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REVIEW OPEN ACCESS

Virulence factors of *Streptococcus agalactiae* relating to neonatal sepsis

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Abstract

Streptococcus agalactiae (Group B *Streptococcus*, GBS) infection is the leading cause of disease in neonates. Infection in the neonate can occur via vertical transmission or ascension through the vaginal tract. After ascension, infection of the amniotic fluid occurs via several virulence factors which allow for invasion of the placental membrane and chorioamniotic membranes. Neonatal infection is categorized into either early-onset sepsis or late-onset sepsis, with both being able to result in severe illness or even death. Prevention of GBS infection in the mother and neonate to this point includes neonatal screening at 35-37 weeks and treatment in GBS positive patients with IV penicillin. In those with allergies, cefazolin or clindamycin may be substituted. Documented increases in antibiotic resistance have led to the need for further research surrounding virulence factors. This outline summarizes several virulence factors including the hemolytic pigment, hyaluronidase, pili, adhesins, and the sialic rich capsular polysaccharide, that have been identified as key to colonization and infection of the neonate. Understanding of the virulence factors and incorporating prior deletion studies allows for advancements in treatment and prevention to be made. Novel theories will open the door for further research towards implementation of a vaccine as a means for preventing colonization.

Keywords: *Streptococcus*; *agalactiae;* neonates; vaginal tract

1. Introduction

Streptococcus agalactiae (Group B Streptococcus, GBS) is a β-hemolytic, gram-positive facultative anaerobic bacterium that frequently inhabits the gastrointestinal and genitourinary tracts. The close proximity of the rectum and vagina often accounts for the contamination of GBS and ascension through the vaginal canal. Vaginal colonization is typically asymptomatic in immunocompetent individuals but can cause major health complications in pregnant patients and their neonates [1]. As many as 18% of pregnant individuals are asymptomatically colonized with GBS [2]. There are two main mechanisms of acquisition of infection in the neonate: 1) ascending infection and 2) vertical transmission during or shortly after delivery. Ascending infection followed by invasion of the placental membrane and chorioamniotic membrane accounts for infection of the amniotic fluid. When amniotic fluid is infected with GBS, neonates are at risk for preterm birth, mortality, and fetal injury [3]. On the other hand, vertical transmission has been linked to increased instances of pneumonia, sepsis and other life-threatening complications. Furthermore, GBS infection has also been implicated in hearing and vision loss, cerebral palsy, and chronic delays in development [4]. Several socioeconomic factors have been related to higher incidence of vaginal colonization during pregnancy. Among these, African American race, young maternal age (<20 years), and diabetes are prominently noted [5].

There are two main presentations of GBS infection in the neonate that pose serious harm and even death: early-onset sepsis and later-onset sepsis. GBS is currently responsible for 38-43% of all bacterial sepsis cases in

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neonates [5]. Early-onset sepsis typically occurs within the first few postnatal days and is characterized by in utero infection, vertically transmitted during delivery. Infection rates are exponentially increased in very-low-birth-weight infants [5]. Early-onset sepsis in infants is characterized by apnea, cyanosis, and bradycardia followed by severe respiratory distress . Cytokine proteins, such as interleukin (IL)-6, IL-8 and tumor necrosing factor alpha (TNF-α) are used as markers in non-specific diagnosis of neonatal sepsis [5]. Late-onset neonatal disease almost always presents within the first three months and cannot be prevented by intrapartum antibiotic prophylaxis like early-onset disease is. Late-onset sepsis is characterized primarily by respiratory illness requiring increased need for respiratory support, along with tachycardia, feeding difficulties and temperature instability [6].

Current treatment guidelines of GBS include late antenatal screening followed by prophylactic antibiotic treatment with IV penicillin, ampicillin, or cefazolin prior to vaginal or cesarean delivery in patients who have positive colonization [7]. Recent increases in antibiotic resistance within the GBS species have interfered with treatment strategies, thus calling for a better understanding of the virulence factors. Accurate identification and summary of virulence factors will allow for novel theories about the current mechanisms of resistance present. This will open the door for further recommendations of treatment strategies including vaccination as a means for preventing colonization.

Virulence factors

1. Hemolytic GBS pigment

The β-hemolytic pigment of GBS plays a large role in their evasion of immune responses and colonization via ascending infection [3]. Ornithine rhamnolipid pigment, also known as hemolytic pigment, is the driving factor for GBS hemolytic activity [3, 8]. The hemolytic GBS pigment may further be distinguished based on two different deletions within the gene, *covR/S* creates hyper-hemolytic and hyper-pigmented versus the *cylE* gene which creates non-hemolytic and non-pigmented [3, 8-10].

Hemolytic GBS pigment binds amnion epithelial cells, neutrophils, mast cells and macrophages causing cytolysis, resistance, degranulation and pyroptosis, respectively [8]. This virulence factor has been found to promote invasion of human amniotic epithelial cells (hAECs) which serves as the final barrier to the amniotic cavity and fetus [1]. Hemolysin is further able to induce activation of proinflammatory mediators in hAECs [1].

In addition to the hemolytic pigment's proinflammatory activity, it also promotes coagulation and is thus prothrombotic [11, 12]. The prothrombotic activities found include activated plasma clotting and blood clotting [11, 12]. The process in which hemolytic GBS pigment with hyper-hemolytic activity affects platelet function is via two steps: 1) activating human platelets and then 2) using necrosis to kill them [12]. Furthermore, GBS strains that were isolated from septic patients bind to surface fibrinogen, inducing synthesis of platelet thromboxane, P-selectin expression as well as platelet aggregation [13].

GBS hemolytic pigment, particularly the hyperhemolytic pigment, is implicated in both ascending infection and evasion of host immune response, leading to increased morbidity and mortality in the infected neonate [14]. Targeting and elimination of this pigment could prove to be a future source of treatment and prevention of neonatal disease.

2. Hyaluronidase enzyme

GBS hyaluronidase enzyme (HylB) facilitates vaginal colonization and preterm birth through its immunosuppressive mechanisms [8, 15]. HylB cleaves host hyaluronic acids/hyaluronan (HA) into disaccharides which subsequently dampen the response from toll like receptors (TLR) 2 and 4 [8, 15, 16]. HA are vital for cell signalizing, cell migration, inflammation regulation as well as preventing infection ascension [17-19]. Hyaluronan receptors such as TLR-2, TLR-4, and cluster of differentiation (CD) 44 have all been found to serve as protection against GBS during pregnancy [16]. Recent research done by Coleman et al., also found increasing levels of interleukin-10 (IL-10) and IL-10 expressing macrophages in mice that were colonized by HylB proficient GBS, further supporting that GBS with HylB has increased rates of dissemination. Another study by Kjems et al showed that out of eighty-one strains of GBS, type three was most often linked with bacteremia and meningitis in infants and 95% of the organisms studied in this strain were hyaluronidase positive [20]. This study implicated the hyaluronidase protein as being a vital part of the type III strain and therefore being a major cause of neonatal disease. Further tracking of the hyaluronidase protein as a major source of infection in neonates with GBS sepsis could provide further benefit regarding prevention strategies.

3. Pili

There have been three different types of pilus proteins isolated in the GBS species and all of which could have potential as a vaccine target [21]. The pilus proteins provide the organism with adhesive properties that lead to vaginal colonization. GBS pili and serine-rich

repeat (Srr) proteins are also responsible for vaginal and cervical host cell attachment. Attachment to epithelial cells requires the glycosylated Srr proteins forming an interaction with the Srr-1 human protein [22]. The GBS cell wall pilus protein has additional responsibility in proper anchoring to the host cell. Two subunits form the pilus protein, with PilA being critical for vaginal cell colonization [22]. A study using a mutant GBS lacking the Srr protein resulted in decreased colonization in comparison to the wild-type GBS strain [22]. Another recent study by Gupalova et al introduced a vaccine that utilized the fimbrial/pili protein of an enterococcus organism and showed both a systemic and local immune response [23]. The question that this study leaves is: Is this vaccine successful in providing an immune response sufficient to protect from ascension and amniotic membrane colonization by GBS? If so, then utilizing enterococcus fimbriae could propose a potential for preventing or decreasing neonatal infection. Furthermore, pilus protein components have been proven to provide protection, when introduced via intranasal vaccination, against *S. pyogenes* species [21].

4. Adhesins

Adhesins are responsible for mediating interaction with host cells, they typically promote bacterial adhesion to epithelial components such as fibrinogen and laminin. These proteins are particularly responsible for initial colonization and invasion of the vaginal canal and placental membranes. Colonization of the placental membrane is a necessary step required in causing neonatal disease. GBS possesses several adhesins that have been linked to colonization of the vagina during pregnancy including Fibrinogen-binding proteins (Fbs), laminin-binding protein (Lmb), group B streptococcal C5a peptidase, and hypervirulent GBS Adhesin (Hvg A) [24].

4.1. ScpB, C5a peptidase

The GBS C5a peptidase is responsible for limiting host immune defenses by inactivating a chemotoxin produced by the human C5a. The molecular structure of GBS's C5a peptidase mimics the human protein yet does not contain all six-amino-acid fragments [25]. In a study comparing a mutant Streptococcus pyogenes, that did not produce the C5a Peptidase, with a wild-type species, it was found that the C5a Peptidase containing organisms had delayed accumulations of leukocytes but no difference in virulence [25]. A possible theory derived from this conclusion involves the idea that prolonged exposure to infectious organisms due to delayed leukocyte response could lead to increased colonization within the amniotic fluid and thus further neonatal infection.

4.2. Hvg A

GBS colonization of the lower genital tract is largely mediated by adhering to epithelial cells through the use of adhesins such as Hvg A [2, 26]. GBS ST-17, a clone of GBS with Hvg A, has been found to be associated with late-onset disease in the neonate further defined by meningitis after one week of life [26].The expression of Hvg A has been found to be necessary for GBS hypervirulence as well as augment its adherence to choroid plexus epithelial cells, intestinal epithelial cells, and the microvascular endothelial cells of the blood-brain barrier (BBB) [26].

4.3 Fibrinogen binding proteins (Fbs)

Fibrinogen receptors for the fibrinogen binding proteins FbsA, FbsB, and FbsC, have all been found to play critical roles in GBS ability to adhere to epithelial cells. When the *fbsA* gene was deleted in various GBS strains, the bacteria's ability to bind fibrinogen, adhere to epithelial cells and thus invade was greatly decreased [27]. FbsC (also known as bacterial surface adhesin or BsaB) plays a similar role to FbsA in adhering to epithelial cells and the extracellular matrix and thus the deletion of both their genes would interfere with the vaginal colonization of GBS [9]. FbsC has also been found to create a biofilm that further enhances GBS ability to remain in hosts [9, 28]. While FbsB also assists GBS in invading epithelial cells, deletion of *fbsB*, however, was found to not affect GBS attachment to fibrinogen [27, 29].

4.4 Laminin binding protein (Lmb)

The laminin-binding protein is a surface protein responsible for adhesion and invasion of host cells in the vaginal canal. Safadi et al outlined the specific genetic element responsible for production of this adhesion protein, the scpB-lmb intergenic region. This region codes for several of the different adhesin proteins, including insertion element IS1548 which was repeatedly found in cases of neonatal meningitis [30]. Subsequent deletion of this particular sequence led to drastically decreased rates of laminin binding protein production. Here again we outline another possible target for vaccination and treatment in neonatal sepsis.

5. Sialic acid rich capsular polysaccharide (CPS)

GBS is one of the few encapsulated streptococci species with a sialylated capsule. The capsule is responsible for inducing a cytokine- specific immune response in the vaginal canal. The immune response in the vaginal canal primarily consists of IgG over IgA and colonization of the vaginal canal with GBS causes increased production of the cytokine IL-17 [31]. The Fc receptor has specifically been linked to adherence and identification of the sialic acid rich CPS of GBS, and mice without this receptor experience prolonged GBS colonization [31]. Another

recent study showed that the GBS CPS III strain induced a response from Jun-N-terminal protein kinase (JNK) and NF-kB [32]. The capsular protein has also been linked to increased internalization and evasion of macrophage phagocytosis [32]. This same study found that differences in expression of this protein led to different responsiveness to macrophage phagocytosis implicating a potential target of a novel vaccine or treatment in GBS associated neonatal disease. Another study by Baker et al explored B-cell-deficient mice with wild-type mice and noted that B-cell deficient mice had a prolonged vaginal colonization [31]. This study suggests that improving immune recognition and reactivity to the GBS sialic rich CPS could provide improved clearance and decreased ascension in the vaginal canal leading to decreased risk for neonatal disease. Baker et al even noted that there is a protective role for anti-capsule antibodies when introduced via intranasal vaccine [31].

3. Potential for future studies

Across the world, in 2015, GBS infection was the cause of disease in 319,000 neonates, and the cause of death in about 90,000 infants [33]. Maternal GBS vaccination could provide the greatest protection against the increasing amounts of GBS infections resistant to antibiotic use, to prevent preterm labor, neonatal disease, and stillbirth [34]. While investigation and research for a GBS vaccine has been ongoing since the 1980s, including phase I and II vaccine studies actively occurring, not one has been federally regulated yet for public use [33, 35]. The inability of vaccines to progress through phase III (efficacy) and phase IV (effectiveness), calls the need for scientists to implement alternative vaccine targets that could lead to the creation of one more readily available to the public. This paper outlines the known virulence factors that GBS possesses that warrant further investigation as guides for potential vaccine development.

The extensive research that has been conducted on the virulence factors of GBS shows promising hope for the development of a vaccine, as current antibiotics are unable to treat late-onset infection [36]. This conclusive outline of virulence factors and subsequent deletion studies can provide great headway in establishing future research against neonatal sepsis, specifically late-onset disease. Although there is a vast amount of research outlining the colonization and infection of placental membranes by GBS, there remains a call for further advancements in preventative vaccinations that extend beyond the already resistant antibiotic regimen.

4. Conclusion

The major cause of neonatal disease and fatality is ascending infection by GBS. In order to cause infection in the neonate, GBS must infiltrate the amniotic fluid through the chorioamniotic membrane. Treatment measures are continuously needed as resistant GBS strains arise. Several countries have now reported resistance to beta-lactams including the current mainstay of treatment, penicillin [37]. Additionally, increased resistance has been documented in not only the second line treatment options (clindamycin and erythromycin) but also both fluoroquinolones and aminoglycosides.

Conflicts of interest

Authors declare no conflicts of interest.

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