



Intrinsic Maturation Neonatal Immune Deficiencies and Susceptibility to Group B *Streptococcus* Infection

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SUMMARY Although a normal member of the gastrointestinal and vaginal microbiota, group B *Streptococcus* (GBS) can also occasionally be the cause of highly invasive neonatal disease and is an emerging pathogen in both elderly and immunocompromised adults. Neonatal GBS infections are typically transmitted from mother to baby either *in utero* or during passage through the birth canal and can lead to pneumonia, sepsis, and meningitis within the first few months of life. Compared to the adult immune system, the neonatal immune system has a number of deficiencies, making neonates more susceptible to infection. Recognition of GBS by the host immune system triggers an inflammatory response to clear the pathogen. However, GBS has developed several mechanisms to evade the host immune response. A comprehensive understanding of this interplay between GBS and the host immune system will aid in the development of new preventative measures and therapeutics.

KEYWORDS group B streptococcus, immunology, inflammation, prevention

INTRODUCTION

Group B *Streptococcus* (GBS) (*Streptococcus agalactiae*) commonly colonizes the human gastrointestinal and/or genitourinary tracts in approximately 30% of healthy adults (1–5). GBS can be found primarily in the outer mucus layer of the colon as well as the small intestine (6). In addition to being a commensal, GBS also causes severe disease in neonates and in elderly and immunocompromised individuals. The Active Bacterial Core Surveillance report estimates that there are 28,550 cases of invasive GBS disease resulting in approximately 1,770 deaths annually in the United States (7).

GBS is a highly diverse species and can be classified by using serotyping and multilocus sequence typing (MLST). Serotyping is based on the capsular polysaccharide (CPS) and categorizes GBS into 10 types: types Ia, Ib, and II through IX (8). These 10

antigenically distinct CPS types play a major role in GBS virulence, with types Ia, Ib, II, III, and V most often resulting in disease. Structural and sequence comparisons of the 10 types indicate that the differences across CPS types are more likely due to horizontal gene transfer rather than gradual mutagenesis (9). MLST uses the allelic profile of seven conserved genes in order to group GBS strains into sequence types (STs), which can be clustered into clonal complexes (CCs) (10). Several studies have shown that ST-17, a serotype III lineage, causes severe neonatal disease more often, indicating that ST-17 may be more virulent than other GBS STs (10–15). Moreover, this lineage has a number of ST-17-specific genes that may contribute to its ability to cause meningitis, a topic that has been reviewed in detail elsewhere (16).

There are two different types of neonatal disease, early-onset disease (EOD) and late-onset disease (LOD), which differ based on the age of the baby at the time of clinical presentation as well as the possible mechanism of transmission. EOD typically presents as pneumonia and sepsis, which occur within hours after birth and up to 1 week of age. Vertical transmission of GBS occurs when the baby inhales infected vaginal fluid during birth or may occur due to ascending GBS infection from the vaginal canal crossing the extraplacental membranes to infect the amniotic fluid (17, 18). LOD typically presents as bloodstream infections leading to meningitis and occurs after 7 days of age but before the first 3 months of life (19). The transmission and pathogenesis of LOD are not well understood, although premature birth has been shown to be a major risk factor (20). In the United States, current rates of EOD are 0.23 cases per 1,000 live births, and LOD rates are 0.34 cases per 1,000 live births (7). Preventative measures against neonatal GBS disease involve intrapartum antibiotic prophylaxis (IAP) given to women who test positive for GBS colonization or those who are in preterm labor in order to reduce the likelihood of transmission to the baby during birth. These practices have successfully reduced the number of cases of EOD; however, the incidence of LOD has remained the same, and overall case rates have plateaued over the years, indicating a need for alternative therapies (21). Because women can remain persistently colonized by GBS even after IAP, they are still able to transmit the bacterium even after birth (22).

The first step in neonatal GBS disease progression is asymptomatic colonization of vaginal epithelial cells in the pregnant mother. Heavy maternal colonization is a primary risk factor for EOD (23). Vertical transmission results in infection via the lungs, where GBS then adheres to and invades lung epithelial cells. From the lungs, GBS can gain access to the bloodstream, causing sepsis. In the most severe cases, GBS is able to breach the blood-brain barrier, resulting in meningitis (17). Although the pathogenesis of LOD is not fully understood, it is possible that the baby acquires the bacterium from the mother (24). A number of case studies, for instance, have identified infected breast milk as a possible source (25–27). However, a number of LOD cases have occurred after the baby was fed formula or in the absence of GBS-infected breast milk (24, 28), suggesting nosocomial, community, or other environmental sources. Since babies can become asymptotically colonized by GBS in their intestines following birth (5), it is also possible that GBS invades across the intestinal epithelium, resulting in LOD.

The severity of disease can be attributed to the susceptibility of the newborn and the ability of GBS to avoid immunological clearance and adapt to changing environments throughout disease progression. Infants generally become infected by GBS during the first 3 months of life, suggesting that the immature state of the immune system contributes to susceptibility to infection. Moreover, GBS infections in nonpregnant adults typically present when the host is in an immunocompromised or relatively compromised state, such as diabetes, cancer, HIV, and others, with diabetes being the predominating underlying condition (29–32). The common theme of GBS infection appears to be that optimal conditions for the pathogenesis of GBS invasion occur when a part of the immune defense system is compromised. A greater understanding of the capacity of GBS to interact with the deficient immune system will aid in the development of novel therapies or preventative measures for invasive disease. Examining which immune cells are deficient in these cases will provide clues about the predominating cell types that keep GBS under control in colonized individuals. The process by

TABLE 1 Deficiencies in neonatal immune cells compared to adult cells

Cell type	Characteristic of neonatal cells relative to adult cells	Reference(s)
Neutrophils	Reduced no. of stored cells	36
	Reduced recruitment to sites of infection	37
	Initially reduced phagocytic ability	38
	Delayed NET response	39, 40
Monocytes	Higher no. of cells	41, 42
	Reduced recruitment to sites of infection	43
	Similar phagocytic ability	38
	Reduced cytokine production in response to stimuli	44
	Diminished antigen presentation capacity	47
Macrophages	Low no. of alveolar macrophages immediately after birth	49
	Reduced antigen processing and presentation	50
	Delayed response to recruit monocytes and neutrophils to site of infection	49
	Similar migration and ROS production	41
Dendritic cells	Reduced capacity to stimulate other immune cells	54
	Reduced IFN- α/β production	56
	Similar level of proinflammatory cytokine production	57
T cells	Higher no. of Th2 cells	63
	Diminished no. of Th1 cells	63
	Diminished no./lack of Th17 cells	64
B cells	Immature development of surface Ig	65
	Deficient signaling through the BCR	66

which GBS transitions from a colonizing state to an invasive pathogen and its interactions with innate immune cells were recently reviewed by Landwehr-Kenzel and Henneke (33). Here, we focus on innate immune deficiencies in the newborn that enhance susceptibility to disease, host immune responses to GBS infection, and mechanisms that GBS uses to evade immune responses.

DEFICIENCIES IN NEONATAL IMMUNITY

The relatively underdeveloped newborn immune system includes a reduced number of available immune cells, resulting in heightened susceptibility to infectious diseases. Moreover, neonatal immune cells can be present in different proportions in different sites relative to adult immune cell populations (34). The general characteristics of neonatal immune cells compared to adult immune cells are listed in Table 1. The neonatal immune system is also relatively naive, resulting in a lack of preexisting memory immune cells, which leads to a dependency on the maternal transfer of antibodies. Furthermore, the newborn immune system produces higher levels of anti-inflammatory cytokines than proinflammatory cytokines. A thorough understanding of these deficiencies and their implications is an important step toward helping to protect neonates from invading pathogens such as GBS. The neonatal immune system was recently reviewed in detail elsewhere (34, 35), and we only briefly discuss neonatal immune deficiencies here.

Innate Immunity Deficiencies

Since the adaptive immune system has limited exposures to antigens *in utero*, resulting in a deficient adaptive immune response, neonates rely mainly on the innate immune response to pathogens. Neutrophils are one of the main phagocyte types found in the blood and are the first cells recruited to the site of infection. The neutrophil storage pool, however, is much smaller than that in adults; also, neonatal rats challenged with GBS developed neutropenia, and neutrophil storage pools rapidly became depleted (36). In addition to the small pool of stored neutrophils, neonatal neutrophils show impaired rolling adhesion, transmigrating, and chemotaxis, resulting in poor recruitment to infection sites (37). Neutrophils from both preterm and term

neonates also show reduced levels of phagocytosis compared to adult neutrophils but become comparable to adult neutrophils by 3 days after birth (38). Neonatal neutrophils are capable of producing functional neutrophil extracellular traps (NETs), but the response is delayed and requires extended stimulation, making them less able to aid in clearing pathogens (39, 40). These findings show not only that there are fewer neutrophils being recruited to the infection site but also that the neutrophils that make it there are deficient in their ability to clear infection, making neonates particularly susceptible to infection within the first few days after birth.

In contrast to neutrophils, the numbers of monocytes are much higher in neonates than in adults, while preterm neonates have even higher numbers of monocytes than do term neonates (41, 42). Although the phagocytic ability of neonatal monocytes is the same as that of adult monocytes (38), monocyte chemotaxis and recruitment to the site of infection are attenuated (43), and cytokine concentrations are lower, resulting in a reduced inflammatory response (44). Additionally, a study that examined differences between adult peripheral and cord blood monocytes in their interactions with GBS found no difference in phagocytic uptake, bacterial degradation, and reactive oxygen species (ROS) production. However, there was a reduced level of cell death following GBS infection in cord blood monocytes compared to adult monocytes (45). Since it is possible that a higher level of apoptosis early during sepsis leads to improved outcomes in patients, this reduced level of apoptosis in cord blood monocytes contributes to poorer outcomes among neonates with sepsis (46). Neonatal monocytes also have reduced levels of major histocompatibility complex (MHC) class II expression on their surface, resulting in a diminished capacity for antigen presentation (47). Toll-like receptor (TLR)-mediated signal transduction pathways are also impaired in neonatal monocytes, resulting in the reduced activation of NF- κ B, which is an important transcription factor involved in immune response regulation (48).

Once monocytes travel to tissues, they differentiate into macrophages. Numbers of alveolar macrophages are much lower in newborns than in adults; however, the number rises to adult levels 24 to 48 h after birth (49). As inhalation of GBS during birth is the main predisposing mechanism for pneumonia, the initial reduction of pulmonary macrophages predisposes newborns to an inability to rapidly clear the infection, resulting in EOD. Relative to adult murine macrophages, neonatal murine macrophages had reduced gene expression levels of MHC class II, CD11b, CD14, CD80, CD86, TLR2, TLR4, and TLR9, all of which are involved in processing and presenting antigens, with a corresponding reduction in the ability to induce T-cell proliferation (50). While neonatal macrophages have a delayed response in recruiting neutrophils and monocytes to the site of infection (49), migration and production of ROS are normal relative to adult macrophages (51). Upon stimulation through TLRs 1, 2, and 4, neonatal macrophages have an enhanced production of interleukin-6 (IL-6), demonstrating the ability to secrete proinflammatory cytokines despite having other deficits (52).

Many different subtypes of dendritic cells (DCs) can be found, which vary in tissue localization as well as surface receptor expression and function. Although DCs are highly specialized, potent antigen-presenting cells (53), cord blood DCs are very immature. Indeed, cord blood DCs are unable to stimulate either adult or cord blood mononuclear or T cells, in contrast to adult DCs, suggesting a deficit in cord blood DCs (54). In addition, cord blood DCs have reduced levels of expression of MHC classes I and II, ICAM-1/CD54, CD40, CD80, CD83, and CD86 relative to adult DCs, which is indicative of immaturity (54, 55). DCs stimulated by TLR7/9 have a reduced ability to produce alpha/beta interferons (IFN- α/β), which are important immune regulators. This deficiency is due to the reduced translocation of the transcription factor interferon regulatory factor 7 (IRF7) into the nucleus (56). However, stimulated cord blood monocyte-derived DCs have similar levels of NF- κ B signaling as well as secretion of the proinflammatory cytokines tumor necrosis factor alpha (TNF- α), IL-6, and IL-8 compared to those of adult DCs (57).

In addition to the reduced numbers and function of neonatal innate immune cells, the complement system is also underdeveloped. Depending on the stimuli, the complement system can be activated through either the classical, alternative, or lectin pathway through a cascade of enzymatic reactions. Regardless of the activation pathway used, the result is the formation of the membrane attack complex (MAC), which creates a channel in cell membranes that results in cell lysis. Additionally, throughout the cascade, a number of enzymatic intermediates and cleavage products are formed, which play a role in immune responses, such as immune cell activation or bacterial cell opsonization (58). Complement proteins cannot be transplacentally transferred from mother to fetus, and the numbers of neonatal complement proteins are only 10 to 80% of those found in adults (59). More specifically, the classical pathway components C1q, C3, and C4 as well as the alternative pathway components properdin and factor B are deficient in neonates (59–61). These deficiencies in the neonatal complement system result in a reduced ability to activate the complement cascade, thereby leading to reduced phagocytosis, a reduced ability to lyse pathogens, and reduced recruitment of immune cells to sites of infection (59).

Adaptive Immunity Deficiencies

Deficiencies in the innate immune system can lead to reduced adaptive immune responses, and there are a number of deficiencies and differences in neonatal adaptive immune cells relative to those of adults. The neonatal adaptive immune response can range from no response to a strong response similar to that of adults (62). Although neonates mainly rely on their innate immune response to pathogens within the timeline of GBS transmission, understanding how neonates differ in their adaptive immune response compared to that of adults may greatly influence vaccine development efforts.

T cells can be classified into different subclasses that play specific roles in the immune response. CD4⁺ T cells, also known as T helper (Th) cells, play an important role in activating or stimulating the maturation of other immune cells and can be further differentiated into other subtypes, with the two major subtypes being Th1 and Th2. Th1 cells aid in the production of inflammatory responses to microbial pathogens, whereas Th2 cells secrete cytokines in response to parasites and allergens. Interestingly, the neonatal immune system has a much larger population of Th2 cells and diminished numbers of Th1 cells (63). In addition to the reduced number of Th1 cells, neonates also have reduced numbers or even a complete lack of Th17 cells, which aid in developing immunity to both bacterial and fungal infections at mucosal surfaces (64).

Neonates have been shown to have defective B-cell responses resulting in deficient humoral immunity as well. This defective response could be due to the immature development of surface immunoglobulins (Igs) and a reduced level of antigen exposure. Additionally, follicular Th (TFH) cells play an important role in developing an antibody response by eliciting the proliferation and maturation of B cells. Neonates have a reduced frequency of TFH cells, which are regulated by IL-4 production by Th2 cells (65). Moreover, B-cell signaling through the B-cell receptor (BCR) is deficient in neonatal B cells, a phenotype that could be caused by higher expression levels of CD22, a negative regulator of BCR signaling in neonatal B cells (66).

Despite these deficiencies in adaptive immunity, neonates have protective antibodies that are passed on from mother to neonate either transplacentally or through breast milk. These maternal antibodies help protect the neonate from infection but can also impact the neonatal immune response to infection and vaccination (67). A study that examined specific antibody concentrations at birth and after immunization found an inverse correlation between birth concentrations and increases in antibody concentrations after immunization. These data suggest that these higher concentrations of antibodies at birth could inhibit the neonatal immune response to vaccines. Nonetheless, most of the neonates in that study developed antibodies, suggesting that there is not a complete inhibition of neonatal antibody development by higher concentrations of maternal antibodies (68).

IMMUNE RESPONSE TO GBS AND MECHANISMS OF IMMUNE EVASION

Recognition of and Cytokine Response to GBS by the Innate Immune System

GBS is able to elicit a strong host inflammatory response. Upon *ex vivo* GBS infection, neonatal monocytes produce proinflammatory cytokines, including TNF and IL-6, but at reduced levels compared to those produced by adult monocytes (69). GBS strains belonging to different sequence types also elicited different cytokine responses in primary human monocyte cells. More specifically, infection by strains belonging to CC17 and -19, both of which are more frequently associated with infection (10), resulted in significantly higher levels of production of TNF- α , IL-6, and IL-8 than those induced by strains of other lineages (70).

GBS activates phagocytes via interactions with TLR2 and TLR6, and this activation is dependent on the TLR adaptor protein myeloid differentiation factor 88 (MyD88) (71, 72). Additionally, GBS-induced activation of inflammatory cytokines requires the c-Jun kinase pathway (73), while phagosomal GBS induces interferon in DCs via TLR7, MyD88, and the transcription factor IRF1 (74). Furthermore, GBS single-stranded RNA (ssRNA) is recognized by monocytes and macrophages via a complex comprising MyD88 and UNC-93B (75). The recognition of GBS ssRNA results in the increased production of nitric oxide (NO) by host cells, which activates macrophages and aids in phagosome acidification (76). The presence of GBS DNA also induces the release of IL-6, IL-12, and TNF- α via TLR9 but does not upregulate IFN- β or NO secretion (77). In contrast, IFN- β production was shown to be induced by GBS DNA in murine bone marrow-derived macrophages as well as THP-1 human monocytes in a TLR-independent manner. Rather, cytoplasmic GBS DNA is sensed by cyclic GMP-AMP synthase (cGAS), which activates stimulator of interferon genes (STING) that leads to IFN- β production (78, 79). GBS also releases cyclic di-AMP (c-di-AMP) into its environment, which can directly activate STING without cGAS; however, a GBS-expressed ectonucleotidase (CdnP) degrades c-di-AMP in order to reduce STING activation (79). Elevated levels of TNF- α occur during GBS sepsis, which is believed to play a role in clinical outcomes and is released from both monocytes and macrophages in response to GBS. The deposition of complement on GBS, more specifically C3 activation via the alternative pathway, triggers TNF- α production by monocytes (80). Monocytes are the most abundant innate immune cells in neonates, which could contribute to the abundance of monocyte-derived TNF- α production (49).

GBS produces a surface-associated beta-hemolysin/cytolysin toxin that is encoded by the *cyl* operon and is a major virulence factor (81). This ornithine rhamnolipid also generates pigmentation in GBS and has been shown to aid in crossing human extra-placental membranes (82). Not only does GBS beta-hemolysin/cytolysin contribute to pathogenicity through its cytolytic properties and by promoting invasion across host cell barriers, it also stimulates a potent proinflammatory cytokine response via the release of IL-1 and IL-6 and NO production in macrophages (83). Moreover, purified beta-hemolysin/cytolysin increased membrane permeabilization, resulting in the osmotic lysis of red blood cells and pyroptosis induction in macrophages (84). Both purified beta-hemolysin/cytolysin and hyperpigmented GBS were also cytotoxic to adult neutrophils but not through apoptosis or pyroptosis (85). Activation of the nucleotide-binding oligomerization domain-like receptor family pyrin domain-containing 3 (NLRP3) inflammasome by GBS is dependent on the expression of beta-hemolysin/cytolysin. Inflammasomes are multiprotein complexes located inside innate immune cells that activate the immune system in response to pathogens through the activation of caspase-1, which leads to an inflammatory response (86). In macrophages, GBS beta-hemolysin/cytolysin can cause leakage of the lysosome containing GBS, which allows the escape of bacterial RNA. This RNA then activates the NLRP3 inflammasome, inducing the production of IL-1 β (87).

GBS Immune System Evasion

GBS employs several mechanisms to resist immune detection and phagocytosis, thereby increasing the chance of survival in the host. These mechanisms are summa-

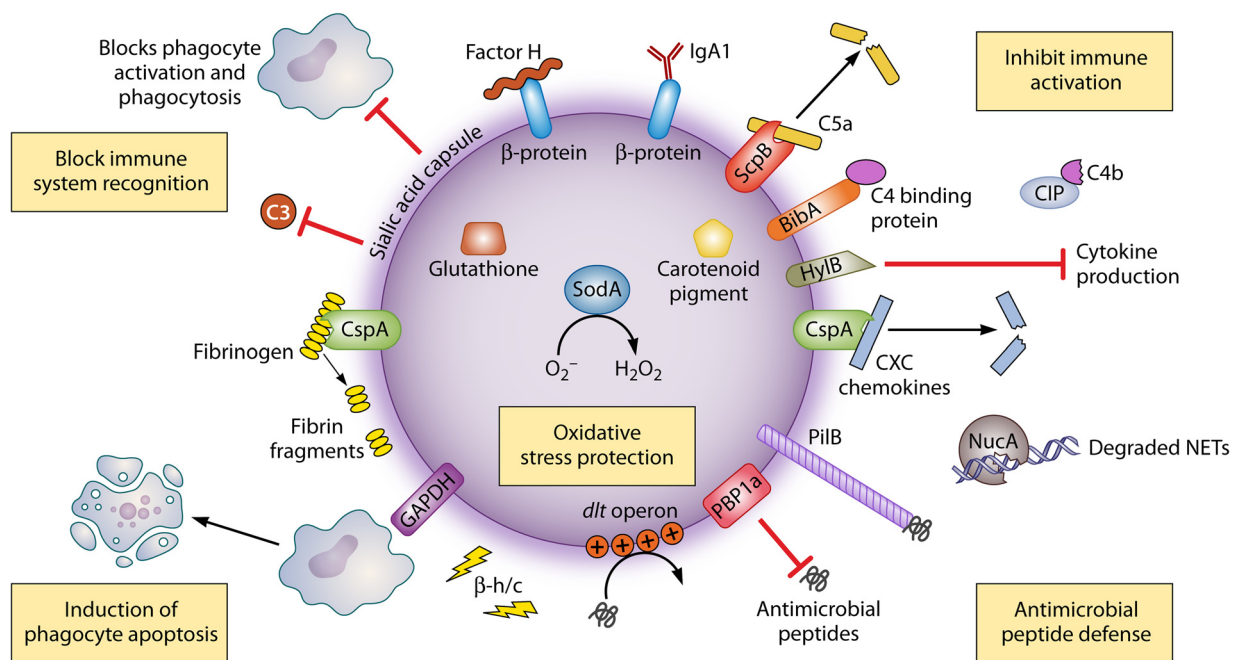


FIG 1 Mechanisms used by GBS to evade the immune system. GBS expresses many factors that help it evade the immune system and increase its survival in the host. The sialic acid capsule and fibrin fragments cleaved by CspA that coat the surface help GBS present as “self” to the immune system. The capsule also blocks C3 deposition and recognition by phagocytes. Sialic acid in the capsule, β-protein, ScpB, CIP, and BibA inhibit the complement system by binding or cleaving complement components. The GBS β-protein also binds the Fc region of IgA1 to inhibit immune activation. HyIB and CspA inhibit or cleave cytokines, while PilB, PBP1a, and proteins encoded by the *dlt* operon assist in resisting antimicrobial peptides. NucA degrades the DNA matrix of neutrophil extracellular traps. Glutathione, carotenoid pigment, and SodA all aid in defense against reactive oxygen species, and both β-hemolysin/cytolysin (β-h/c) and GAPDH aid in inducing apoptosis in phagocytes.

ized in Fig. 1. One example is the expression of the polysaccharide capsule (CPS), which is considered a major virulence factor, as unencapsulated GBS strains are less virulent in animal models (88). The GBS capsule contains a terminal sialic acid (Sia). Since Sia is also present on the surface of vertebrate cells, the Sia on the surface of GBS allows it to mimic host cells and avoid immune detection (89).

Sia-binding immunoglobulin-like lectins (Siglecs) are located primarily on the surface of leukocytes and are responsible for distinguishing between “self” and “nonself” to determine if an immune response should be activated. The Sia in the GBS capsule binds to Siglecs in order to reduce the activation of NF-κB and mitogen-activated protein kinase (MAPK) signaling, thus inhibiting the immune response. Siglec-9 expressed by human neutrophils recognizes Sia on the surface of GBS and dampens the immune response (90). Additionally, the surface-expressed β-protein of GBS binds to both Siglec-5 and Siglec-14 on the surface of neutrophils (91, 92). Interestingly, ligand binding to Siglec-5 elicits an inhibitory response in phagocytes, whereas Siglec-14 binding elicits an activating response. Since both Siglecs have similar ligand-binding motifs, it has been suggested that they are paired receptors that play a role in balancing the immune response to invading bacteria. Moreover, Siglec-5/14 expression was found on the surface of the amniotic membrane in human extraplacental membranes (92). This unusual location for Siglec expression is of particular interest, since GBS is capable of crossing extraplacental membranes (18). GBS binding to Siglecs results in the impairment of phagocytosis, reduced ROS generation, and poor extracellular trap formation in leukocytes (90, 91). Macrophages lacking Siglecs show enhanced production of proinflammatory cytokines, phagocytosis, and bacterial killing of GBS (93). Macrophages also express sialoadhesin on their surface, which is a unique type of Siglec that contains an elongated extracellular portion capable of recognizing Sia on the surface of pathogens and mounting an inflammatory response. Sialoadhesin aids in clearing GBS infection and blocking dissemination to organs in mice (94).

Another mechanism of host cell mimicry employed by GBS is coating itself with the highly adhesive fibrin breakdown product of fibrinogen. GBS uses the cell surface protein CspA to cleave fibrinogen similarly to thrombin, which results in the exposure of the regions responsible for fibrinogen polymerization, leading to the aggregation of GBS and coating of the bacterial surface with fibrin. This fibrin coating allows GBS to appear as “self” to host immune cells and reduces the access of opsonins to the surface, thereby inhibiting opsonophagocytosis (95).

The CPS can also inhibit opsonophagocytosis by blocking the deposition of C3b on the surface of GBS. Both unencapsulated and encapsulated strains lacking sialic acid, for instance, bound more C3 molecules than did a wild-type (WT) strain (96). GBS also expresses other surface components that prevent opsonophagocytosis as well as the activation of the complement cascade. BibA, for example, resists opsonophagocytic killing by neutrophils via the specific binding of the C4-binding protein, which is a regulator of the complement pathway (97). The secreted complement-interfering protein (CIP) binds to C4b, inhibiting its interaction with C2 to reduce complement activation through the classical and lectin pathways but not the alternative pathway (98). Similarly, the GBS β -protein binds the soluble complement inhibitor factor H to the bacterial surface in a way that inhibits C3b deposition and opsonophagocytosis (99); the Sia residues in the CPS can also bind factor H (100). Another important factor is a serine protease, ScpB, which is a C5a peptidase that proteolytically cleaves complement-activated C5a, a powerful chemoattractant involved in the recruitment of inflammatory cells (101). In addition to its ability to cleave fibrinogen, CspA is also capable of cleaving and, therefore, inactivating CXC chemokines that recruit neutrophils to different infection sites (102).

In a pregnant mouse model, GBS was shown to ascend the vaginal tract to infect the decidua, placenta, and fetus. This invasion was marked by a large recruitment of neutrophils to the infection site in the decidua and placenta, which is similar to what is seen in chorioamnionitis in human patients. Neutrophils isolated from mice also produced NETs in response to GBS (103); similar results were observed in a nonhuman primate model of amniotic cavity infection by GBS (85). NETs are produced by neutrophils in response to invading bacteria and consist of DNA and antimicrobial peptides (AMPs). These NETs ensnare bacteria and eliminate them to help clear infections (104). High expression levels of beta-hemolysin/cytolysin, as well as purified beta-hemolysin/cytolysin, induce NET formation in adult neutrophils, although beta-hemolysin/cytolysin also conferred resistance to killing by these NETs (85). GBS-induced NETs contain lactoferrin, which sequesters iron, preventing invading pathogens from using it as a nutrient source. Lactoferrin is capable of repressing GBS growth and could be one way in which these NETs prevent some GBS strains from invading (103). Nonetheless, GBS also produces nuclease A (NucA), which degrades the DNA in the NETs to allow GBS to escape. In a previous study, NucA was needed for GBS persistence in lung tissue, and a *nucA* mutant was less virulent than the WT in a mouse model, suggesting that NucA is important for both initial infection as well as dissemination (105).

In response to tissue injury following pathogen invasion, hyaluronan (HA), a component of the extracellular matrix, is quickly degraded by host hyaluronidases and ROS (106). The small cleavage products are recognized by TLR2 and/or TLR4 to stimulate an inflammatory response to clear the pathogen as well as initiate wound healing (107, 108). GBS secretes hyaluronidase, encoded by *hylB*, to degrade HA to assist in dissemination. Interestingly, HylB plays roles in enhancing survival inside macrophages, inhibiting proinflammatory cytokine expression, and utilizing HA as a carbon source in the host (109). The GBS hyaluronidase degrades HA into disaccharides instead of 4- to 16-mer fragments that produce a proinflammatory response. These HA disaccharides are capable of blocking TLR2/4 signaling, resulting in reduced proinflammatory cytokine production (110).

Phagocytic Uptake of GBS

Despite the above-described mechanisms employed by GBS to avoid immune detection and phagocytosis, GBS is easily phagocytosed and killed by phagocytic cells

in the presence of serotype-specific antibodies via Fc receptors (111). Internalization of GBS can also occur through complement receptor 3 (CR3) in the presence of other opsonins like lectins and L-ficolin (112). Since GBS elicits a poor antibody response and neonates have low levels of complement, opsonin-independent pathways of phagocytosis would be the more likely mechanism of uptake of GBS. Additionally, GBS is rapidly taken up by macrophages in the absence of opsonins (111). Because CR3 is important for opsonin-independent phagocytosis by macrophages, GBS was suggested to interact with CR3 in a C3-independent manner (113). Furthermore, the uptake of GBS requires actin (111) and varies by strain type (114). Besides the complement-binding domain, CR3 also contains a lectin domain that is able to bind the type III CPS to initiate phagocytosis in neutrophils (115).

GBS Induction of Apoptosis in Macrophages

One strategy used to avoid immune activation after a pathogen is taken up by a phagocyte and to persist at the site of infection is to induce the apoptosis of immune cells before they become activated (116). Apoptosis is a process of programmed cell death that is less likely to elicit a strong inflammatory response, such as that seen with necrosis or pyroptosis. However, there are certain cases in which apoptosis can be inflammatory (117). Since apoptosis plays a role in the maintenance of cell populations in tissues as well as during development and aging, it is a tightly regulated process. This process involves protein kinase C (PKC) activity and modulation of cytoplasmic calcium levels and is regulated by the caspase family of cysteine-directed proteases (caspase-dependent pathway) or calpains (caspase-independent pathway) as well as Bcl-2 family regulators (118).

GBS is capable of inducing apoptosis in macrophages, which requires internalization and is bacterial dose dependent (119). During induction, GBS stimulates the sustained activation of c-Jun NH₂-terminal kinase (JNK) and p38 but inhibits extracellular signal-regulated kinase (ERK), all of which are members of the MAPK family (120). Moreover, GBS infection of macrophages also induces the expression of TNF- α , IL-1, and inducible nitric oxide synthase (iNOS), leading to apoptosis. Inhibition of iNOS expression inhibited GBS-induced apoptosis, but inhibition of TNF- α and IL-1 did not. Also, the addition of NO alone without infection induced apoptosis, indicating a direct effect of GBS-induced NO production on apoptosis (121).

The role of caspases in GBS-induced apoptosis is not clear. One study showed that GBS-induced apoptosis was independent of caspase-1 and -3 (119), whereas another study showed that caspase-3 and -9 were important for this process (121). These contradictory results could be due to the use of different GBS strains in those studies: both studies used serotype III strains, but different strains of the same serotype have been shown to have various host-pathogen interactions (122). Therefore, it is possible that diverse strains of GBS are capable of using different mechanisms for inducing apoptosis. Moreover, those studies were done by using cell culture, making it difficult to fully understand the mechanism of GBS-induced apoptosis *in vivo*. Interestingly, one study used an *ex vivo* fetal rat lung model to show that caspase-3 activation results in apoptosis in macrophages and erythroblasts in the lung interstitium following GBS infection (123).

Through beta-hemolysin/cytolysin-induced plasma membrane permeability, GBS is able to cause a massive increase in calcium levels inside macrophages leading to the activation of the calcium-sensitive calpains, which leads to the degradation of structural and regulatory cytoskeletal proteins as well as the induction of apoptosis (124, 125). GBS-induced calcium influx also results in PKC activation (119) as well as the activation of gelsolin, an important regulator of the actin cytoskeleton and apoptosis (126). Glyceraldehyde-3-phosphate dehydrogenases (GAPDHs) are surface-localized enzymes that are capable of binding to host cell components and have immunomodulatory effects. Interestingly, GAPDHs from GBS and other pathogens, including *Streptococcus pyogenes* and *Staphylococcus aureus*, can induce apoptosis in macrophages, indicating yet another role of bacterial GAPDH in pathogenesis (127).

GBS Survival inside Phagocytes

Once a bacterium is taken up by a phagocytic cell, it gets trapped within a vacuole that goes through phagosomal maturation, in which the vacuole fuses with various compartments in the endocytic pathway. The end product is a fully mature phagolysosome, which consists of a harsh, highly acidic, and nutrient-limiting environment where AMPs, ROS, and reactive nitrogen species (RNS) are generated to kill the bacterium (128). Although most bacteria are efficiently killed by this process, many pathogens have developed ways to overcome these defense mechanisms. For instance, some pathogens can disrupt cellular signaling to prevent or slow down the phagosome maturation process and live inside the phagosome. Other pathogens can escape from the phagosome by lysing the membrane to replicate in the cytosol, while others can remain inside the phagolysosome, defending against the many stressors (129).

GBS is capable of persisting within macrophages and remains inside the phagosome, which recruits late endosomal markers. This recruitment indicates that GBS does not inhibit phagosome maturation as a survival strategy and likely uses a phagosomal stress defense mechanism (111, 130). This ability to survive inside innate immune cells allows GBS to avoid immune detection, protect against antibiotics, and facilitate dissemination to other sites of the body, making it a particularly important topic of study (131, 132). Indeed, opsonization of GBS significantly reduces the ability of GBS to survive intracellularly (111). Although the CPS helps GBS avoid phagocytosis, it does not aid in intracellular survival, as unencapsulated mutants were internalized at a higher rate in a previous study; the time of survival intracellularly, however, was no different than that for the encapsulated WT strain (133).

GBS has several strategies to help it survive under the antimicrobial conditions of the phagosome. Upon infection, macrophages undergo a number of changes in protein expression that result in the decreased expression of enzymes that impact ROS production and NO synthesis, both of which are important for antimicrobial responses. Since these changes were not observed in macrophages infected with heat-inactivated GBS, it is likely that GBS actively induces these changes (134). In addition to its ability to inhibit ROS production, GBS also has the ability to inactivate ROS through the use of superoxide dismutase (SodA), which functions to convert superoxide into oxygen and hydrogen peroxide (135). Although GBS is catalase negative, sequencing shows that the GBS genome contains NADH peroxidase, a thiol peroxidase, and an alkylhydroperoxide reductase, all of which could possibly be used to detoxify hydrogen peroxide (136). Moreover, GBS has been shown to produce glutathione (137), which protects the bacterial cell from oxidative stress, low pH, as well as other stresses (138). In addition to its immunomodulatory effects and cytolytic properties, beta-hemolysin/cytolysin also produces an orange carotenoid pigment, which has also been shown to protect GBS from oxidative damage (139).

In addition to ROS and RNS production, a number of AMPs and hydrolases are present in the phagosome to kill bacteria (140). Penicillin-binding protein 1a (PBP1a), for example, is important for resisting host AMPs (141). One mechanism used to avoid the effect of cationic AMPs used by GBS is to increase the number of D-alanine residues in lipoteichoic acids, which is regulated by the *dlt* operon (142). Initially, it was thought that D-alanylation would reduce the electronegativity of the cell wall and therefore decrease the affinity of cationic AMPs. However, a previous study showed that D-alanylation altered the rigidity and permeability of the cell wall, which blocked certain cationic AMPs from crossing it (143).

Additionally, GBS pili have been shown to mediate resistance to AMPs in addition to aiding in host cell attachment. There are three distinct pilus islands (PIs), PI-1, PI-2a, and PI-2b, that encode structurally different pili in GBS (144). PilB, a pilus protein subunit, was shown to play a role in intracellular survival in murine macrophages and human neutrophils by conferring resistance to cathelicidin and defensin families of AMPs and facilitates bloodstream survival in a mouse model. Moreover, the expression of GBS PilB in *Lactococcus lactis*, which is susceptible to AMPs, conferred resistance to AMPs (145).

Contradictory to these results, one study found no significant difference in survival inside murine macrophages between WT and $\Delta pilB$ strains (146). One possible explanation for this difference could be that different strains were used, which may vary in the mechanism used to survive inside the phagosome (114). The pilus backbone protein specific for ST-17 lineages, Spb1, was also shown to enhance both phagocytosis and intracellular survival of GBS. Additionally, the presence of *spb1* in GBS strains did not alter NO or TNF- α responses in macrophages (147). Another ST-17-specific gene, *srr2*, plays a role in binding both fibrinogen and plasminogen but has also been shown to increase phagocytic uptake and intracellular survival in macrophages and neutrophils (148). Although having a protein that would enhance the phagocytic uptake of the pathogen seems counterintuitive, that same protein can also be used to enhance survival inside macrophages while promoting dissemination. These proteins, along with several other ST-17-specific virulence factors, may partly explain the enhanced ability of ST-17 strains to survive inside macrophages as well as their increased virulence and association with neonatal infections (16).

As a lactic acid-producing bacterium, GBS has mechanisms to withstand low pH and should be expected to withstand the low pH of the phagosome. Indeed, a previous study demonstrated that ~18% of the genes in the GBS genome were differentially expressed at pH 5.5 relative to pH 7.0, and most of these genes are regulated by the CovR/S (also known as CsrRS) two-component regulatory system (149). In addition to regulating many virulence factors, this CovR/S acid response regulator was found to be required for GBS to survive inside macrophages. Some of the genes upregulated at low pH encode transporters, which may allow GBS to increase its scavenging ability to facilitate survival under the nutrient-limiting conditions of the phagosome (130). Moreover, inhibition of the acidification of the phagosome significantly reduced the ability of GBS to survive in macrophages, suggesting that acidic pH is needed for GBS to survive phagosomal stress. This reduced survival, however, was not observed in all of the strains examined, suggesting that diverse strains of GBS are using alternative mechanisms to withstand phagosomal stress (114).

Antibody Response to GBS and Vaccine Development

Because of the large number of deficits in the neonatal innate immune system, maternal antibody transfer is very important in passive immune protection of the newborn. A deficiency in maternal antibody responses targeting GBS has been considered to be important for neonatal infections (150). Moreover, CPS type III strains induce a lower antibody response than those induced by strains of other CPS types (151). This finding is consistent with data from our previous study showing that CPS type III strains representing multiple STs survived better in a multiple-stress medium comprising key phagosomal stressors than did strains of other genotypes with various CPS types. Indeed, enhanced survival in macrophages could result in decreased bacterial killing and presentation of CPS antigens to the adaptive immune system (114).

Since human colostrum and milk contain high concentrations of secretory IgA, it is probable that IgA plays an important role in neonatal protective immunity. Known roles of IgA include recognizing pathogens and triggering a response to eliminate them. Once IgA recognizes a pathogen, it interacts with CD89 on the surface of phagocytes to induce phagocytosis, ROS production, and the production of inflammatory mediators (152). The GBS surface-expressed β -protein also plays a role in binding to the Fc region of IgA, which inhibits IgA binding to CD89 and blocks proactive immunity from maternal IgA (153).

Due to the high level of diversity across GBS strains, vaccine development efforts have been difficult. Since CPS types are both antigenically and structurally unique, CPS-based vaccines do not offer protection against other CPS types (154). Studies have switched toward examining conserved antigenic proteins as vaccine candidates. Interestingly, one study found that both mothers and their newborns naturally produced antibodies against the GBS surface protein Sip. This finding suggests not only that mothers can produce Sip antibodies but also that these antibodies are transferred

transplacentally and can persist in the infant (155). Another study found that GBS-colonized mothers who delivered healthy babies had higher levels of naturally occurring antibodies against both CPS and pilus proteins than did mothers whose babies developed GBS infection or noncolonized mothers (156). This finding further supports the role of maternal antibodies in protecting neonates from GBS infections and suggests that a vaccine strategy targeting pregnant women has potential merit and warrants further investigation. The possibilities of such a vaccine as well as the status of vaccine development have been reviewed elsewhere (157, 158). Current efforts have also focused on developing both CPS-protein conjugate vaccines and protein-based vaccines that target conserved GBS proteins (159).

CONCLUDING REMARKS AND FUTURE DIRECTIONS

The neonatal immune system has several deficiencies and limitations that render neonates more susceptible to infection. Furthermore, GBS has an arsenal of immune evasion strategies and virulence factors that make it an extremely successful pathogen in neonates. Although previous studies examined the interaction between GBS and the immune system, many of those studies were conducted *in vitro* by using cell lines or primary cells, and hence, it is difficult to know how these findings correlate with those of *in vivo* studies. Additionally, many studies have used immune cells derived from adults, which have properties and functions distinct from those of neonatal cells. It would therefore be interesting and informative to explore more of these interactions using neonatal or deficient immune cells. Similarly, most *in vivo* studies utilize murine models, which have important differences from humans (160) and also limit our ability to correlate findings to natural human infections. The development of humanized strains of mice has helped overcome a number of these differences and has become a popular method for studying specific aspects of the immune system (161). Interestingly, humanized mice have deficiencies in several immune cells and the complement system, which are similar to those found in neonates, making humanized mice a promising model to study neonatal responses to infections. The use of specific-pathogen-free or germfree murine models will also be useful to mimic the naive nature of the neonatal immune system. Indeed, Ernst et al. recently introduced a neonatal humanized model of GBS sepsis, which represents an intriguing system to further explore neonatal infections (162).

Despite the large number of advancements in our understanding of neonatal GBS infections, there are still many areas left to be explored. Although a number of studies have begun to explore variation across GBS strains, it is important to further examine these differences to determine why certain strains/lineages have a greater capacity to cause disease than do others. Some aspects of the phagocytic uptake of GBS in the absence of opsonins have been explored; however, more details of the precise mechanisms still need to be elucidated. Moreover, the mechanism by which GBS induces apoptosis *in vivo* is another interesting area to be explored, as most previous studies were performed *in vitro*. Although GBS survives inside a mature phagolysosome and likely uses a stress defense mechanism, only a few bacterial factors have been identified to be important for this process to date. Future studies should therefore focus on identifying additional mechanisms that are important for resisting phagosomal stress, particularly in those genotypes that more commonly cause neonatal infections.

GBS is a highly versatile organism that causes invasive disease in neonates in addition to elderly and immunocompromised adults. Since GBS is a leading cause of neonatal sepsis and meningitis, many studies have focused on these infections. The steady rate of EOD in neonates despite preventative measures, as well as high frequencies of antibiotic resistance, emphasizes the need to find additional or alternative therapeutics and preventatives. Additionally, the current preventative practice of IAP has not had an effect on the incidence of LOD. In order to better tailor efforts in developing new therapeutic and preventive measures, a more thorough understanding of the how GBS interacts with the immune system is required.

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