MCP-1), Granulocyte-Macrophage Colony-Stimulating Factor (GM-CSF), and Interleukin-8 (IL-8) was observed when compared to cultures treated with infant formula (Noel et al., 2021). These findings suggest that EGF, in conjunction with other immunological factors in breast milk, acts synergistically to enhance mucosal defense

mechanisms while mitigating potentially harmful local inflammatory responses. Thus, the presence of EGF in breast milk not only supports tissue growth but also plays a crucial immunoprotected role during the neonatal period (Grases-Pintó et al., 2024; Noel et al., 2021; Torres-Castro et al., 2020).

Osteopontin is a multifunctional glycoprotein naturally found in high concentrations in human breast milk, particularly during the early stages of lactation (Ceroni et al., 2025; Jiang et al., 2023; Sørensen & Christensen, 2023). OPN plays a significant role in modulating both innate and adaptive immune responses. One of its key mechanisms of action involves influencing the activation and function of T cells and macrophages—two central components of the mucosal immune system (Sørensen & Christensen, 2023). In addition, OPN contributes to the maintenance of intestinal mucosal homeostasis, ensuring the stability of the epithelial environment that is essential for the physiological and protective functions of the gastrointestinal tract (McClanahan et al., 2025).

Experimental studies in animal models have shown that milk-derived OPN can influence the development of intraepithelial lymphocytes (IELs), particularly the CD8αα subset, which plays a crucial role in maintaining tolerance to dietary and environmental antigens. However, the direct effects of OPN on susceptibility to inflammatory diseases such as colitis remain to be fully elucidated, as current findings are still inconclusive (McClanahan et al., 2025). Overall, OPN in breast milk appears to contribute to the establishment of a mucosal environment that promotes immunological tolerance, accelerates the healing of microscopic epithelial injuries, and supports the balance of the gut microbiota. Studies have highlighted that neonatal exposure to OPN may exert long-term effects on immune system resilience and the stability of the gastrointestinal mucosa (Ceroni et al., 2025; Jiang et al., 2023; Sørensen & Christensen, 2023).

TGF-β, EGF, and OPN play synergistic roles in enhancing intestinal mucosal function and promoting immunological tolerance in neonates (Grases-Pintó et al., 2024; Nolan et al., 2019; Torres-Castro et al., 2020). By binding to specific receptors on the surface of intestinal epithelial and immune cells, these proteins activate diverse intracellular signaling pathways that regulate cell proliferation, differentiation, and immune modulation. Through these mechanisms, they reinforce epithelial barrier integrity by upregulating tight junction proteins, and they support the development of oral tolerance by dampening excessive inflammatory responses to dietary and microbial antigens. Moreover, these bioactive proteins facilitate neonatal adaptation to the external environment after birth by preserving mucosal homeostasis and enhancing resilience against infections and inflammatory insults, thereby ensuring optimal maturation of the gastrointestinal and immune systems during this critical developmental window.

Beyond these key proteins, the interaction of TGF-β, EGF, and OPN with other bioactive constituents in human breast milk such as oligosaccharides, beneficial microbes, and extracellular vesicles (e.g., exosomes) creates a complex and synergistic microenvironment that shapes the neonatal mucosal immune system (Dawod et al., 2021). HMOs, for example, not only act as prebiotics that promote the growth of commensal microbiota but also modulate gene expression in mast cells and goblet cells, both of which are integral to mucosal immune function and tolerance induction. Osteopontin, in particular, has been associated with improved infant growth and immune competence; clinical studies have shown that higher OPN concentrations in breast milk correlate with a reduced incidence of fever and fewer infection-related hospitalizations during the first three months of life (Aksan et al., 2021). This intricate interplay among milk-derived bioactive factors underscores the role of breast milk not merely as a source of nutrition, but as a dynamic immunological and developmental modulator in early life.

Human breast milk plays a critical role not only in nutrition but also in enhancing mucosal immunity and protecting against disease during early life. It stimulates the production of DEFA5, a key antimicrobial peptide produced by both Paneth and goblet cells, thereby strengthening mucosal defenses and supporting intestinal homeostasis (Noel et al., 2021). Beyond its antimicrobial effects, breast milk contains immunomodulatory components such as TGF-β, which is strongly associated with reduced incidence of neonatal

inflammatory conditions, including NEC, respiratory infections, and allergic diseases. Higher levels of TGF-β in breast milk have been shown to suppress the production of pro-inflammatory cytokines such as interleukin-6 (IL-6) and interferon-gamma (IFN-γ), contributing to a more regulated immune environment (Quitadamo et al., 2021). These synergistic effects demonstrate that breast milk functions as a multifaceted defense system—preserving the integrity of the intestinal barrier, promoting immune tolerance, reducing inflammation, modulating epithelial gene expression, and offering protection from environmental pathogens during the critical neonatal period.

Quantitative evidence provides further insight into the role of these proteins in mucosal maturation. Reported concentrations of TGF-β in colostrum range from 2–7 ng/mL, with levels decreasing as lactation progresses, while EGF levels are highest in colostrum (~200– 300 ng/mL) and decline steadily in mature milk (Miller, 2024). OPN is present in particularly high concentrations (~100–250 mg/L), nearly 100-fold higher than in bovine milk (Sørensen & Christensen, 2023). Clinical studies further highlight their functional significance: higher TGF-β levels have been associated with reduced incidence of NEC and allergic manifestations in infants (Aksan et al., 2021), EGF supplementation in animal models accelerates intestinal healing (Torres-Castro et al., 2020), and elevated OPN concentrations correlate with lower rates of fever and infection-related hospitalizations in the first months of life (Hård et al., 2019). These findings emphasize the translational potential of breast milk proteins as natural immunomodulators with measurable clinical benefits.

Despite these promising findings, current evidence has important limitations. The concentrations of TGF-β, EGF, and OPN vary considerably depending on maternal genetics, nutrition, stage of lactation, and even preterm versus term delivery, making it difficult to establish universal reference ranges. Moreover, many human studies remain observational, and randomized controlled trials are still limited. Future research should address these gaps to better define the dose-response effects and long-term outcomes of these proteins on mucosal development and immune regulation. From a practical standpoint, this knowledge underscores the importance of breastfeeding promotion while also guiding innovations in infant nutrition. Fortification of formula with recombinant TGF-β, EGF, or OPN analogs represents a potential industrial application to more closely mimic the bioactivity of human milk for infants who cannot be exclusively breastfed.

3.3 Nutritional and enzymatic proteins: Nutrition, bioactivity, and microbiota colonization

Breast milk proteins also serve as an essential nutritional source and bioactive agents that support microbiota colonization and metabolic efficiency in infants. Key components in this category include α-lactalbumin, β-casein, and bile salt-stimulated lipase (BSSL). α-Lactalbumin is the main whey protein, rich in essential amino acids, and crucial for protein synthesis and infant growth. Additionally, α-lactalbumin exhibits antibacterial activity and supports the maturation of enterocytes in the intestine (Layman et al., 2018; Menglu et al., 2023; Semënova et al., 2022). A study by Layman et al. (2018) highlighted α-lactalbumin as one of the primary whey proteins in human breast milk, with an amino acid profile highly suited to infant needs. α-Lactalbumin accounts for approximately 22% of the total protein in human milk, compared to only around 3.5% in cow's milk, with high levels of essential amino acids such as tryptophan, lysine, and BCAAs (leucine, isoleucine, valine) as well as sulfur-containing amino acids (Layman et al., 2018). α-Lactalbumin contains high concentrations of tryptophan, which is essential for serotonin synthesis, as well as BCAAs that support tissue growth and central nervous system development.

α-Lactalbumin plays a vital role in the formation of lactose in the mammary glands through the lactose synthase complex—a process that triggers an increase in milk volume. In terms of nutrition formulation, α-lactalbumin allows for the reduction of total protein in infant formulas while maintaining optimal nutritional value for the infant. In addition to being a source of essential amino acids, α-lactalbumin generates bioactive peptides with

antibacterial and prebiotic activity and can support neurological functions and sleep in adults due to its high tryptophan content (Layman et al., 2018).

The β-caseomorphin (BCM) peptides (BCM-8, -9, -10, and -11) are derived from the breakdown of β-casein in breast milk through endogenous proteolysis mediated by natural protease enzymes in the milk. According to Enjapoori et al. (2019), these peptides are naturally present in human breast milk and exhibit biological activity as weak opioid agonists. Their effects include modulation of gastrointestinal motility, regulation of satiety, and potential pain reduction. Furthermore, BCM is also suspected of having immunomodulatory and antioxidant functions, which synergistically protect the infant from oxidative stress and systemic inflammation. These findings support the understanding that naturally hydrolyzed milk proteins are not only a source of amino acids but also provide bioactive peptides that play a crucial role in physiological regulation during early life. Therefore, β-casein in breast milk not only contributes to structural nutrition but also mediates vital biological responses that are essential for the holistic development of the infant.

Research by Holgersen et al. (2024) revealed that the composition of β-casein in infant formulas directly affects gut health and neonatal immune responses. In a neonatal pig model, formulas with β-casein levels resembling the natural composition of breast milk (65% of total casein) were clinically safe—without inducing inflammation, impairing growth, or causing gastrointestinal complications. However, when β-casein levels were increased to ≥91%, an increase in inflammatory marker expression and disruption of gut mucosal integrity were observed. This highlights the importance of protein balance in formula, as excessive β-casein may trigger dysbiosis and intestinal inflammation, potentially interfering with immune system and metabolic development in infants.

As the main component of casein, β-casein releases bioactive peptides during digestion. These peptides have prebiotic effects that support the growth of beneficial microbiota such as Bifidobacterium, while also enhancing mucosal immune responses (Enjapoori et al., 2019; Holgersen, 2024). In a study of mother–infant pairs, Menglu et al. found a positive correlation between functional protein content in breast milk (sIgA, κ-casein, osteopontin, α-lactalbumin) and the presence of certain microbiota species such as Veillonella parvula, Clostridium butyricum, and Parabacteroides distasonis in the infant gut at 42 days of age. Proteins like OPN and κ-casein are also associated with key metabolic pathways (amino acids, propionate, linoleic acid, α-linolenic acid), suggesting that breast milk proteins influence both the composition and function of the infant's microbiota in the early stages of life (Menglu et al., 2023).

Meanwhile, Koh et al. (2020) investigated the impact of pasteurization methods on digestive enzyme activity in donor breast milk, specifically bile salt-stimulated lipase (BSSL), which plays an important role in the digestion of triglycerides and absorption of long-chain fatty acids in infants. Conventional pasteurization using low temperature and long time (Low-Temperature Long-Time, LTLT or Holder pasteurization) drastically reduces BSSL activity, leaving only about 0.3% of its original activity. In contrast, alternative techniques such as high-pressure processing (HPP) and ultraviolet (UV) pasteurization proved to better preserve BSSL activity, at around 60–62%. As infants rely heavily on this enzymatic activity for efficient fat digestion, the selection of an appropriate pasteurization method is crucial for maintaining the nutritional value of donor breast milk for infants who do not receive direct breast milk. BSSL is a critical lipolytic enzyme in breast milk that breaks down triglycerides into free fatty acids and monoglycerides, especially during the neonatal period when pancreatic lipase is not yet fully functional. In addition to its role in digestion, BSSL is also believed to be involved in the adaptation of the intestinal mucosa to fat metabolism (Koh, 2020; Wada & Lönnerdal, 2015).

The integration of nutrient aspects, enzymatic activity, and bioactivity of proteins in breast milk forms a crucial physiological foundation for infant development, particularly in supporting gut microbiota colonization and immune system maturation. Proteins such as α-lactalbumin and β-casein not only provide essential amino acids needed for growth but also serve as precursors for bioactive peptides that regulate various neonatal biological

processes. These degradation products can act as immunomodulators, antioxidants, and support symbiotic interactions between the host and commensal microbes in the digestive tract (Layman et al., 2018; Enjapoori et al., 2019).

The role of proteins in breast milk also depends on the stability of their structure and function, which is influenced by storage and processing techniques. For example, donor breast milk pasteurized using high temperature and long time (Holder) may inactivate essential enzymes like BSSL, which are involved in fat digestion in infants. In contrast, non-thermal methods like HPP are more capable of maintaining enzymatic activity, thereby more closely mimicking the physiological function of fresh breast milk. The stability of enzymes and protein bioactivity is crucial for breast milk to effectively support infant metabolism and immune defenses (Koh et al., 2020).

In the case of infant formulas, adjustments to protein composition such as β-casein must also consider biological balance. Recent studies show that a high ratio of β-casein in formulas increases the risk of inflammation and gut mucosal disturbance, while a composition resembling that of breast milk supports a more adaptive immune response and a healthy microbiota. This suggests that the success of infant formula is not determined solely by its nutritional content but also by how the protein structure interacts with the infant's digestive and immune systems (Layman et al., 2018).

α-Lactalbumin has an amino acid profile that is highly suited to infant needs and is involved in lactose synthesis, thereby supporting osmotic balance and the growth of probiotic microbes. Meanwhile, β-casein produces peptides like β-casomorphins (BCM) that exhibit biological activity as mild opioid agonists, with the potential to regulate intestinal motility, satiety, and even protect against oxidative stress. These activities demonstrate that breast milk proteins function not only as macronutrients but also as signaling molecules in the regulation of infant physiology (Enjapoori et al., 2019).

In the context of neonatal physiology, breast milk proteins can be viewed as multifunctional elements involved in the regulation of various biological systems. Their availability as substrates for proteolysis generates active peptides with both local and systemic effects, including immune tolerance formation, infection protection, and digestive system maturation. Therefore, protein degradation is not a passive process but a directed biological mechanism that refines the function of breast milk as the ideal food for infants. It is also important to emphasize that the effectiveness of breast milk proteins in supporting microbiota colonization is influenced by their interaction with other components such as oligosaccharides and immunoglobulins. Partially hydrolyzed proteins can create a microenvironment that supports the growth of symbiotic microorganisms like Bifidobacterium and Lactobacillus, collectively enhancing resistance to pathogens and maintaining intestinal mucosal integrity (Layman et al., 2018; Menglu et al., 2023).

Thus, understanding the structure, function, and enzymatic dynamics of breast milk proteins is crucial in the development of evidence-based neonatal nutrition interventions. Whether optimizing infant formulas or managing donor breast milk, scientific principles regarding protein bioactivity and stability must form the foundation of considerations. This holistic approach not only focuses on meeting macronutrient needs but also supports the overall biological and immunological development of the infant. Overall, proteins in breast milk are not merely nutritional components but bioactive agents with complex regulatory functions. The integration of nutritional, enzymatic, and bioactive aspects make breast milk unique and irreplaceable, serving as an ideal model for the development of safe, effective, and physiologically aligned neonatal nutrition products (Layman et al., 2018; Holgersen et al., 2024).

Quantitative analyses indicate that α-lactalbumin constitutes approximately 22–25% of total breast milk protein, while β-casein accounts for nearly 30–35% of the protein fraction, reflecting a whey-to-casein ratio that favors infant digestibility (Ponchon et al., 2024; Holgersen et al., 2024). BSSL, although a minor component by weight, demonstrates remarkable functional potency; its enzymatic activity in fresh milk has been measured at 500–700 U/mL, with a drastic decline following Holder pasteurization to below 1% of its native activity (Sari et al., 2023; Davis & Perrin, 2023). Clinical studies further show that α-

lactalbumin-enriched formulas improve nitrogen retention, promote sleep quality, and reduce gastrointestinal distress, while β-casein-balanced formulations support favorable microbiota colonization and reduce pro-inflammatory markers (Holgersen et al., 2024; Nagel et al., 2023). Similarly, observational evidence suggests that infants fed donor milk with higher residual BSSL activity demonstrate better fat absorption and improved weight gain compared to those receiving pasteurized milk with inactivated enzymes (Sari et al., 2023; Davis & Perrin, 2023).

Several gaps remain: protein levels and enzymatic activity vary across mothers, lactation stages, and diets, making universal references difficult. Evidence on bioactive peptides such as β-casomorphins and α-lactalbumin fragments is largely preclinical, with long-term effects still unclear. Although high-pressure and UV pasteurization preserve activity better than conventional methods, large-scale clinical trials are lacking. These insights emphasize the importance of prioritizing breastfeeding, adopting advanced processing in donor milk banks, and refining infant formula to better replicate the bioactive environment of human milk, highlighting breast milk proteins as multifunctional regulators of metabolism, immunity, and microbiota.

4. Conclusions

Breast milk proteins such as lactoferrin, sIgA, lysozyme, EGF, TGF-β, α-lactalbumin, β-casein, and BSSL work synergistically to support gastrointestinal maturation, immune protection, and microbiota balance, making them indispensable for early life nutrition. These insights have direct implications for clinical practice in pediatrics and neonatology, emphasizing the role of breastfeeding in infection prevention and growth optimization, while also guiding the development of infant formulas that more closely replicate the structural and functional properties of human milk proteins. Beyond nutritional considerations, this knowledge reinforces the urgency of promoting exclusive breastfeeding as the optimal feeding strategy in the first six months of life. Future research should further explore population-specific variability in protein composition, the long-term physiological impact of bioactive peptides, and innovations in donor milk processing or formula technology to preserve protein bioactivity, ensuring both scientific progress and tangible health benefits for infants worldwide.

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