The Double Life of Group B Streptococcus: Asymptomatic Colonizer and Potent Pathogen

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Abstract

Group B streptococcus (GBS) is a β-hemolytic gram-positive bacterium that colonizes the lower genital tract of approximately 18% of women globally as an asymptomatic member of the gastrointestinal and/or vaginal flora. If established in other host niches, however, GBS is highly pathogenic. During pregnancy, ascending GBS infection from the vagina to the intrauterine space is associated with preterm birth, stillbirth, and fetal injury. In addition, vertical transmission of GBS during or after birth results in life-threatening neonatal infections, including pneumonia, sepsis, and meningitis. Although the mechanisms by which GBS traffics from the lower genital tract to vulnerable host niches are not well understood, recent advances have revealed that many of the same bacterial factors that promote asymptomatic vaginal carriage also facilitate dissemination and virulence. Furthermore, highly pathogenic GBS strains have acquired unique factors that enhance survival in invasive niches. Several host factors also exist that either subdue GBS upon vaginal colonization or alternatively permit invasive infection. This review summarizes the GBS and host factors involved in GBS's state as both an asymptomatic colonizer and an invasive pathogen. Gaining a better understanding of these mechanisms is key to overcoming the challenges associated with vaccine development and identification of novel strategies to mitigate GBS virulence.

Introduction

Group B streptococcus (GBS, or *Streptococcus agalactiae*) is a leading cause of neonatal infectious morbidity and mortality globally. GBS is a β-hemolytic, gram-positive coccus that asymptomatically colonizes the lower genital and gastrointestinal tracts but is an invasive pathogen in other host niches. Fetuses and neonates are uniquely susceptible to GBS infections, which most commonly include sepsis, pneumonia, meningitis, and encephalopathy (Fig. 1) [2]. Maternal colonization with GBS is associated with stillbirth and preterm birth, and thus the sequelae of prematurity in the neonate (e.g., bronchopulmonary dysplasia) [3,4]. Rarely, GBS causes maternal sepsis [5]. In newborns, the timing of GBS bacteremia or sepsis may be early onset (within the first week), which is thought be associated with either an *in utero* infection or exposure during vaginal delivery. Alternatively, late-onset GBS disease presents after the first week, but within the first 3 months. To date, the only clinical intervention to prevent early- and late-onset disease in neonates is the administration of intravenous antibiotics to pregnant women during birth, known as intrapartum antibiotic prophylaxis (IAP).

As a colonizing microbe, GBS is detected in the gastrointestinal and genital tracts of approximately

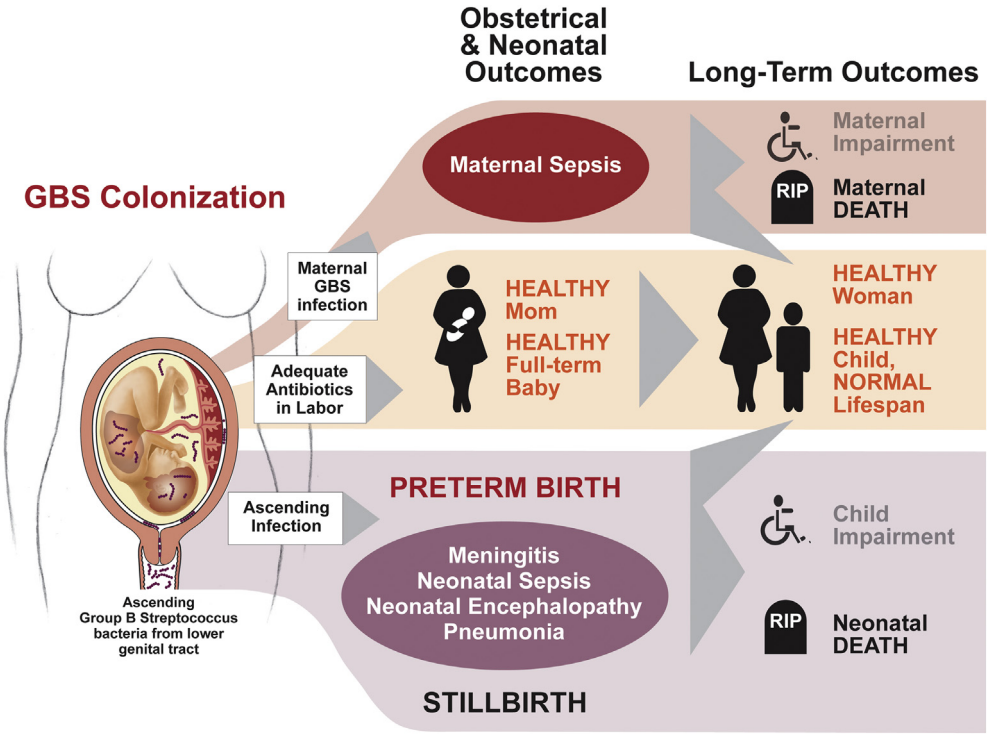

Fig. 1. Clinical pathways in maternal GBS colonization. GBS is associated with several perinatal outcomes. In the case of adequate prophylaxis, most mothers and babies are healthy with normal life span. Virulent ascending GBS is associated with significant morbidity and mortality, some of which is not preventable with intrapartum prophylaxis. With adequate treatment of ascending infection, normal and healthy outcomes may be achieved. Figure adapted from Lawn *et al.* [1].

18% of pregnant women globally; rates of colonization range from 1 in 3 pregnant women in the Caribbean to 1 in 6 women in Southern and Eastern Asia [6]. Interestingly, only a small proportion of people colonized with GBS experience invasive disease, suggesting that host-specific factors potentially play a role in determining an individual's susceptibility to invasive disease. The most comprehensive systematic review and meta-analysis to date estimated the pooled incidence of neonatal morbidity and mortality to be 0.49 per 1000 worldwide, which includes GBS-associated preterm birth, stillbirth, and neonatal GBS infection [7]. Low- and middle-income countries experience increased rates of invasive GBS disease in neonates, and this is correlated with the lack of availability of IAP [8,9]. In countries that have adopted screening protocols for maternal colonization and IAP, the burden of early-onset GBS disease has diminished dramatically; when data were first collected in the United States in 1997, the rate was 0.7 per 1000 and in 2016 was 0.22 per 1000 newborns [9,10]. However, IAP appears to have little to no effect on the rate of late-onset disease in newborns or preterm birth and stillbirth, which are estimated to collectively affect 97,000 to 4 million pregnancies per year [2–4,7,8,11]. In addition, the widespread use of IAP has increased concern over antibiotic resistance in GBS. One multi-state study in the United States—where screening and IAP are standard of care—showed that 32% and 15% of GBS isolates were resistant to erythromycin and clindamycin, respectively [12]. In contrast, a study in South Africa—where IAP is not regularly implemented—found that only 4% of GBS isolates were resistant to erythromycin and 2% were resistant to clindamycin [13].

Overall, GBS remains a significant etiological agent of neonatal and maternal morbidity and mortality worldwide [2–4,7,8], and improved, rationally designed strategies to prevent invasive GBS disease are an urgent global health priority. Understanding the mechanisms that underlie GBS's transition from asymptomatic colonizer to invasive pathogen is key to this effort. In this review, we discuss how GBS utilizes the same bacterial factors for both facets of its lifestyle, including how GBS regulates their expression using signal transduction systems. We also discuss unique factors acquired by GBS strains that are most commonly associated with disease. Finally, we review the several host processes known to either subdue GBS upon vaginal colonization or alternatively permit invasive infection.

GBS Factors that Promote Vaginal Colonization and Virulence

The main host niche where GBS persists as an asymptomatic colonizer is the female rectovaginal tract. Many of the same strategies that GBS uses to colonize the vagina, such as binding host surfaces and subverting immune defenses, also promote dissemination and damage to vulnerable host niches such as newborns. Some GBS strains exhibit increased pathogenic potential due to the evolutionary acquisition of specified virulence factors that increase efficiency of dissemination, immune evasion, and tissue damage. At the cellular level, GBS tightly regulates the expression of virulence factors through signal transduction systems, which sense and respond to various host environments and subsequently enhance survival in host environments outside the lower genital tract. This section begins with a brief introduction of known signal transduction systems in GBS, and then summarizes specific factors that GBS uses to promote vaginal colonization as well as dissemination.

Signal transduction systems

In its transition from a commensal to pathogen, GBS encounters diverse host environments, which requires coordinated transcriptional control of genes involved in growth, cell wall components, stress response, metabolism, cellular processes, and virulence. To sense and respond to environmental changes, bacteria have evolved specialized signal transduction systems, such as the two-component systems (TCSs). TCSs comprise a membrane-associated sensor histidine kinase and a corresponding response regulator; the latter usually has DNA-binding abilities. Upon recognizing an external signal *via* the N-terminal domain, the C-terminal transmitter domain of the histidine kinase phosphorylates its cognate response regulator at a conserved active site aspartate residue, thereby causing conformational changes that alter the response regulator's binding affinity to target DNA. Whole genomic analysis has revealed that GBS encode between 17 and 21 TCSs [14,15], although the function of just 7 TCSs, namely, CovR/S, DltR/S, RgfA/C, CiaR/H, FspR/S, LrdR/S, and SaeR/S, are described [15–25]. The most studied TCS in GBS is CovR/S, which is highly conserved in *Streptococcus* spp. [16]. In GBS, CovR/S serves as a global regulatory system, either activating or repressing the expression of over 100 genes, many of which are involved in virulence [16,17,26]. CovR/S regulation of several virulence genes is highly conserved among various strains of GBS (known as the "core regulon") [27]. However, many regulatory targets of CovR/S differ among strains, suggesting that variability in pathogenic potential of GBS may be in part due to differences in sensing and responding to various host environments [27]. GBS also encodes one-component transcriptional regulators, such as MtaR, RovS, and RogB, which respond to signals from the cytosol (e.g., small molecules) or external environment *via* unknown sensing mechanisms [28–33]. In addition, GBS encodes a eukaryotic-like signaling system comprising a serine/threonine kinase and its cognate phosphate Stp1, which overlaps with the CovR/S regulon [34,35]. A membrane-bound protein known as Abx1 was also shown to mediate CovS function [33]. The bacterial-encoded factors that are under the control of many of these and other signaling systems are discussed in the sections below and summarized in Table 1 and Fig. 2.

Adhesins

A major strategy that GBS employs to colonize the lower genital tract is adherence to epithelial cells *via* surface-associated adhesins. These include fibrinogen-binding proteins (Fbs), the hypervirulent GBS adhesin (HvgA), laminin-binding protein (Lmb), C5a peptidase, pili, plasminogen-binding surface protein (PbsP), fibronectin-binding protein (SfbA), and the immunogenic bacterial adhesin (BibA) (summarized in Table 1, Fig. 2). A common ability conferred by these adhesins is GBS binding to components of the extracellular matrix (ECM). Some GBS adhesins execute additional functions, such as cellular invasion and immune evasion. Importantly, contact between GBS and host cells *via* adhesins is vital for establishing a niche in the gastrointestinal and vaginal mucosa and for invasion into other host compartments. In general, binding to surfaces improves GBS's ability to cross host barriers, which is most likely why the enhanced ability to bind host cells increases GBS's pathogenic potential [91].

Ssr1/Ssr2

Some highly virulent GBS clonal lineages have accomplished enhanced binding ability by evolving surface-associated adhesins that confer greater binding affinity to host cellular components than less pathogenic strains. Take, for example, the highly virulent clonal complex 17 (CC17) strains (capsular serotype III), which are frequently associated with neonatal meningitis. The GBS serine-rich repeat glycoproteins are surface-associated Fbs that bind to a single tandem repeat region of human fibrinogen *via* a "lock, dock, and latch" mechanism [92]. Most GBS strains express Ssr1 and use it to bind to fibrinogen on vaginal and cervical epithelial cells *via* the "latch" domain, which promotes persistent vaginal colonization [36,37,93,94]. CC17 strains, on the other hand, produce a homolog of Ssr1, known as Ssr2 [92,93]. In a mouse model of systemic infection,

Table 1. Summary of GBS colonization and virulence factors and their mechanisms of regulation in order of their appearance in the main text

Factor	Promotes colonization by:	Promotes invasive infection by:	Mechanism of regulation
Adhesins			
Serine rich-repeat glycoproteins	Fibrinogen binding on vaginal and cervical epithelial cells (Ssr1) [36,37]	Enhancing fibrinogen binding affinity (Ssr2) [38] Plasmin and plasminogen binding (Ssr2) [38]	Unknown
HvgA	Unknown	Adherence to cells that make up the intestinal and BBB [39]	CovR/S [39]
FbsA	Adherence to epithelial cells (?—not tested in vaginal model)	Adherence to epithelial cells [40]	CovR/S [17] RgfA/C [20,21] RogB (in serotype III) [32] RovS [31]
FbsB	Unknown	Invading human epithelial cells [41]	CovR/S [42] RgfA/C [21] MtaR [30]
FbsC	Fibrinogen binding on vaginal and cervical epithelial cells [43] Promoting biofilm formation [43]	Invasion of brain endothelial cells and crossing the BBB [44]	CovR/S [43]
Lmb	Binding laminin [45] (?—not tested in vaginal model) Acquiring environmental zinc [46] (?—not tested *in vivo*)	Binding laminin on basement membranes of damaged cells [45,47] Acquiring environmental zinc [46] (?—not tested *in vivo*)	Unknown Some strains contain mobile genetic element that enhances expression [48]
C5a peptidase (ScpB)	Binding fibronectin [49–52] (?—not tested in vaginal model) Cleaving C5a [53,54] (?—not tested in vaginal model)	Binding fibronectin [49–52] Cleaving C5a [53,54]	RgfA/C (strain-specific) [20] CovR/S (strain-specific) [16]
Pili	Adhesion to vaginal and cervical epithelial cells [36] Resisting macrophage and neutrophil killing [55] (?—not tested in vaginal model) Resisting host antimicrobial peptides [55] (?—not tested in vaginal model) Biofilm formation [56] (?—not tested *in vivo*)	Resisting macrophage and neutrophil killing [55] Disrupting barrier function of the lung and BBB [57–60] Resisting host antimicrobial peptides [55]	CovR/S (PI-1) [61] Ape1 (PI-1) [61] Rga (PI-2a) [62,63] RogB (PI-2a) [57] Upstream hairpin structures (PI-2b, strain-specific) [64]
PbsP	Unknown, but up-regulated during vaginal colonization [25]	Adhering to and invading endothelial cells that make up the BBB [65,66]	CovR/S [65] SaeR/S in the vagina [25]
SfbA	Invading vaginal and cervical cells [67]	Invading endothelial cells that make up the BBB [67] Invading astrocytes [68]	Unknown
BibA	Binding to vaginal and cervical epithelial cells [69] Interfering with complement regulator C4bp [69] (?—not tested in vaginal model)	Binding lung and intestinal epithelial cells [69] Interfering with complement regulator C4bp [69]	CovR/S—dependent on pH [70]
Other virulence factors			
Hemolytic pigment	Resisting ROS and neutrophil response [71,72]	Disrupting barrier function of placental membranes [73,74], lungs [75–77], and BBB [26,78] Resisting NETs [74] Inflammasome induction [79]	CovR/S [17] Stk1 [80] Abx1 [33] RovS [31]
Superoxide dismutase (SodA)	Resisting ROS [81] (?—not tested in vaginal model)	Resisting ROS [81]	RovS [31]
Hyaluronidase (HylB)	Dampening TLR2/4 recognition and signaling [82]	Dampening TLR2/4 recognition and signaling [82,83]	CovR/S in strain A909 (serotype Ia) [26]
Sialic acid-rich CPS	Interfering with complement pathway [84,85] (?—not tested in vaginal model) Binding host Siglecs to prevent neutrophil activation [86,87] (?—not tested in vaginal model)	Interfering with complement pathway [84,85] Binding host Siglecs to prevent neutrophil activation [86,87]	RogB (strain-specific) [32] CovR/S (strain-specific) [16,17]
Cyclic di-AMP	Osmotic homeostasis [88] (?—not	Osmotic homeostasis [88] (?—not	CdnP [88]

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Table 1 (*continued*)

Factor	Promotes colonization by:	Promotes invasive infection by:	Mechanism of regulation
	tested *in vivo*)	tested *in vivo*)	
CdnP	Unknown	Degrading cyclic di-AMP to avoid cGAS/STING recognition and signaling [89]	Unknown
D-alanylation of LTA	Resisting host anti-microbial compounds and leukocyte killing [19,90] (?—not tested in vaginal model)	Resisting host anti-microbial compounds and leukocyte killing [19,90]	DltR/S [18]

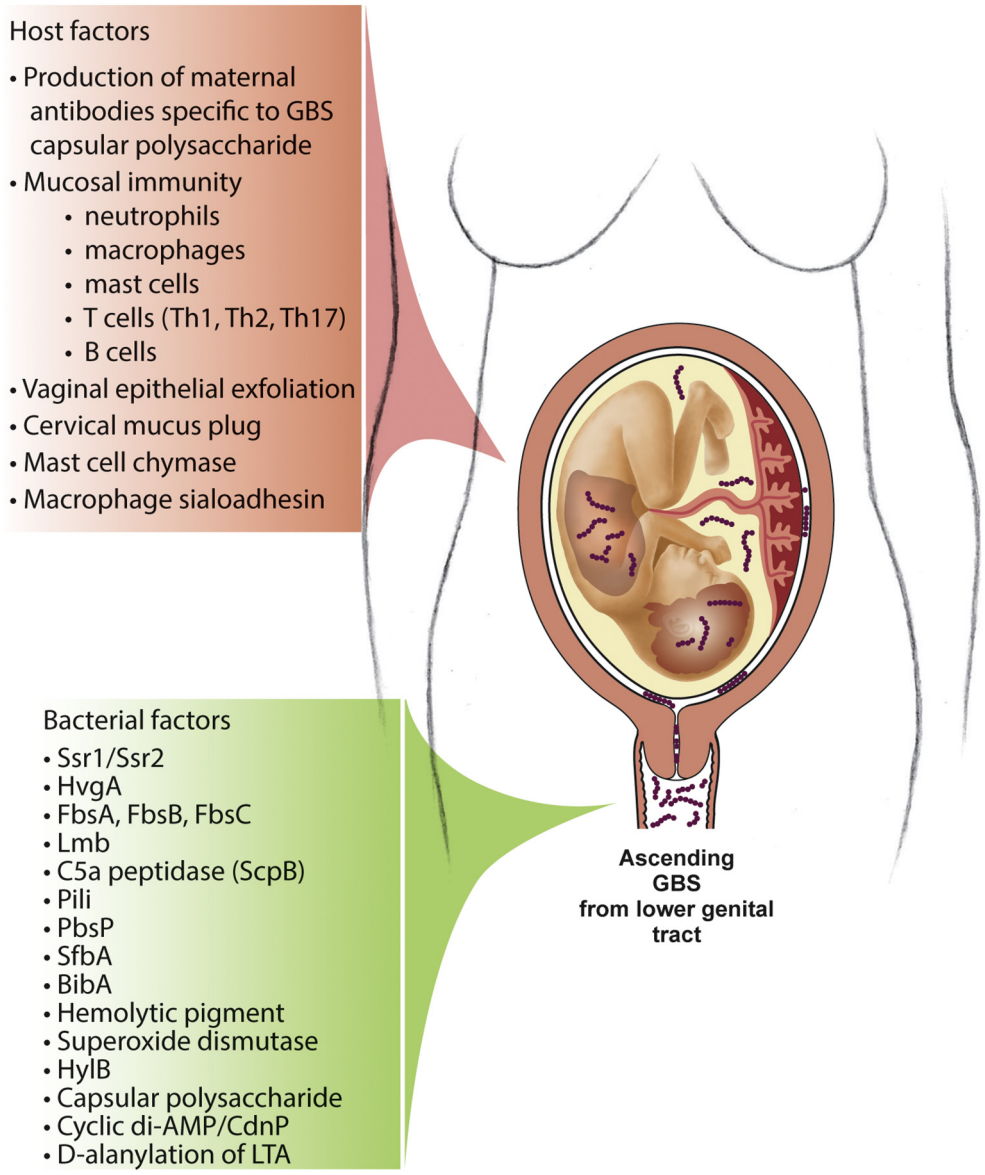

Fig. 2. Host and bacterial factors that contribute to GBS's status as either an asymptomatic colonizer or an invasive pathogen. GBS typically colonizes the gastrointestinal/vaginal tract asymptomatically but is highly pathogenic in other host compartments. The host responses to GBS are multifaceted and can promote asymptomatic colonization and clearance or alternatively permit invasive infection and disease. Many of the bacterial factors that promote colonization are also involved in dissemination and tissue damage. GBS tightly regulates the expression of these factors using signal transduction systems, which sense and respond to variations in the external environment.

a CC17 strain lacking Ssr2 was less able to persist in the liver and brain, indicating that this adhesin promoted dissemination to peripheral organs [38]. Interestingly, Ssr2 was found to have greater binding affinity to fibrinogen compared to Ssr1 and could also bind immobilized plasmin and plasminogen, which Ssr1 could not [38]. Enhanced fibrinogen binding by Ssr2 likely provides CC17 strains with a competitive advantage in the vaginal niche while also promoting binding to epithelial and endothelial cells in invasive niches. In addition, Ssr2-mediated plasminogen and plasmin binding could be a mechanism by which CC17 strains usurp host coagulation mechanisms to disseminate to peripheral organs, thereby making them more pathogenic than other GBS strains typically associated with asymptomatic colonization.

HvgA

Another surface-associated adhesin exclusively encoded in hyper-virulent CC17 strains is the HvgA, which is negatively regulated by the CovR/S TCS [39,95]. Although the host ligand for HvgA has not been discovered, expression of this adhesin augmented adherence to intestinal epithelial cells, choroid epithelial cells, and microvascular endothelial cells which make up the blood–brain barrier (BBB) [39]. Adherence to these cells *via* HvgA promoted intestinal and meningeal tropism, as HvgA-expressing GBS was better able to colonize and cross the intestinal barrier and BBB than a strain lacking this adhesin in orally inoculated mice [39].

FbsA, FbsB, FbsC

In addition to Ssr1/Ssr2, other Fbs encoded by GBS include FbsA, FbsB, and FbsC (also known as BsaB). FbsA and FbsC are central to GBS's ability to adhere to human epithelial cells, which likely promotes vaginal colonization [40,43]. In addition, FbsC mediates biofilm formation, which could facilitate vaginal colonization by binding to other commensal microbes in the gastrointestinal and/or vaginal tract [43]. Studies have also shown a role for FbsC in facilitating GBS dissemination; deletion of FbsC reduced GBS's ability to bind to and invade human brain endothelial cells *in vitro* and colonize the brain in a murine model of systemic infection [44]. FbsB is also thought to be important for GBS dissemination by promoting bacterial invasion of human epithelial cells [41]. The signaling systems that regulate the expression of *fbs* genes are multifaceted and complex and appear to show strain dependence. For instance, FbsA expression is negatively regulated by CovR/S, RgfA/C, and RovS [17,20,21,31], and in serotype III strains, positively regulated by RogB [32]. FbsB, on the other hand, is regulated by CovR/S (represses expression), RgfA/C (activates expression), and in some strains, MtaR [21,30,42]. Finally, FbsC is negatively regulated CovR/S [43].

Lmb

The *lmb* gene encodes the Lmb of GBS, which mediates binding to laminin, a major component of the basement membrane in tissues throughout the human body [45,48]. The *lmb* gene is present in almost all human isolates of GBS [48], which suggests that this factor is crucial for colonization in humans. In addition, the ability to bind laminin on the basement membrane of damaged tissues may be a key mechanism that GBS uses to disseminate in the human host. Indeed, deletion of *lmb* results in decreased invasion into human brain microvascular endothelial cells [47], indicating that Lmb contributes to GBS's neurotropism. To bind laminin, the Lmb adhesin utilizes a zinc-binding pocket, which likely keeps Lmb protein in a structural confirmation conducive to laminin binding and/or enhances affinity to zinc finger motifs present in laminin [96–98]. Intriguingly, the zinc-binding pocket of Lmb also plays a role in zinc acquisition from the environment, which is important for bacterial survival and growth in various host niches [46]. Whether Lmb is under the control of a signal transduction system is unknown. However, increased Lmb surface expression and enhanced laminin-binding ability and has been reported in GBS strains harboring the mobile genetic element IS*1548* upstream of the putative *lmb* promoter [48], indicating that some GBS strains have evolved to constitutively up-regulate Lmb.

C5a peptidase

Upstream of the *lmb* gene is the *scpB* gene, which encodes a surface-associated serine protease known as C5a peptidase or ScpB [99,100]. C5a peptidase was first described to facilitate immune evasion by cleaving the human complement component C5a, a chemotaxin involved in neutrophil recruitment [53,54]. Later, C5a peptidase was shown to promote GBS adherence to and invasion of human epithelial cells by binding fibronectin independently of its peptidase activity [49–52]. Although the regulation of C5a peptidase by RgfA/C and CovR/S is strain-specific [27,101,102], both response regulators in these TCS repress expression of the *scpB* gene [16,20].

Pili

GBS encodes cell wall-anchored appendages known as pili, which protrude from the bacterial surface and comprise three structural subunit proteins: the pilus shaft backbone protein (PilB), the pilus tip (PilA, the pilus-associated adhesin), and the pilus base (PilC) [57,103–105]. Targeted mutagenesis has revealed

distinct roles of the pili components in colonization and virulence. GBS strains lacking PilA were less able to adhere to vaginal and cervical epithelial cells *in vitro* and were out-competed by wild-type GBS in a vaginal model of colonization [36]. Furthermore, PilA knockouts were significantly impaired in their ability to adhere to human pulmonary epithelial cells and brain microvascular endothelial cells *in vitro* [57,58], and in systemic mouse models, caused less neutrophil infiltration and bacterial dissemination to the brain [59]. PilB knockouts, on the other hand, were less able to form biofilms [56], transcytose monolayers of immortalized cervical epithelial cells [60], resist macrophage and neutrophil killing, and combat the effects of host antimicrobial peptides compared to wild-type strains [55]. Unsurprisingly, systemic infection with GBS lacking PilB resulted in enhanced clearance and reduced mortality [55].

The genetic loci that encode GBS pili are pilus islands 1 and 2, (PI-1 and PI-2, respectively) [103,106,107], and the PI-2 locus exists in two variants: PI-2a (present in the majority of GBS isolates belonging to all capsular serotypes) and PI-2b (restricted to CC17 and a few other clinical isolates) [103]. All characterized GBS strains contain at least one variant or a combination of the two pilus islands [103,106,107], and variation among pili likely account for differential ability to colonize and invade among GBS strains. Variation also exists in the regulatory mechanisms for the pili genetic loci. PI-1 expression is repressed by CovR/S TCS and activated by Ape1, an Arac-type regulator encoded immediately upstream of the PI-1 locus [61]. Interestingly, acidic pH activated the expression of PI-1 independently of CovR/S and Ape1 [61], suggesting that another unidentified signal transduction system is involved in PI-1 regulation. On the other hand, PI-2a is positively regulated by the transcriptional regulators RogB, which is encoded upstream of the PI-2a locus, and Rga, which binds to the PI-2a promoter region [57,62,63]. Intriguingly, differential expression of PI-2b among strains may be due to variations in DNA folding; a recent study by Périchon *et al*. [64] found that decreased expression of PI-2b in GBS strain BM110 compared to strain A909 was due to the presence of a 43-base-pair DNA hairpin structure upstream of the PI-2b operon in BM110. Further studies on the variations in regulatory mechanisms of GBS pili will improve our understanding of how GBS pili contribute to adhesion, biofilm formation, and resistance to host immune mechanisms in GBS isolates.

PbsP

PbsP is a cell wall-associated surface protein that binds to the ECM component plasminogen [65]. PbsP expression is highly conserved among GBS strains, but strains vary in the level of PbsP surface expression [65]. GBS employs PbsP to promote vaginal colonization; in a murine model, the *pbsP* transcript was significantly increased in the vaginal tract compared to GBS grown in culturally defined medium, and a GBS strain lacking PbsP was a deficient vaginal colonizer compared to wild-type GBS [25]. Interestingly, vaginal lavage fluid from mice induced SaerR/S-dependent up-regulation of *pbsP* [25], indicating that the histidine kinase SaeS senses a signal in the vaginal milieu to control the expression of *pbsP* through the SaeR response regulator. Expression of the *pbsP* locus is also repressed by CovR/S [65,108], but direct binding of CovR to the *pbsP* promoter could not be proven, suggesting that this regulation may be indirect [65]. Plasminogen-binding *via* PbsP also promotes invasive infection, as PbsP is required for GBS to adhere to brain endothelial cells and cross the BBB [65,66]. In addition, PbsP confers GBS the ability to bind human vitronectin, a glycoprotein abundant in the ECM and in serum, which promotes GBS adherence to and invasion of epithelial cells [109]. Because PbsP is surface-associated, highly conserved, up-regulated *in vivo*, and is important for both vaginal colonization and invasion, it represents a promising target for vaccine development.

SfbA

The Streptococcal SfbA is well conserved among GBS strains and promotes invasion of human vaginal and cervical cells, brain microvascular endothelial cells, and astrocytes [67,68]. Thus, SfbA is likely involved in bacterial tropism to the gastrointestinal/vaginal tract and central nervous system. In a murine model of hematogenous GBS meningitis, a SfbA-deficient GBS strain was less able to colonize the brain, indicating that SfbA mediates GBS translocation of the BBB [67]. The mechanisms that regulate SfbA expression in GBS remain unexplored. Interestingly, hosts have evolved a strategy to counteract GBS fibronectin binding to prevent bacterial dissemination. A recent study revealed that in response to GBS infection, mast cells release a protease known as chymase, which degrades fibronectin and thus impairs bacterial adherence to fibronectin and downstream dissemination [110]. Mice lacking mast cell chymase experienced greater rates of preterm birth and bacterial dissemination when infected with wild-type GBS compared to chymase-sufficient mice. Furthermore, this effect was abrogated when chymase-deficient mice were infected with GBS lacking SfbA [110]. Together, these findings indicate that fibronectin degradation by host mast cell chymase prevents SfbA-mediated dissemination.

BibA

The GBS BibA is another highly conserved, multifunctional, cell wall-tethered protein that GBS

uses to promote colonization at mucosal surfaces and invasion to peripheral compartments [69,111]. BibA is involved in adherence to human cervical, lung, and intestinal epithelial cells, and was proposed to interfere with host antimicrobial defense mechanisms by binding to the human complement regulator C4-binding protein [69,111]. Recombinant BibA protein was shown to induce protective, opsonizing antibodies against GBS in mice [111], and high surface expression of BibA on GBS enhanced opsonophagocytic killing of GBS *in vitro* [70]. These findings indicate that BibA is a target of effective host immune responses to GBS and could be a potential vaccine candidate. Expression of BibA, however, is repressed by the CovR/S TCS and dependent on pH [17,70]; BibA expression is up-regulated in neutral *versus* acidic environmental conditions [70]. Consequently, a vaccine targeting BibA may be more effective in protecting against GBS infection of the blood, where pH is close to neutral, *versus* the lower genital tract, where pH is low.

Hemolytic pigment

Hemolytic activity in GBS is due to the ornithine rhamnolipid pigment (hereafter called "hemolytic pigment" or "pigment") [73], which is produced by the gene products of the *cyl* operon [112,113]. Several genes within the *cyl* operon have homology to those involved in fatty acid biosynthesis, and the *cylE* gene, which encodes an N-acyl transferase, is necessary for pigment production [73,112,113]. Transcription of *cyl* genes is negatively regulated by the CovR/S TCS, so loss of function in this TCS results in a hyper-hemolytic/hyper-pigmented phenotype in GBS [17]. The hemolytic pigment contains a polyene chain consisting of 12 double-bonds [73,114], and serves both as a potent cytotoxin [73,79] and an antioxidant with reactive oxygen species (ROS)-quenching properties [71]. In a murine model of vaginal colonization, a GBS strain lacking the hemolytic pigment was a deficient vaginal colonizer compared to wild-type GBS and was associated with amplified neutrophil response in the vagina [115]. However, GBS over-expressing the hemolytic pigment was more readily cleared from the murine vagina [72,116], likely due to an inflammatory response resulting in an infiltration of neutrophils [72] and/or mast cell degranulation [116]. The finely tuned expression of the hemolytic pigment in the lower genital tract, therefore, is essential to GBS survival; GBS must achieve an optimum level of pigment production to resist ROS but also avoid inflammation and subsequent clearance.

In addition to modulating vaginal colonization, the hemolytic pigment permits GBS dissemination to host niches outside the vagina and facilitates tissue damage. In a murine model of vaginal colonization, non-hemolytic GBS was less likely to disseminate or cause fetal injury [117], suggesting that the hemolytic pigment plays a role in GBS's ability to breach the placental barrier. Indeed, hyper-hemolytic GBS strains lacking CovR were found to more readily invade amniotic epithelial cells, cause amniotic epithelial cell barrier disruption, and penetrate the human placenta compared to wild-type GBS and non-hemolytic GBS also lacking CovR (GBSΔ*covR*Δ*cylE*) [73]. Furthermore, hyper-hemolytic GBS with CovR/S mutations have been isolated from women in preterm labor [73] and non-pregnant adults with invasive infections [118–121], suggesting that some GBS isolates have evolved the ability to constitutively over express the hemolytic pigment and that these are associated with human disease. In a non-human primate study of GBS infection during pregnancy, GBS lacking CovR invaded the amniotic cavity more efficiently, resulting in uterine contractions and inflammation indicative of preterm labor [74]. Notably, although hyper-hemolytic GBS caused an infiltration of neutrophils to the chorioamniotic (placental) membranes of non-human primate and the subsequent release of neutrophil extracellular traps (NETs), hyper-hemolytic GBS were able to resist killing by NETs [74]. Other inflammatory cascades initiated by the pigment during pregnancy, such as the NLRP3 inflammasome, can likewise result in fetal injury and preterm birth [79].

Neonates, particularly those born prematurely, are especially susceptible to the toxic effects of the hemolytic pigment. Many infants acquire early-onset GBS by aspirating infected vaginal or amniotic fluid, resulting in colonization of the lung. Higher expression of the hemolytic pigment correlates to greater cytotoxicity of the very cells that constitute the mucosal barrier of the lung: alveolar epithelial cells [75] and capillary endothelial cells [76]. In addition, mutant GBS strains lacking the *cylE* gene are less capable of adhering to, invading, and initiating inflammatory cascades in lung epithelial cells [77]. Once GBS breaches the lung's barrier, it deploys several virulence factors (such as adhesins and invasins described elsewhere in this review) to disseminate to peripheral organs, resulting in septicemia and neurotropism. At the BBB, the hemolytic pigment facilitates bacterial penetration into the brain and promotes acute inflammatory responses in microvascular endothelial cells, thus contributing to the severity of GBS meningitis [26,78].

Beyond pH [70], little is known about the stimuli sensed by CovS to induce or relieve expression of the *cyl* genes *via* CovR. Other strain-specific regulators have been shown to alter expression of *cyl* genes, such as the sensor kinase Stk1 [80,122] and the transmembrane protein Abx1 [33], which interact with CovR and CovS, respectively. In addition, the one-component transcriptional regulator RovS can bind to and activate genes in the *cyl* operon [31]. In short, the

signal transduction mechanisms responsible for regulating expression of the hemolytic pigment are complex. Further studies on how the hemolytic pigment is regulated in the vagina and in vulnerable niches are critical to understanding GBS's conversion from commensal to pathogen.

Superoxide dismutase

In addition to the hemolytic pigment, GBS resists host ROS during infection using the Mn^{2+}-cofactored superoxide dismutase, SodA, which detoxifies superoxide radical species (O^{2-}) [81,123]. A GBS mutant deficient in SodA was more susceptible to killing following macrophage internalization compared to wild-type GBS, and in a systemic murine model of infection, was attenuated for virulence [81]. The transcriptional regulator RovS was shown to bind directly to the promoter region of the *sodA* gene, and a *rovS* deletion mutant exhibited over a 2-fold decrease in *sodA* expression [31]. These data indicate that RovS is an activator of *sodA*, but the signals that activate or repress sodA expression through RovS are unknown and warrant further study.

HylB

The GBS hyaluronidase, also known as HylB, is an endoglycosidase that cleaves glycosaminoglycan chains, such as hyaluronic acid (HA) into disaccharides [124]. HylB activity has long been demonstrated to correlate to GBS's invasive potential; in a 1978 study, increased expression of HylB was observed in GBS strains isolated from infected neonates [125,126]. Similarly, in 1989, the highly virulent GBS clonal type now known as CC17 was shown to have increased expression of hyaluronidase compared to strains less often associated with invasive disease in neonates [126–128]. Even in a GBS strain that lacks the potent hemolytic pigment toxin, over-expression of HylB confers hypervirulence [129].

GBS mutants lacking HylB induce more pro-inflammatory cytokines *in vitro* and *in vivo* [130], and the precise mechanism of HylB-mediated immune suppression was elucidated in a recent study by Kolar *et al*. [82]. HylB's substrate, HA, is present in almost all human tissues and is important for stability and structure of the ECM as well as immune surveillance [131,132]. Upon sterile or microbial-induced tissue injury, HA is degraded into multimeric fragments by ROS, which act as damage-associated molecular patterns that bind to pattern recognition receptors such as TLRs on innate immune cells to initiate a pro-inflammatory response. The HA dimers generated by GBS HylB inhibit TLR2 and TLR4 inflammatory pathways by blocking the direct binding of stimulatory HA fragments and other pathogen-associated molecular patterns to TLR2 and TLR4 [82]. Using a non-pregnant mouse model of vaginal inoculation, Kolar *et al*. [82] established that the immune-suppressing properties of HylB facilitate vaginal colonization of GBS. In pregnant mice, wild-type GBS caused increased ascending infection into the uterus, decreased uterine inflammation, and higher rates of fetal demise compared to HylB-deficient GBS [83], indicating that suppression of inflammatory responses in the uterus also facilitates GBS-associated fetal injury. These findings show that like many other virulence factors, GBS uses HylB to promote both vaginal colonization and invasive disease. GBS therefore likely regulates expression of the *hylB* gene to modulate the immune response during colonizing and invasive states. One study found that CovR activated *hylB* expression in GBS strain A909 (capsular serotype Ia), suggesting that the CovR/S TCS may be involved in *hylB* gene regulation [26]. Beyond this, little evidence exists about how, when, and where GBS modulates *hylB* expression during colonization or invasive infection.

Sialic acid-rich capsular polysaccharide

GBS is encased by a capsular polysaccharide (CPS) rich in sialic acid and made up of 1 of the 10 capsular serotypes: Ia, Ib, or II-IX. The CPS is assembled by the gene products of the *cps* operon [133–135] and is known to promote virulence; CPS-deficient GBS strains have attenuated virulence in systemic models of infection [136–138]. The sialic acid present in the CPS is a nine-carbon monosaccharide that is also widely distributed on the terminal glycan structures present on host cell surfaces. Because of this, the CPS sialic acid facilitates GBS innate immune evasion by molecular mimicry. For instance, CPS sialic acid prevents deposition of the C3b complement component on the surface of GBS and subsequent opsonophagocytosis [84,85], likely by interfering with regulatory complement enzymes. In addition, CPS sialic acid binds to host inhibitory Siglecs (Sialic acid-binding immunoglobulin-type lectins), which reduces neutrophil activation and inflammation of the placental membranes [86,87,139], dampens pro-inflammatory signaling *in vivo* [140], and facilitates bacterial dissemination [140]. Interestingly, diminished CPS expression enhances GBS's ability to invade epithelial and endothelial cells *in vitro* [141,142], and acapsular, hyper-hemolytic GBS more readily disseminates to peripheral organs and crosses the BBB *in vivo* [143]. Also, sialoadhesin present in splenic macrophages binds CPS sialic acid and promotes phagocytic uptake, thereby restricting GBS dissemination [144]. These findings indicate that up- and down-regulation of CPS confers differential advantages to GBS in certain situations during infection. RogB, a transcriptional regulator of the RofA-like protein family, suppresses expression of the first gene in the *cps*

operon, *cpsA* [32], which encodes a cell-wall anchored protein involved in surface attachment of CPS [135]. However, the gene locus encoding RogB is absent in some GBS strains, including A909 (serotype Ia) and COH1 (serotype III), indicating that its regulation is strain-specific. CovR/S activation of the *cps* genes is also strain-specific [16,17]. To date, our understanding of gene regulation by the *cps* operon is limited, and further research will lend valuable clues into the mechanisms that GBS uses to survive various host defenses.

Cyclic di-AMP and CndP

GBS synthesizes the signaling nucleotide cyclic di-AMP, which plays a critical role in maintaining osmotic homeostasis [88]. Osmotic regulation through cyclic di-AMP is likely a key strategy that GBS uses to survive osmotic stress encountered in various host niches, such as the acidic vagina or more alkaline blood. However, the cyclic di-AMP synthesized by GBS is recognized by intracellular host pattern recognition receptors c-GAS and STING, leading to the production of type 1 interferons (e.g., IFN-β) [89], which promote neutrophil recruitment and subsequent bacterial clearance [89,145,146]. It was recently discovered that GBS encodes a cell wall-anchored ectonucleotidase, CdnP, which degrades extracellular cyclic di-AMP [89]. Interestingly, inactivation of CdnP led to increased IFN-β and reduced bacteremia in wild-type mice in a murine model of intravenous GBS infection. This effect was not observed in mice lacking cGAS or STING, indicating that GBS expresses CdnP to dampen STING-mediated detection of cyclic di-AMP and subsequent pro-inflammatory cascades to promote invasive infection [89]. Whether GBS fine-tunes the expression of CdnP to promote cyclic di-AMP-mediated osmotic homeostasis in some host environments and immune evasion in others remains unknown.

D-alanylation of LTA

Like many gram-positive bacteria, GBS contains a cell wall densely packed with lipoteichoic acid (LTA), a phosphate-containing polymer that contains glycosyl and D-alanine ester substituents. LTA mediates ligand binding and surface charge, and the incorporation of D-alanine to LTAs can modify these properties in bacteria. For instance, D-alanylation of LTAs decreases the net negative charge on the bacterial surface, which confers improved resistance to cationic antimicrobial compounds [147]. In GBS, D-alanylation of LTAs is regulated by the *dltRSABCD* operon, which encodes the DltR/S TCS and structural genes *dltA-D* [18]. GBS strains lacking *dltA* cannot produce the D-alanine-D-alanyl carrier ligase and are severely attenuated for virulence compared to wild-type GBS due to increased susceptibility to host cationic defensins and leukocyte killing [19,90]. GBS uses the DltR/S TCS to control the level of D-alanine esters in LTAs; upon activation by DltS, DltR binds to promoter DNA to induce the expression of the *dlt* operon [18]. Although the specific signal sensed by DltS is unknown, up-regulation of the *dlt* operon is observed when amounts of D-alanine esters in LTAs decrease [18], suggesting that GBS may detect changes in their own surface charge or cell wall composition. In addition, the presence of host cationic antimicrobial peptides may trigger activation of *dlt* genes by the DltR/S TCS. In fact, antimicrobial peptides have been shown to trigger TCS regulation of virulence-associated genes in group A streptococci [148,149].

Host factors that mediate GBS's transition from asymptomatic colonizer to invasive pathogen

Although some host defenses are effective at clearing GBS, others are permissive to or exploited by GBS, allowing GBS to gain a foothold in invasive niches. Thus, many host responses to GBS are promising targets for therapeutic intervention. Only a small proportion of people who are colonized with GBS experience invasive infection, indicating that host-specific responses to GBS may vary by individual. More research on GBS–host interactions as well as the variations or deficiencies in the host response to GBS are critical to identifying individuals most at risk for invasive GBS disease and developing effective therapies. The known host factors involved in GBS colonization and disease are discussed below and summarized in Table 2.

Production of CPS-specific antibodies

The adaptive immune response to the GBS CPS plays an important role in GBS's state as an asymptomatic colonizer or invasive pathogen. Clinical studies have suggested a correlation between naturally acquired maternal CPS-specific antibodies and asymptomatic vaginal carriage and/or clearance [150,151], and maternal titers of CPS-specific antibodies >1 μg/mL at birth were associated with a 70% reduction in the risk of early-onset disease in newborns [152]. Given the CPS-dependent antibody protection observed in these studies, the CPS is a frequent target for GBS vaccine candidates, and several multivalent CPS conjugate vaccines have shown promising safety and immunogenicity outcomes in phase I and II clinical trials [153–159]. However, the diversity of GBS CPS types and CPS switching are significant challenges to the development of broadly efficacious GBS vaccines targeting the CPS [167,168].

Table 2. Host factors that mediate GBS's transition from asymptomatic colonizer to invasive pathogen in order of their appearance in the main text

Factor	Effect on GBS colonization/virulence	Intervention
Maternal CPS-specific antibody production	Associated with asymptomatic vaginal colonization [150,151] Associated with enhanced vaginal clearance [151] Associated with reduced risk of early-onset disease in newborns [152]	CPS-based conjugate vaccines [153–159]
Mucosal immunity	Neutrophils [72,115,160], macrophages [115], mast cells [116] promote clearance of GBS from the vagina Soluble inflammatory mediators promote clearance of GBS from the vagina [72,115,160] IL-17 and IL-17+ cells promoted clearance of persistent strain [160] Humoral immunity in vaginal mucosa promotes clearance of GBS from the vagina [161] Deficiencies may increase risk for invasive disease	Mucosal vaccination tested using *in vivo* mouse models [161]
Vaginal epithelial exfoliation	Disrupts barrier function of vaginal epithelia, promotes ascending GBS infection [162]	None known
Cervical mucus plug	Physical barrier between lower genital tract and uterus Can secrete pro-inflammatory cytokines, chemokines (not tested *in vivo*) [163–165] Can retain antibiotic drugs that kill GBS [166] Antimicrobial compounds within CMPs insufficient to kill GBS [166]	None known
Mast cell chymase	Inhibits GBS's ability to bind fibronectin [110]	None known
Macrophage sialoadhesin	Binds GBS sialic acid, promotes phagocytic uptake and clearance [144]	None known

Mucosal immune response

Recently, *in vivo* models have revealed that GBS vaginal colonization invokes the recruitment of neutrophils [72,115,160], macrophages [115], and mast cells [116], which promote clearance. In addition, several soluble inflammatory mediators, including histamine, 1β, IL-6, IL-8, IL-23, and IL-17, have been shown to be important for decreased colonization [72,115,160]. The mucosal T-cell response to GBS colonization is poorly defined, although one study found that cytokines involved in Th1, Th2, and Th17 differentiation pathways are important for decreased colonization [115]. Similarly, another study found that IL-17 and IL-17-producing cells in the vaginal tract promote clearance of a persistent GBS strain [160]. The mucosal humoral response to GBS is also incompletely understood, but a recent study showed that B cell-deficient mice vaginally inoculated with GBS demonstrated prolonged time to colonization clearance compared to wild-type mice [161]. This trend was recapitulated immunoglobulin-deficient mice, suggesting a role for GBS-specific antibodies in clearing GBS from the vagina [161]. Furthermore, mice immunized intranasally with heat-killed GBS more readily cleared GBS from the vagina in a serotype-specific manner [161]. Together, these data indicate that specialized immune responses to GBS at the mucosal surface mediate vaginal clearance and thus have implications for designing effective delivery routes for vaccines and/or therapeutics. Some evidence suggests that deficiencies in mucosal immune response may render some hosts less able to combat persistent vaginal colonization and/or invasive GBS disease. For instance, a recent study found that HIV-positive women vaginally colonized with GBS experienced a 19%–39% reduction in the concentration of transplacental transfer of GBS surface protein antibodies compared to HIV-negative women [169]. This could, in part, explain increased susceptibility to GBS infection in HIV-exposed uninfected infants [170].

Vaginal epithelial exfoliation

A common host defense mechanism use to clear mucosal surfaces of adherent pathogenic bacteria is epithelial exfoliation, a process in which epithelial cells lose their tight junctions and detach from the basement membrane [171]. In fact, *Neisseria gonorrheae* and uropathogenic *Escherichia coli* have evolved specific mechanisms to prevent clearance from the lower genital tract by epithelial exfoliation [172–175]. A recent study by Vornhagen *et al.* [162] showed that GBS induce exfoliation of vaginal epithelial cells by activating β-catenin signaling to induce epithelial-to-mesenchymal transition (EMT). Surprisingly, GBS-induced exfoliation of vaginal epithelial cells had no effect on vaginal colonization within the short time window of the study (3 days), but instead permitted ascending infection to the intrauterine space, resulting in fetal demise and preterm birth in pregnant mice [162]. The specific GBS factors involved in binding integrin to induce EMT and how EMT (or lack thereof) impacts long-term vaginal colonization are unknown and warrant further study.

CMP composition

During pregnancy, a dense and viscous mucoid structure known as the cervical mucus plug (CMP) forms in the cervical canal. In addition to acting as a physical barrier, CMPs can harbor pro-inflammatory cytokines as well as a variety of antimicrobial compounds, including lysozyme, lactoferrin, calprotectin, and secretory leukoprotease inhibitor 1, which vary by individual [163–165]. Although our understanding of the CMP's role in defending against pathogenic bacteria is poor, a recent study examining 60 human CMPs showed that CMP-associated proteins activated leukocytes in whole blood, resulting in increased GBS killing [166]. However, the concentration of antimicrobial factors was insufficient to kill several strains of GBS except in those CMPs still harboring antibiotics administered during cesarean section and prior to CMP collection [166]. These findings suggest that GBS have evolved mechanisms to resist the antibiotic properties of CMPs. Resisting the antimicrobial effects of CMPs may be a mechanism GBS employ to promote vaginal colonization during pregnancy, a host state that has unique immune characteristics. In addition, CMP antimicrobial resistance may be a factor in permitting GBS to cross the cervix, although the mechanisms by which GBS bind to and ascend the CMP remain unknown.

Conclusion

GBS leads a double life as both an asymptomatic colonizer and a potent pathogen. Although most people who are colonized with GBS do not experience invasive disease, invasion of GBS into host niches outside of the gastrointestinal and/or vaginal mucosa causes severe damage to the host, resulting in devastating clinical outcomes in pregnant women and newborns. Even with the development of screening and IAP protocols in some countries, the global burden of GBS disease remains high. New, rationally designed strategies to prevent GBS colonization and disease are an urgent priority and require a comprehensive understanding of the dynamics of commensal *versus* invasive states. The factors mediating GBS's transition from commensal to pathogen are derived both from the bacteria themselves and from the host. Many of the same strategies that GBS uses to reside in the lower genital tract also allow it to wreak havoc in invasive niches. Furthermore, GBS tightly regulates the expression of bacterial products such as adhesins, the hemolytic pigment, and immune modulators, to best equip itself for its surroundings. While several host defenses to GBS have been identified, they vary by individual and can be inadequate to suppress GBS or are exploited by GBS for its dissemination. Despite prolific research in recent years, our understanding of GBS's dual lifestyle as a commensal and pathogen is perforated with many knowledge gaps. More basic research revealing the molecular underpinnings of signal transduction systems, bacterial products, and the host response to GBS is essential to developing novel therapeutic interventions that keep GBS at bay.

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Abbreviations used:
GBS, group B streptococcus; IAP, intrapartum antibiotic prophylaxis; TCS, two-component system; Fbs, fibrinogen-binding protein; HvgA, hypervirulent GBS adhesin; Lmb, laminin-binding protein; PbsP, plasminogen-binding surface protein; SfbA, fibronectin-binding protein; BibA, immunogenic bacterial adhesin; ECM, extracellular matrix; BBB, blood–brain barrier; ROS, reactive oxygen species; NETs, neutrophil extracellular traps; HA, hyaluronic acid; CPS, capsular polysaccharide; LTA, lipoteichoic acid; EMT, epithelial-to-mesenchymal transition; CMP, cervical mucus plug.

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