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Review article

Ameliorating adverse perinatal outcomes with Lactoferrin: An intriguing chemotherapeutic intervention

Julie A. Talbert [a], Jacky Lu [b], Sabrina K. Spicer [a], Rebecca E. Moore [c], Steven D. Townsend [a,*], Jennifer A. Gaddy [b,c,d,e,*]

[a] Department of Chemistry, Vanderbilt University, Nashville, TN, USA
[b] Department of Pathology, Microbiology and Immunology, Vanderbilt University Medical Center, Nashville, TN, USA
[c] Department of Medicine, Vanderbilt University Medical Center, Nashville, TN, USA
[d] Tennessee Valley Healthcare Systems, Department of Veterans Affairs, Nashville, TN, USA
[e] Center for Medicine, Health, and Society, Vanderbilt University, Nashville, TN, USA

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ABSTRACT

Adverse pregnancy outcomes affect 54 million people globally per year, with at least 50% of these attributed to infection during gestation. These include inflammation of the membranes surrounding the growing fetus (chorioamnionitis), preterm prelabor rupture of membranes (PPROM), preterm birth (PTB), early-onset disease (EOD) and late-onset disease (LOD), neonatal and maternal sepsis, and maternal or fetal demise. Although universal screening and implementation of intrapartum antibiotic prophylaxis (IAP) has improved EOD outcomes, these interventions have not reduced the incidences of LOD or complications occurring early on during pregnancy such as PPROM and PTB. Thus, novel therapies are needed to prevent adverse pregnancy outcomes and to ameliorate disease risk in vulnerable populations. Lactoferrin has recently been explored as a potential therapeutic as it demonstrates strong antimicrobial and anti-biofilm activity. Lactoferrin is a glycoprotein capable of iron chelation found in a variety of human tissues and is produced in high concentrations in human breast milk. In recent studies, lactoferrin has shown promise inhibiting growth and biofilm formation of streptococcal species, including Group B *Streptococcus* (GBS), a prominent perinatal pathogen. Understanding the interactions between lactoferrin and GBS could elucidate a novel treatment strategy for adverse pregnancy outcomes caused by GBS infection.

1. Introduction

1.1. Adverse pregnancy outcomes

Over 50 million people experience an adverse pregnancy outcome each year according to the World Health Organization (WHO).[1–7] These adverse pregnancy outcomes include chorioamnionitis, preterm birth (PTB), preterm prelabor rupture of membranes (PPROM), early-onset (EOD) and late-onset disease (LOD) neonatal sepsis, maternal sepsis, necrotizing enterocolitis and maternal or fetal demise. Often, these outcomes stem from *Streptococcus agalactiae* or group B *Streptococcus* (GBS) infection. GBS is often a commensal bacterium in healthy adults, but can become infectious in those that are pregnant, older, or immunocompromised. Treatment for GBS infection includes intrapartum antibiotic prophylaxis (IAP) during labor as infants most often contract GBS through vertical transmission, although, they can also acquire GBS through skin-to-skin contact or from hospital settings. While implementation of vaginal and rectal GBS screening and IAP has improved statistics, there are still over half a million preterm births annually leading to about 100,000 neonatal deaths and 46,000 still births attributable to GBS infection.[8] Additionally, with the rise in antibiotic resistance and the detrimental effects of antibiotics to the immature infant microbiota, novel remedies are desperately needed. Fig. 1. Fig. 2. Table 1.

* Corresponding authors at: Vanderbilt University School of Medicine- Division of Infectious Diseases, A2200 Medical Center North, 1161 21st Avenue South, Nashville, Tennessee 37232, USA (J.A. Gaddy) and Vanderbilt University, Department of Chemistry, 7330 Stevenson Center, Station B 351822, Nashville, Tennessee 37235, USA (S.D. Townsend).
E-mail addresses: Steven.d.townsend@vanderbilt.edu, jennifer.a.gaddy@vumc.org (J.A. Gaddy).

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Table 1
Adverse pregnancy outcomes and how they manifest in the patient.

Adverse Pregnancy Outcome	Manifestation
Chorioamnionitis	• Inflammation and/or infection of the placenta and fetal membranes • Can result in stillbirth, PTB, PPROM, neonatal sepsis, and long-term brain and lung injury
Preterm Birth (PTB)	• Delivery before 37 weeks gestation • Often caused by chorioamnionitis or inflammation and/or infection of the placenta and fetal membranes
Preterm Prelabor Rupture of Membranes (PPROM)	• Rupture of membranes before 37 weeks gestation • Major risks include respiratory preterm birth, sepsis necrotizing enterocolitis, and syncytial virus (RSV).
Early-Onset Disease (EOD)	• Transmitted vertically from the mother during delivery through the birth canal • Can result in sepsis, pneumonia, and meningitis
Late-Onset Disease (LOD)	• Transmitted vertically, horizontally from hospital environment, or contaminated breast milk • Can result in meningitis, sepsis, bacteremia
Neonatal Sepsis	• Life-threatening blood infections during the first 90 days of life
Necrotizing Enterocolitis	
Maternal Sepsis	• Risk factors include Caesarean section, early labor, obesity, diabetes, and prolonged rupture of membranes

1.2. Chorioamnionitis

Chorioamnionitis is the acute inflammation of the amniochorionic membrane and is often associated with PPROM and PTB. *Ureaplasma urealyticum, Chlamydia trachomatis, Neisseria gonorrhoea, Mycoplasma hominins,* GBS, *Trichomonas vaginalis,* and some gram negative anaerobes are the usual culprits of this inflammation.[9–13] This complication can cause significant maternal, perinatal, and long-term adverse effects. Poor patient outcomes include postpartum infections and sepsis while poor infant outcomes include stillbirth, PTB, neonatal sepsis, chronic lung disease, and brain injury.[14–20] Current treatment methods include the use of broad-spectrum antibiotics. Most studies used intravenous ampicillin (2 g) every 6 hours for gram positive organisms, gentamicin (1.5 mg/kg) every 8 hours for gram negative organisms, and clindamycin (900 mg) every 8 hours for additional coverage during any necessary caesarean sections.[21–23] The utility of antibiotics is confounded as they can alter the infant's gut microbiota, and are steadily being rendered ineffective with the emergence of multi-drug resistant bacteria.[24–25] Although there is some deviation from this antibiotic regimen in the literature, consensus on the best protocol is not evident. Chorioamnionitis plays a role in approximately 40–70 % premature births, highlighting the need for alternative methods to prevent and treat this inflammation.[26]

1.3. Preterm prelabor rupture of membranes (PPROM)

PROM (prelabor rupture of membranes) is defined as the rupture of membranes before the start of labor. Furthermore, PPROM is the rupture of membranes before 37 weeks of gestation and is responsible for 3–4% of pregnancies and one-third of all preterm births.[27–29] PPROM is a major contributor to perinatal mortality due, in part, to its immediate initiation of labor post rupture. Gestational age is the main variable that dictates the severity of complications after PPROM, but severity can increase with perinatal infection, placental abruption, and umbilical cord compression.[27] The two most common complications after PPROM are respiratory syncytial virus (RSV) and PTB. Other morbidities, include sepsis, necrotizing enterocolitis, and intraventricular hemorrhage.[27–29] There are several risk factors for PPROM, including PPROM in a prior pregnancy, chronic steroid therapy, cigarette smoking, low body mass index, low socioeconomic status, nutritional deficiencies, chorioamnionitis, and placental abruption.[30] When the membranes rupture, it is recommended to deliver the baby immediately if the risk of ascending infection outweighs the risk of prematurity. If immediate delivery is unnecessary and the gestational age is less than 34 weeks, options include administering antenatal corticosteroids or broad-spectrum antibiotics.[30–32] Although protocols to diagnose and treat PPROM exist, PPROM still affects 3–4% of all pregnancies and precedes 40% to 50% of preterm births,[33–34] demonstrating the need for novel therapeutic strategies.

1.4. Preterm birth (PTB)

PTB is a delivery that occurs prior to 37 weeks' gestation. In 2018, PTB was the second leading cause of death in infants and in 2020, 1 in every 10 infants born in the U.S. were affected by PTB.[35] Even more alarming are the racial and ethnic disparities amongst PTB rates. The PTB rate for African American women in 2020 was 13.8%, which is higher than the global PTB rate of 12%.[35] Risk factors of PTB include race, socioeconomic background, maternal nutritional status, and the bacterial dysbiosis of the vaginal microbiome.[36–41] Historically, the hallmark of vaginal health has consisted of a *Lactobacillus*-dominated vaginal microbiome and disruption of this dominance can cause bacterial vaginosis, a higher risk for acquiring sexually transmitted infections, PTB, and pelvic inflammatory disease.[42–44] For example, *Lactobacillus crispatus* prevalence has shown association with a lower risk of PTB.[45–49]

The lifelong implications of PTB on the infant can include asthma, cerebral palsy, vision and hearing impairments, and some learning disabilities, such as attention deficit hyperactivity disorder (ADHD) and increased anxiety.[50–52] Studies examining prevention of PTB include administering progesterone, a hormone that increases over the course of gestation. Progesterone protects fetal membranes, prevents uterine contractions, and has anti-inflammatory properties. Makena, an intramuscular injection of synthetic progestin hydroxyprogesterone caproate in oil, is the only current FDA approved drug for PTB prevention.[53–55] Due to several studies reporting mixed results on Makena, the Center for Drug Evaluation and Research (CDER) recommended that the Food and Drug Administration (FDA) withdraw Makena's approval.[56] Studies have been conducted to investigate the efficacy of vaginally and orally administered progesterone in preventing PTB, yet no FDA approved treatment has been made available.[57–64] The use of aspirin to combat PTB has also been researched, but more trials are needed to better understand its potential benefits.[65–66] Clearly, PTB is a serious complication of pregnancy and although extensive studies and trials have been conducted, novel methods are required to decrease the mortality and morbidity linked to PTB.

1.5. Neonatal sepsis

Sepsis is defined as a life-endangering infection caused by a dysregulated host response to infection.[67] Although difficult to determine, the global burden of sepsis in 2017 was estimated to consist of 48.9 million cases resulting in 11 million deaths with almost half of all cases occurring among children.[68] More alarming though, is that 2.9 million of these deaths are attributed to children under the age of five and roughly 85% of sepsis cases occurred in low to middle income countries.[68] Most commonly, *Escherichia coli* (*E. coli*) and GBS are the etiological pathogens of sepsis, but *Listeria monocytogenes,* non-typeable *Haemophilus influenzae,* gram negative enteric bacilli other than *E. coli, Candida* spp., coagulase negative staphylococci, *Streptococcus pneumoniae,* and *Streptococcus pyogenes* have also been implicated in neonatal sepsis.[69–74] Risk factors for neonatal sepsis include invasive medical devices, PROM, chorioamnionitis, PTB, maternal fever, and low birth weight.[75–77]

Recognition of sepsis, though, is difficult as the symptoms and signs are subtle. Although vague, some symptoms include hypothermia in preterm babies, fever in term babies, tachycardia, bradycardia, cool or pale extremities, respiratory symptoms, such as nasal flaring or grunting, neurological symptoms, such as seizures or abnormal primitive reflexes, gastrointestinal symptoms, such as vomiting or jaundice, and skin abnormalities.[78]

Diagnostic testing is performed in any neonate with risk factors or any concerning symptoms to determine the need for antibiotics and observation. Such testing includes blood cell counts, blood cultures, swab cultures, placental cultures, polymerase chain reaction (PCR), and matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry.[78] All current treatment options require antibiotic interventions, underscoring the need for novel remedies to combat the rise in antibiotic resistance.

1.6. Early-Onset disease (EOD)

Most consistently, early-onset disease (EOD) is defined as occurring during the first 7 days of life and is typically caused by vertical transmission of bacterial pathogens from mother to infant before or during delivery.[79] Often, causative pathogens of EOD are colonizers of the maternal genitourinary tract, which leads to amniotic membrane rupture or an intra-amniotic infection that allows the transmission of the pathogen vertically to the infant.[79–81] The most common colonizers of the maternal genitourinary tract that are responsible for EOD include GBS and *Escherichia coli*. Combined, these two pathogens account for over 70% of EOD cases worldwide. Among these, other causative bacteria include *Enterococcus* spp., *Haemophilius influenzae*, *Listeria monocytogenes*, *staphylococcus aureus*, gram negative *Enterobacter* spp., and other streptococci. While historically GBS is the most common culprit for EOD, disease burden is increasingly becoming attributable to *E. coli* and other gram negative rods. In fact, gram negative neonatal sepsis is steadily increasing, which is worrisome due to difficulties in treating gram negative infections.[82]

As previously mentioned, GBS remains the current, most frequently implicated bacteria causing EOD in infants.[83] EOD accounts for 60–70% of GBS disease in infants with GBS serotypes Ia, III, and IV predominately associated with this outcome.[84–85] Briefly, diagnosis of a GBS infection is done through a rectovaginal swab of the pregnant person between 36- and 37-weeks gestation. If GBS cultures are positive, treatment includes IAP during labor to prevent transmission. Antibiotic selection is based on any maternal allergies as well as GBS strain sequencing for resistance. Although IAP has been shown to prevent EOD with up to 90% efficiency,[86] EOD is still associated with the highest rates of mortality for GBS infections.

1.7. Late-Onset disease (LOD)

Late-onset disease (LOD) occurs after the first 7 days of life and can be vertically or horizontally acquired. LOD is normally less fatal than EOD although it can be acquired horizontally several ways, including skin-to-skin contact with mothers during breast feeding or contact with external sources like infected surfaces, hospital settings, and contact with other adults and infants.[87–89] The NICHD Neonatal Research Network shows that 70% of LOD infections are caused by gram positive bacteria. These bacteria include coagulase negative *Staphylococcus* spp. (CoNS), *Staphylococcus aureus*, *Enterococcus* spp. and GBS.[90–91] Gram negative organisms, including *E. coli*, *Klebsiella*, and *Pseudomonas* species, account for 18% of all LOD infections, with the remaining 12% of infections attributable to fungi and other non-culturable bacteria.

While many LOD cases are caused by nosocomially acquired pathogens, studies have shown that GBS can be transmitted through breast milk, although the relationship between GBS colonization in infants from GBS-containing breast milk is not fully understood.[92] While IAP has been highly effective in preventing EOD, rates of LOD have remained relatively constant. It is also important to note that while IAP has proven effective against GBS-induced EOD, it has also been linked to increased susceptibility to antibiotic resistant infections leading to LOD.[93]

1.8. Necrotizing enterocolitis

Necrotizing enterocolitis (NEC) is the most common etiology of gastrointestinal emergencies in infants.[94] Characterized by mucosal and, in invasive cases, transmural necrosis of the gastrointestinal tract, specifically the intestines, NEC is the leading cause of neonatal mortality and morbidity.[95] NEC infections begin with an impressive inflammatory cascade within the highly immunoreactive infant intestine and extend systemically, ultimately affecting other organs including the brain. While the pathophysiology of NEC is poorly understood, intestinal immaturity is a cornerstone of disease predisposition. As such, the preterm infant is at an increased risk. Further, very low birth weight infants, who are unable to breast feed, have less species diversity within their intestinal microbiota, often causing dysbiosis of the primitive microbiome. This dysbiosis opens the premature host up to numerous opportunistic infections and pathogenic bacteria ultimately increasing the risk of NEC.

Because our current understanding of the molecular underpinnings of NEC is lacking, the current therapeutic strategies employed are more preventative in nature. These strategies include avoiding/withholding enteral feedings and administration of various probiotic agents to the premature infant. Recent therapeutic advances have explored a more immunological approach to treatment. This includes pharmacological modulation of toll-like receptor 4 (TLR4), a first line of defense against pathogens.[96] Ultimately, these efforts have been futile and a larger understanding of NEC is required to strategize better treatment options.

1.9. Maternal sepsis

During pregnancy, the fetus is semi-allogenic to the maternal host, thus immune tolerance must be maintained for fetal development to progress. To maintain a tolerogenic state, anti-inflammatory signaling pathways are initiated, and this leads to higher susceptibility to infections, including sepsis in pregnant patients compared to their non-pregnant counterparts. *E. coli,* beta-hemolytic streptococci*, Staphylococcus aureus,* and *Listeria monocytogenes* are organisms associated with maternal sepsis.[85,97] Roughly 11% of maternal deaths arise from infection.[98] The risk factors for maternal sepsis differ depending on the availability of resources. In high-income countries, risk factors include delivery by Caesarean section, early labor, obesity, diabetes, and prolonged rupture of membranes.[99–100] Risk factors in low-income countries include poverty, unhygienic birth conditions, lack of skilled birth assistants, unavailable medical supplies, distance to healthcare facility, young age, and HIV.[101–102] Although the inequity is clear, sepsis is still a major contributor of maternal deaths in all countries and novel mechanisms of recognition and treatment are necessary.

2. Novel chemotherapeutic strategies are desperately needed for these disease outcomes

Although screening for GBS infection in the third trimester has significantly improved the EOD burden, disease outcomes that occur earlier in pregnancy are not alleviated by this screening. Furthermore, treatment with IAP has reduced the incidence of EOD, but cannot prevent LOD. Other complications of IAP or any antibiotic regimen include the rise of antibiotic resistance and the potential devastating impact on the developing neonatal microbiome. Novel strategies are crucial for the reduction of these adverse pregnancy outcomes. Vaccine administration to women early in pregnancy could provide protection against adverse pregnancy outcomes attributed to GBS; however, no current vaccines are available due to the difficulties in this area of research. Most vaccine candidates to date were developed to target the streptococcal polysaccharide capsule, but because of serotype variability and capsular switching, these vaccines have not proven functionally active.[103–104]

3. Human breast milk components in protection against disease

A large body of literature underscores the benefits of human breast milk nutrition in improving infant health, including protection against infectious diseases. Recent studies have supported that human breast milk components can prevent bacterial infections, including diarrheal diseases,[105–106] urinary tract infections,[107–108] and other diseases associated with bacterial infection.[109] More studies have revealed that components of human breast milk, including maternal immunoglobulins, transforming growth factor beta (TGF-β), proteins and milk oligosaccharides aid in improving infant health.[110–112] Components of human breast milk also influence the nascent microbiome to protect against invading pathogens,[113] and many of these components can directly inhibit the pathogenic bacteria from establishing a replicative niche within the host. In fact, studies have shown that human milk components can suppress bacterial growth and biofilm formation and enhance the efficacy of antibiotics.[114–115]

One antimicrobial glycoprotein secreted in high concentrations (up to 20% of the proteinaceous composition) in human breast milk is lactoferrin.[116–118] Lactoferrin concentrations within milk vary with gestational age at delivery and time since parturition.[117] For example, lactoferrin concentrations are higher in the first milk called colostrum, which tapers in production as the infant matures. Generally, lactoferrin concentrations in colostrum at term parturition average 7–9 g/L and decrease to 1–3 g/L at 6–12 months after parturition.[117–118] Additionally, neonatal male sex has been associated with lower lactoferrin levels, a result that coincides with enhanced susceptibility to neonatal infections, such as necrotizing enterocolitis.[119–120] Interestingly, lactoferrin levels in human breast milk from preterm infants are significantly higher and remain higher for up to 2 months post-parturition.[118,121]

4. Lactoferrin: a glycoprotein in human breast milk

4.1. Structure and function of lactoferrin

Lactoferrin is an 80-kDa single-chain glycoprotein comprised of 703 amino acids folded into two globular lobes. Because of its intrinsic ability to bind iron, lactoferrin can exist in two primary isoforms: an apo- form (lacking iron within its binding sites) or a holo- form (where iron inhabits the binding sites).

Lactoferrin exerts antimicrobial activity against a wide range of viral, fungal, and bacterial pathogens.[122–125] Lactoferrin has two high-affinity iron binding sites[104] that participate in chelation of essential nutrient iron and starvation of an invading microorganism; a process commonly called “nutritional immunity”.[122,126] Iron, in particular, is crucial for bacterial survival within the host niche by acting as a cofactor for enzymes driving bacterial DNA replication, transcription, and central metabolism.[127] In addition to iron-scavenging activity, lactoferrin binds directly to bacterial cell walls causing destabilization in an iron-independent mechanism.[128] Thus, due to its potent broad antimicrobial activity, several groups have studied lactoferrin for therapeutic use. In a systematic review of available literature, it was found that enteral lactoferrin supplementation decreases late-onset sepsis.[117] Additionally, combining lactoferrin with probiotics may decrease necrotizing enterocolitis in preterm infants.[129] Lactoferrin also shows promise as an agent against streptococcal infections,[130] including a study demonstrating lactoferrin decreases biofilm formation in *Streptococcus mutans*.[131] Biofilms are multicellular structures that are critical for bacterial pathogenesis, specifically in GBS, to circumnavigate host defenses and persist in the hostile environment.[132–133]

In previous studies, lactoferrin has been purified from donor human breast milk and utilized to demonstrate that human milk lactoferrin has the capacity to inhibit bacterial growth, survival, and biofilm formation by GBS *in vitro*.[134] Furthermore, human milk lactoferrin has the capacity to inhibit GBS adherence to primary human gestational membrane

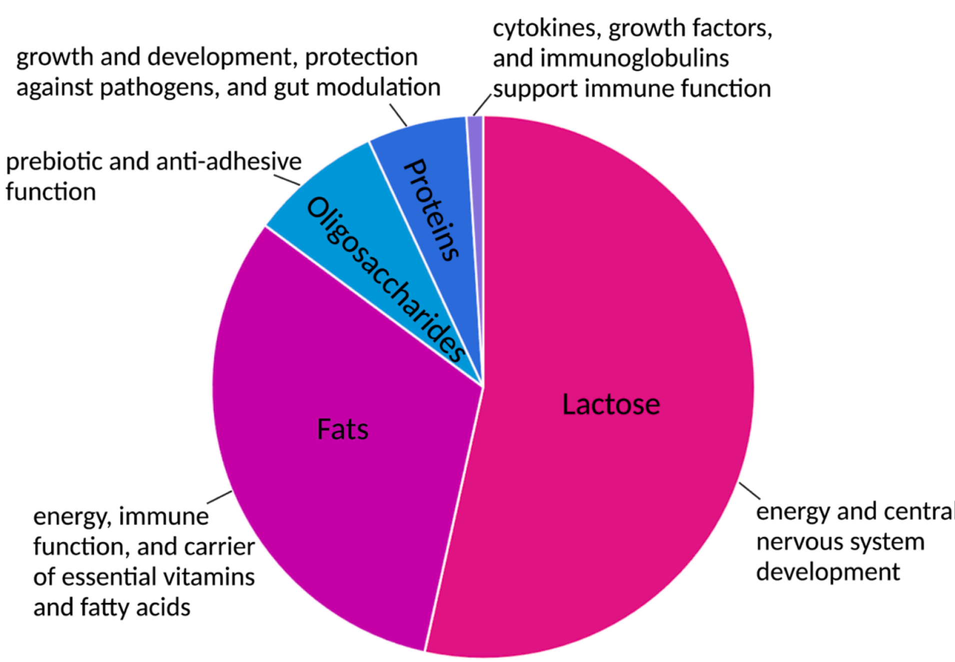

Fig. 1. Composition of human breast milk broken down into five main components, including lactose, fats, oligosaccharides, proteins, and cytokines, growth factors, and immunoglobulins.

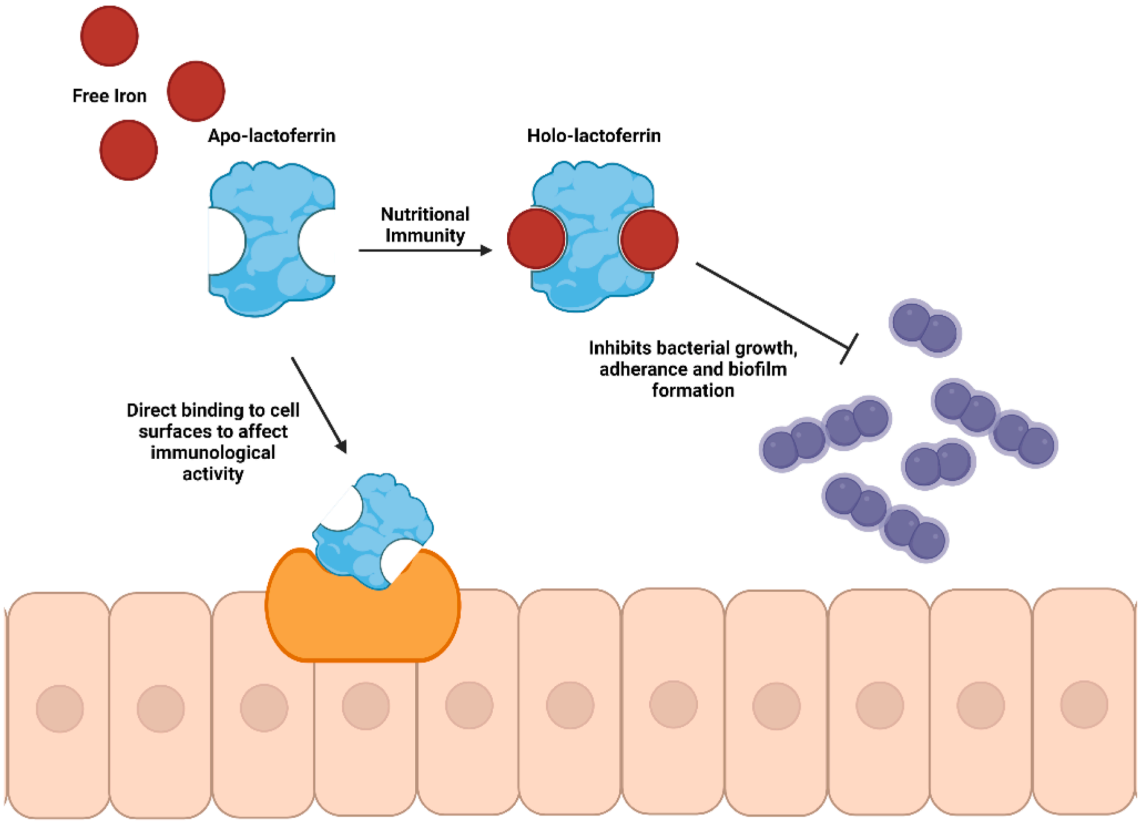

Fig. 2. Proposed mechanisms by which lactoferrin inhibits activity of GBS.

tissues *ex vivo*, and the antimicrobial properties are enhanced when the glycoprotein is capable of binding iron (in the apo- isoform).[135]

4.2. Lactoferrin and Escherichia coli

E. coli is a gram negative, facultative anaerobic, rod-shaped bacterium that is commonly found in the intestine of humans. Some strains can cause severe intestinal infections with complicating symptoms, while others are harmless. *E. coli* O157:H7 is a strain that infects individuals causing symptoms, such as severe abdominal cramps and bloody diarrhea, that can lead to renal malfunctions with prolonged infection. Transmission of this infectious bacterium often comes from eating contaminated food, or swimming in or drinking contaminated water.[136] Although there are some methods to reduce the occurrence of infectious *E. coli* breakouts, they have not fully eliminated the risk of *E. coli* infection.

In 1993, Dionysius, Grieve, and Milne studied the effects of apo- and holo- lactoferrin on enterotoxigenic strains of *E. coli*.[137] It was observed that apo-lactoferrin inhibited growth of all strains at 1.0 mg/mL, while holo-lactoferrin had no effect on bacterial growth. Furthermore, Gnezda, Franklin, and McKillip found that lactoferrin from raw bovine milk inhibited the growth of *E. coli* O157:H7 at concentrations greater than 14.05 mg/mL.[138] It has recently been discovered that oral administration of lactoferrin B, derived from lactoferrin in whey, protected mice against *E. coli* O157:H7 infection.[139] In this study lactoferrin B improved epithelial barrier function, relieved inflammation, and induced regulation of gut microbiota. Many more studies exist in which lactoferrin inhibits the growth of pathogenic *E. coli* strains.[140–142]

4.3. Lactoferrin and Acinetobacter baumannii

Acinetobacter baumannii is a gram negative bacterium that causes a wide variety of diseases, such as sepsis, meningitis, and pneumonia.[143] Most often, *A. baumannii* infections are nocosomially acquired and seen in ventilated patients in intensive care unit facilities. *A. baumannii* is associated with 80% of all hospital-acquired pneumonia and ventilator-associated pneumonia exposing how threatening *A. baumannii* is in a hospital setting.[144] The rise in multidrug resistant *A. baumannii* strains coupled with the lack of an available vaccine makes battling *A. baumannii* infection difficult. Since lactoferrin binds available iron, it has shown antimicrobial activity against *A. baumannii* and could be useful as a potential therapeutic.

Our group has investigated bovine and human lactoferrin in combination with *A. baumannii*.[145] We found that both human and bovine lactoferrin inhibited bacterial growth of *A. baumannii* isolated from wounds at concentrations of 250 μg/mL and above. *A. baumannii* isolated from sputum collected from patients with respiratory infections was inhibited by human lactoferrin at concentrations of 250 μg/mL and above and by bovine lactoferrin at concentrations of 500 μg/mL and above. Human lactoferrin at concentrations as low as 62.5 μg/mL and bovine lactoferrin at concentrations of 250 μg/mL and above inhibited growth of *A. baumannii* isolated from blood. Finally, isolates of *A. baumannii* from urinary tract or abdominal cavity infections were susceptible to growth inhibition by both human and bovine lactoferrin starting at 62.5 μg/mL.

Mahdi *et al.* have also studied the combination of lactoferrin and *A. baumannii*.[146] Using purified lactoferrin extracted from camel colostrum milk, this study investigated the antibacterial activity of lactoferrin against 14 isolates of multidrug resistant *A. baumannii*. They found that purified camel lactoferrin at concentrations of 8, 16, 32, and 64 μg/mL inhibited the growth of *A. baumannii in vitro*. Additionally, lactoferrin significantly reduced the number of bacteria in lung and blood cultures in a mouse model. With these two studies, it is apparent that different types of lactoferrin have inhibitory properties against *A. baumannii* and should be explored further.

4.4. Lactoferrin and commensal microbes

The hallmark of a normal or healthy vaginal microbiome includes the dominance of *Lactobacillus* species, especially, *L. crispatus, L. gasseri, L. jensenii, and L. iners*.[147–149] *Lactobacillus* species protect the vaginal environment from pathogens through the production of lactic acid, resulting in a low pH of 3.5–4.5.[150–153] Moreover, *Lactobacillus* species display antimicrobial activity by production of target-specific bacteriocins,[154–155] which fend off the growth of pathogenic organisms.

Several studies show the benefit of utilizing lactoferrin to enhance *Lactobacillus* spp. growth. Pino *et al.* found that lactoferrin modified the vaginal microbiota composition in patients with bacterial vaginosis.[156] The treatment group saw decreases in occurrence of bacteria associated with bacterial vaginosis, including *Gardnerella*, *Prevotella*, and *Lachnospira*. Further, lactoferrin increased the occurrence of *Lactobacillus* species. One study by Otsuki and colleagues found that oral and vaginal administration of lactoferrin allowed a woman with three consecutive PPROMs to have a successful cesarean section.[157] *Lactobacillus* spp. were dominant in the vaginal flora only until discontinuing the use of lactoferrin after the delivery. In 2017, Otsuki & Imai conducted a study including six women with a history of adverse pregnancy outcomes.[158] The results showed that orally and vaginally administered lactoferrin allowed each of these women to have a successful birth. *Lactobacillus* spp. dominated the vaginal microbiomes of these women only after one month of the lactoferrin therapy.

4.5. Immunomodulatory actions of lactoferrin

Adverse pregnancy outcomes are frequently related to inflammation.[159] One mechanism by which lactoferrin acts is by interfering with recognition of pathogens associated molecular patterns (PAMPs) by toll-like receptions (TLRs). A consequence of this receptor binding is the downregulation of proinflammatory cytokines by the immune cells. Lactoferrin has been shown to bind to bacterial LPS, the ligand for TLR4, thus mitigating TLR4 mediated pro-inflammatory cytokine production by macrophages.[160] Another study revealed that lactoferrin can bind to soluble CD14 (sCD14), which normally complexes with LPS to induce production of IL-8, resulting in the inhibition of IL-8 production by epithelial cells and macrophages, ultimately reducing recruitment of neutrophils to the site of infection.[161] Lactoferrin is also known to bind DNA, so internalization of the peptide into immune cells by receptors such as nucleolin[162] can inhibit NF-κB binding to the TNF-α promoter and downregulate LPS-induced cytokine production.[160]

The glycosaminoglycans of membrane proteoglycans on cell surfaces account for 80% of binding by lactoferrin with low affinity (10^{-5}-10^{-6} M).[163] Proteoglycans are important because they interact with cytokines to activate its immune properties. For instance, IL-8 is a chemokine that activates LFA-1 integrins, therefore playing a role in cellular chemotaxis. Proteoglycans can bind IL-8, and other cytokines, therefore concentrating the local IL-8. Elass *et al.* in 2002 revealed that human lactoferrin disrupted the interaction between immobilized heparin and IL-8 *in vitro*, thereby modulating cell migration to the cite of inflammation.[164] Proteoglycans can also bind to other cytokines such as IL-2[165] and IL-7.[166] As such, lactoferrin may disrupt the interactions between these cytokines and the cell.

In a study conducted by Wisgill and colleagues, lactoferrin demonstrated weakening of the proinflammatory response of neonatal monocyte-derived macrophages.[167] Specifically, human lactoferrin treatment on LPS-activated neonatal macrophages isolated from heparinized cord and peripheral blood resulted in a decrease production of cytokines, including TNF, IL-β, IL-6, IL-8, and IL-10. Furthermore, lactoferrin treatment reduced expression of activation marker and phagocytosis by these macrophages. Taken together, lactoferrin may be a prime candidate for improving adverse pregnancy outcomes not only by its antimicrobial activity, but also by its ability to modulate immune cells and reduce inflammation.

5. Conclusions

Millions of people suffer every year from adverse pregnancy outcomes. Although universal screenings and antibiotic treatments are available, these only decrease the risk of some adverse pregnancy and birth outcomes, leaving many patients without proper care. While vaccine research is ongoing, it is imperative we look for alternative therapeutics for treating perinatal infections. As outlined here, utilizing the beneficial properties of lactoferrin could prevent or alleviate the risk of infections associated with microbial pathogenesis.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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