

9 Group B *Streptococcus* Meningitis

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9.1 Introduction

Streptococcus agalactiae (Group B *Streptococcus*, GBS) is a Gram-positive encapsulated bacterium possessing an array of virulence factors that render it capable of producing serious disease in susceptible hosts, in particular the human newborn (Maisey *et al.*, 2008a). Notably, GBS is the leading cause of meningitis in the neonatal period (Brouwer *et al.*, 2010; Thigpen *et al.*, 2011). Although advances in intensive care management and antibiotic therapy have changed GBS meningitis from a uniformly fatal disease to a frequently curable one, the overall outcome remains unfavourable. Morbidity is high; 25–50% of surviving infants suffer neurological sequelae of varying severity, including cerebral palsy, mental retardation, blindness, deafness or seizures.

The pathogenesis of neonatal GBS infection begins with the asymptomatic colonization of the female genital tract. Approximately 20–30% of healthy women are colonized with GBS on their vaginal or rectal mucosa, and 50–70% of infants born to these women will themselves become colonized with the bacterium (Baker and Edwards, 2001). For the purposes of epidemiological classification, neonatal GBS

infections are traditionally divided among two forms: early-onset disease (EoD) and late-onset disease (LoD). Early-onset infections are described to occur through the first 7 days of life, but in fact have a median onset of only 6–8 h of life, presenting acutely with pneumonia and respiratory failure complicated by bloodstream infection and septicaemia. GBS EoD cases result from ascending infection of the bacterium through the placental membranes to initiate infection *in utero*, or, alternatively, by aspiration of infected vaginal fluids during the birth process. Premature, low-birth-weight infants are at increased risk of developing early-onset infection, with GBS placental infection itself often the critical factor triggering premature labour. In contrast, GBS LoD occurs in infants up to 7 months of age, with more indolent symptom progression related to bacteraemia, absence of lung involvement and a high incidence (~50%) of meningitis (Baker and Edwards, 2001). Universal screening of pregnant women at 35–37 weeks gestation and intrapartum antibiotic prophylaxis has resulted in a decline in early-onset GBS invasive disease in the USA (Phares *et al.*, 2008; Van Dyke *et al.*, 2009). However, this treatment has not eliminated the incidence of GBS meningitis, and concern has

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been raised about concurrent increases in non-GBS early-onset bacterial infections, especially in pre-term infants as a result of increased antibiotic use (Stoll *et al.*, 2002a,b). Furthermore, the occurrence of GBS meningitis in older children or adults is more commonly appreciated, with an approximate 4% increase in total number of cases reported between 1997 and 2007 in the USA (Thigpen *et al.*, 2011). No vaccination strategies are currently in place to prevent GBS infections, but if ultimately achieved they would be anticipated to reduce the number of meningitis cases (Thigpen *et al.*, 2011). Here we review the current understanding of the pathogenesis of GBS meningitis, highlighting important bacterial virulence factors and host interactions that promote disease progression.

9.2 Pathophysiology of GBS Meningitis

The pathophysiology of GBS meningitis varies according to age of onset. In EoD, autopsy studies demonstrate little or no evidence of leptomeningeal inflammation, despite the presence of abundant bacteria, vascular thrombosis and parenchymal haemorrhage (Quirante *et al.*, 1974). By contrast, infants with LoD usually have diffuse purulent arachnoiditis with prominent involvement of the base of the brain (Berman and Banker, 1966). Similar age-related differences in central nervous system (CNS) pathology are evident in the infant rat model of invasive disease (Ferrieri *et al.*, 1980). These histopathological differences reflect underdevelopment of the host immunological response in the immediate neonatal period, with a higher proportion of deaths resulting from overwhelming septicaemia. Clinical and neuropathological studies have documented the clear association between bacterial meningitis and brain oedema formation, increased intracranial pressure (ICP), seizure activity, arterial and venous cerebral vascular insults, and other neurological sequelae (Schedl *et al.*, 2002).

To produce meningitis, blood-borne GBS must typically penetrate the blood–brain barrier (BBB) and/or the blood–cerebrospinal

fluid barrier (BCSFB). For the purposes of this review, the BBB and BCSFB are interchangeable concepts with respect to vessel endothelial cell penetration by GBS. Disruption of BBB integrity is a hallmark event in the pathophysiology of bacterial meningitis. This disruption may be due to the combined effect of bacterial entry and penetration of brain microvascular endothelial cells (BMECs), direct cellular injury by bacterial cytotoxins, and/or activation of host inflammatory pathways that compromise BMEC barrier function. It is apparent that the host immune response is not only incapable of controlling infection within the CNS but also may be responsible for many adverse events during bacterial meningitis (Tunkel and Scheld, 1995). A very complex and integrated series of events involving host cytokines, chemokines, proteolytic enzymes and oxidants appears to be responsible for meningitis-induced brain dysfunction. The development of GBS meningitis progresses through phases including: (i) bloodstream survival and the development of bacteraemia; (ii) direct GBS invasion and disruption of the BBB/BCSFB; and (iii) GBS multiplication in the CSF-containing subarachnoid and ventricular spaces, which induces inflammation with associated pathophysiological alterations leading to the development of neural damage. Brain injury results mainly from cerebrovascular involvement leading to cerebral ischaemia, brain oedema, hydrocephalus and increased ICP.

9.2.1 Bloodstream survival and the development of bacteraemia

An association between sustained high-level bacteraemia and the development of GBS meningitis has been suggested in humans and in experimental models of haematogenous meningitis (Ferrieri *et al.*, 1980; Doran *et al.*, 2002a). This observation implies that GBS bloodstream survival is an important virulence trait to avoid immune clearance by phagocytic killing by host immune cells, prior to CNS penetration. Neonates are particularly prone to invasive disease because of their quantitative or qualitative deficiencies in

phagocytic cell function, specific antibody, or the classical and alternative complement pathways. In addition to these newborn host susceptibilities, GBS possess a number of virulence determinants that promote bloodstream survival by thwarting key components of effective opsonophagocytic killing such as complement (Fig. 9.1). For example, the surface-anchored GBS β -protein prevents opsonophagocytosis by binding short consensus repeats found in the middle region of factor H, a host counter-regulator of complement (Maruvada *et al.*, 2008), enabling the unbound active region to block C3b deposition on the bacterial cell surface (Jarva *et al.*, 2004). In addition, the cell-surface GBS immunogenic bacterial adhesin (BibA) binds human C3bp, promoting resistance to phagocytic killing and contributing to virulence in the mouse model (Santi *et al.*,

2007). The β -antigen of C protein binds human IgA antibody (Jerlstrom *et al.*, 1991), and non-specific deposition of IgA on the bacterial surface probably inhibits interactions with complement. Finally, a cell-surface protease, CspA, targets host fibrinogen, producing adherent fibrin-like cleavage products that coat the bacterial surface and interfere with complement-mediated opsonophagocytic clearance (Harris *et al.*, 2003).

The profile of GBS gene transcription changes dramatically during growth in human blood, resulting in an altered cell morphology and increased expression of complement regulatory proteins (Santi *et al.*, 2007; Mereghetti *et al.*, 2008). The sialylated GBS capsular polysaccharide (CPS) represents one of the most critical factors for limiting the effectiveness of host complement and phagocytic defence. Passage of GBS in animals

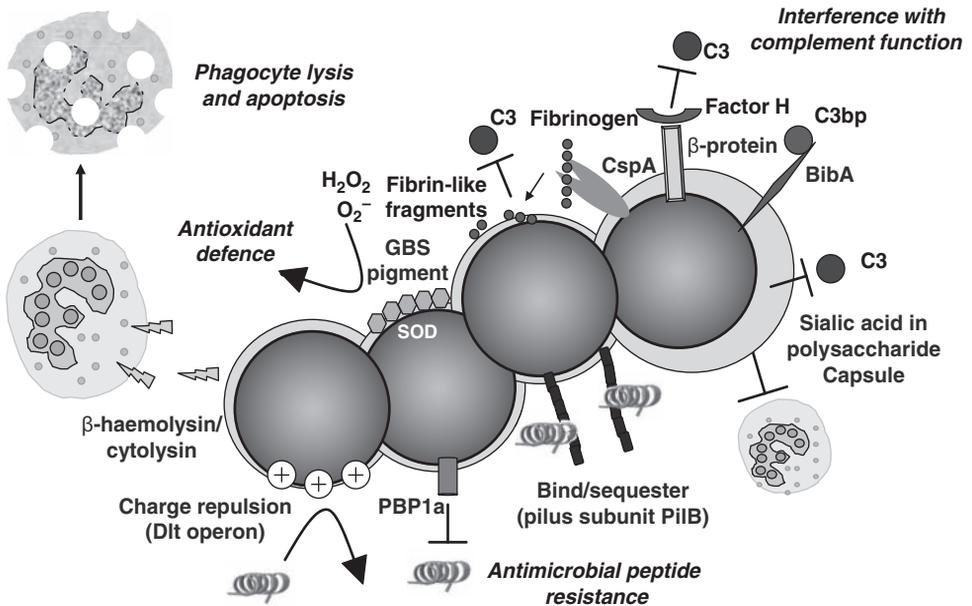


Fig. 9.1. Mechanisms of GBS immune evasion. GBS express multiple surface-exposed or secreted factors to evade host immune defences and promote bloodstream survival. The PBP1a and the PilB subunit of GBS pili contribute to antimicrobial peptide resistance. The Dlt operon is responsible for increasing incorporation of D-alanine residues in cell-wall teichoic acids, thereby reducing electronegativity and affinity for cationic antimicrobial peptides. ScpB, the sialic acid capsule, BibA, β -protein and CspA all inhibit host clearance of GBS by interfering with complement components C5a, C3 and C3bp. Superoxide dismutase (SOD) properties of the orange carotenoid pigment shield GBS from killing by phagocyte-generated reactive oxygen species. Alternatively, β -haemolysin/cytolysin can boost GBS survival by cytolytic or pro-apoptotic injury to host phagocytes.

increases capsulation, while serial *in vitro* passage leads to reduced capsule expression (Hakansson *et al.*, 1988), and strains obtained from infants with septicemia or meningitis have increased encapsulation compared with vaginal colonizing strains (Hakansson *et al.*, 1987). Thus, it appears that GBS capsule expression is induced during bloodstream replication and repressed while on mucosal or endothelial cell surfaces, a feature common to other meningeal pathogens. Currently, ten GBS capsular serotypes have been identified (Ia, Ib, II–IX) based on the different arrangements of four monosaccharides (glucose, galactose, *N*-acetylglucosamine and sialic acid) into unique repeating units. Serotype III GBS strains have accounted for a majority of LoD and meningitis (Baker and Edwards, 2001; Tazi *et al.*, 2010), but all serotypes contain a terminal-linked sialic acid bound to galactose in an $\alpha 2 \rightarrow 3$ linkage (Cieslewicz *et al.*, 2005). The sialic acid moiety provides antiphagocytic protection by impairing deposition of opsonically active complement C3 on the bacterial surface. Isogenic GBS mutants lacking CPS or capsular sialylation are more susceptible to neutrophil killing and are less virulent in animal models of infection (Campbell *et al.*, 1991; Marques *et al.*, 1992). Furthermore, the conserved GBS terminal $\alpha 2 \rightarrow 3$ linked sialic acid capsular component is identical to a sugar epitope widely displayed on the surface of all mammalian cells. Thus, bacterial surface sialylation may have evolved to mimic host ‘self’ antigens, allowing GBS to avoid immune detection, manipulate phagocyte function and dampen the immune response to GBS infection (Carlin *et al.*, 2007).

When GBS are engulfed and contained within the phagosome, a rapid release of toxic reactive oxygen species (ROS) is produced through the phagocyte oxidative burst. Although GBS do not produce catalase, they are nevertheless able to resist ROS killing and survive inside macrophage phagolysosomes (Wilson and Weaver, 1985; Cornacchione *et al.*, 1998; Teixeira *et al.*, 2001). GBS possess an endogenous source of the oxygen-metabolite scavenger glutathione (Wilson and Weaver, 1985), and the GBS SodA enzyme can neutralize superoxide anions (Poyart *et al.*, 2001b). GBS also produce an orange

carotenoid pigment, a property unique to GBS among haemolytic streptococci, associated with the *cyl* operon encoding the β -haemolysin/cytolysin cytotoxin (Spellerberg *et al.*, 2000). The free-radical scavenging properties of this carotenoid neutralize hydrogen peroxide, superoxide, hypochlorite and singlet oxygen, and thereby provide a shield against several elements of phagocyte ROS killing (Liu *et al.*, 2004). Other GBS factors that have been linked to survival inside phagocytic cells and/or dendritic cells include CPS (Lemire *et al.*, 2012), a pilin protein (Maisey *et al.*, 2008b) and transcriptional response regulators CovR (Cumley *et al.*, 2012) and CiaR (Quach *et al.*, 2009), which may coordinate expression of acid and stress survival genes.

Another important host defence mechanism inherent to many immune cells is the production of small cationic antimicrobial peptides (AMPs), such as cathelicidins and defensins. These peptides are attracted electrostatically to negatively charged microbial cell surfaces, followed by their self-assembly to form membrane pores or otherwise disrupt membrane integrity. GBS increase their intrinsic resistance to AMPs by incorporation of positively charged *D*-alanine residues into their cell wall teichoic acids, thereby reducing surface electronegative charge and affinity for the cationic peptides (Poyart *et al.*, 2001a). A surface-anchored penicillin-binding protein, PBP1a, enhances GBS resistance to cathelicidins and defensins, thereby reducing GBS susceptibility to killing by alveolar macrophages and neutrophils and promoting bacterial survival in a neonatal rat model of GBS infection (Jones *et al.*, 2007). Similarly, expression of the pilus backbone protein PilB and the action of the two-component regulator CiaR both render GBS more resistant to killing by cathelicidin AMPs (Maisey *et al.*, 2008b; Quach *et al.*, 2009).

9.2.2 GBS invasion of the BBB

Following bloodstream survival, GBS interacts directly with BBB endothelium, which can result in bacterial invasion of the BBB with subsequent infection of the CNS.

This process can result from increased permeability of the BBB and/or the direct invasion of BMECs by the pathogen. Microbial interaction with the BBB may involve crossing the brain endothelium by direct intracellular invasion and vacuole transit (transcytosis), by passage through the intercellular junctional spaces (paracytosis) or by transport inside another host cell (phagocyte-facilitated invasion). With the availability of *in vitro* tissue culture models of human (H)BMECs (Stins *et al.*, 1994; Nizet *et al.*, 1997) and animal models of GBS infection (Doran *et al.*, 2003; Tazi *et al.*, 2010), significant progress has been made in identifying and characterizing the molecular determinants that promote GBS–BBB interaction (Fig. 9.2).

Intracellular invasion (transcytosis)

GBS enter or ‘invade’ brain endothelium apically and exit the cell on the basolateral side, thereby crossing the BBB transcellularly (Nizet *et al.*, 1997; Lembo *et al.*, 2010). Electron microscopy has demonstrated the presence of the meningeal pathogen in membrane-bound vacuoles within HBMECs (Nizet *et al.*, 1997), suggesting the involvement of endocytic pathways as well as avoidance of lysosomal fusion for BBB traversal. Further HBMEC invasion can be blocked by inhibition of actin polymerization, suggesting that GBS trigger rearrangement of the host cytoskeleton and induce their own uptake (Nizet *et al.*, 1997). This process may be accomplished, at least in

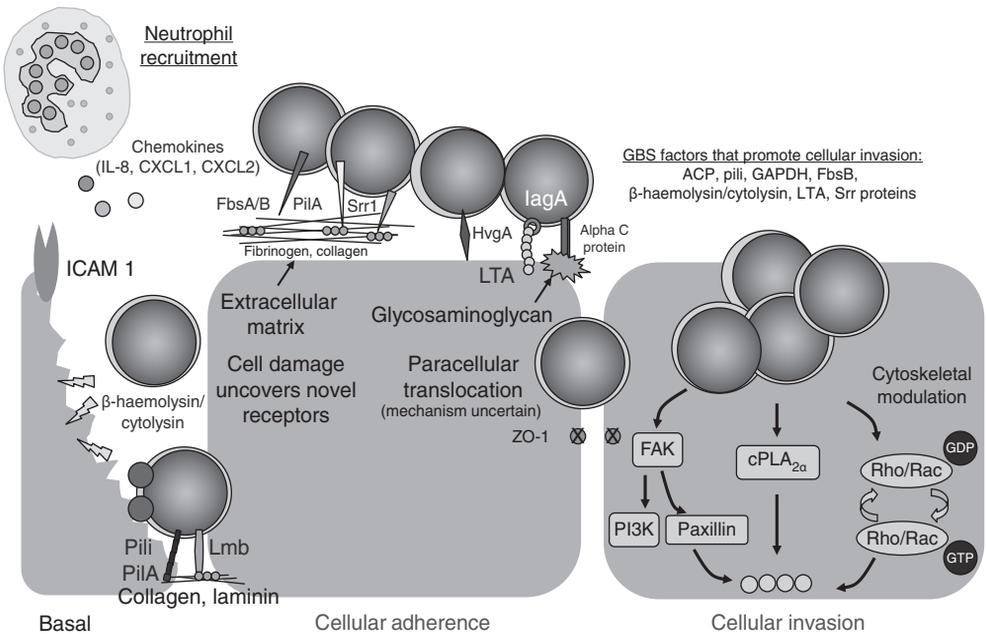


Fig. 9.2. Mechanisms of GBS penetration of the BBB. Surface-expressed proteins FbsA/B, Srr1, PiA, HvgA, lipoteichoic acid (LTA) and alpha C protein (ACP) mediate GBS binding to host cells and extracellular matrix (ECM) components, such as fibrinogen and collagen. Secreted β -haemolysin/cytolysin promotes GBS invasion, possibly by breaking down host barriers to reveal novel receptors on the basement membrane, such as laminin and collagen, as well as promoting neutrophil influx that contributes to barrier disruption. GBS also use glyceraldehyde 3-phosphate dehydrogenase (GAPDH) to activate host plasminogen and degrade the ECM. Intracellular GBS invasion is enhanced by bacterial-dependent cytoskeletal rearrangements triggered by host PI3K/AKT- and FAK-signalling pathways and the Rho family of GTPases. Alternatively, GBS can also disrupt tight junction complexes to cross the barrier by a paracellular route. Several GBS adhesins, including FbsB, pili, LTA and ACP, also contribute to cellular invasion.

part, by tyrosine phosphorylation of focal adhesion kinase (FAK), which occurs upon GBS infection. Phosphorylation of FAK induces its association with PI3K and paxillin, an actin filament adaptor protein (Shin *et al.*, 2006), and is required for efficient invasion of HBMECs by GBS. GBS-infected HBMECs also exhibit increased levels of activated Rho family members RhoA and Rac1. Rho family GTPase inhibitors and dominant-negative expression of RhoA and Rac1 are effective in blocking GBS invasion (Shin and Kim, 2006).

To elucidate the GBS determinants involved in the pathogenesis of meningitis, many groups have focused on the characterization of GBS isolates responsible for CNS disease. Clinical isolates of serotype III GBS, which are over-represented in LoD, appear to belong to two distinct evolutionary clusters (Musser *et al.*, 1989), which have now been shown through multilocus sequence typing to represent a limited number of clonal complexes (Jones *et al.*, 2003). Of these clones, sequence type (ST)-17 is strongly associated with neonatal meningitis and has been designated as the hypervirulent clone (Lamy *et al.*, 2006). Screening of a GBS ST-17 mutant library revealed a unique requirement for the novel 'invasion associated gene', *iagA*, in BBB penetration by GBS (Doran *et al.*, 2005). Decreased invasion of HBMECs by the GBS *ΔiagA* mutant *in vitro* was correlated with a reduced risk for development of meningitis and markedly diminished lethality *in vivo*. Deletion of *iagA* did not affect other key steps in the pathogenesis of GBS meningitis, including bloodstream survival, HBMEC adherence and intracellular survival. Thus, the *iagA*-encoded phenotype of GBS has a specific function in promoting HBMEC uptake of the pathogen. The *iagA* gene encodes an enzyme for the biosynthesis of diglycosyldiacylglycerol, a membrane glycolipid that functions as an anchor for lipoteichoic acid (LTA), indicating that proper LTA anchoring is important to facilitate penetration of the BBB by GBS (Doran *et al.*, 2005). The host cell receptor for GBS LTA that mediates these interactions has yet to be identified. While it is known that LTA is a molecule recognized by Toll-like receptor

(TLR) 2, the data strongly suggest that the attenuated phenotype of the *ΔiagA* mutant is not dependent on TLR2 (Doran *et al.*, 2005). The evidence that the LTA surface polymer mediates unique host cell interactions is consistent with early epidemiological studies. Clinical isolates of GBS from infants with EoD or LoD possess higher quantities of cell-associated LTA than strains isolated from mucosal surfaces of asymptotically colonized infants (Nealon and Mattingly, 1983). Furthermore, longer LTA polymer length is characteristic of isolates from carriers with invasive GBS disease compared with asymptomatic carriers. LTA is common to all GBS sero- and sequence types, but it remains to be determined if ST-17 clones contain longer or structurally distinct LTA polymers, which may account for their increased virulence.

More recently, the availability of GBS genome sequences has enabled the identification of genes restricted to the ST-17 lineage (Tettelin *et al.*, 2005; Brochet *et al.*, 2006). Mosaic variants were identified at a single genomic locus encoding a cell wall-anchored protein, with two main variants displaying 38% overall amino acid identity, namely BibA (Santi *et al.*, 2007), and a second gene to be strictly specific to the ST-17 clone (Lamy *et al.*, 2006). This gene, now called hypervirulent GBS adhesin (HvgA), was shown to be required for GBS hypervirulence (Tazi *et al.*, 2010). GBS strains that express HvgA are more efficient in gut colonization and in crossing the intestinal-blood barrier and BBB in neonates, including choroid plexus epithelial cells and brain microvascular endothelium (Tazi *et al.*, 2010). Furthermore, heterologous expression of HvgA in non-adhesive bacteria conferred the ability to adhere to intestinal barrier and BBB-constituting cells.

Serotypes Ia, Ib and V are also commonly isolated from neonates, children and adult patients with meningitis (Phares *et al.*, 2008), suggesting that other GBS determinants prevalent among these serotypes are also relevant for the pathogenesis of meningitis. Proteins targeted for cell surface expression in GBS are predicted to share a C-terminal

sequence (L/IPXTG) for sortase recognition and anchoring to the Gram-positive cell wall. Several cell wall-anchored proteins promoting GBS BBB penetration have been identified and characterized. In a paradigm-shifting study, it was discovered that GBS express surface-associated pili (Lauer *et al.*, 2005). Among the sequenced GBS genomes, two genetic loci encoding pili have been identified, pilus island (PI)-1 and PI-2, the second existing in one of two variants (PI-2a and PI-2b), and not all genomes contain both loci (Rosini *et al.*, 2006). GBS PI-2a includes the genes encoding PilB, an LP(x)TG-motif-containing protein that polymerizes to form a pilus backbone, and accessory pilus proteins PilA and PilC that are incorporated in the pilus (Dramsai *et al.*, 2006). Both PilA and PilB promote adherence to and invasion of brain endothelium, respectively (Maisey *et al.*, 2007), and PilA has been implicated in BBB penetration *in vivo* using a mouse model of haematogenous GBS meningitis (Banerjee *et al.*, 2011). Analysis of the PilA protein sequence revealed an integrin I-like domain resembling the A3 domain of human von Willebrand factor, a molecule known to interact with collagens. PilA also binds the extracellular matrix (ECM) component collagen, and collagen binding enhanced GBS attachment as well as uptake into HBMECs in a dose-dependent manner (Banerjee *et al.*, 2011). The PilA-collagen complex engages $\alpha 2$ - $\beta 1$ integrins on brain endothelium to promote bacterial attachment and pro-inflammatory chemokine release. As a result, increased neutrophil infiltration was correlated with increased BBB permeability and higher levels of bacterial CNS penetration *in vivo*. This study reveals the deleterious role of the neutrophil response to the development of GBS meningitis, and indicates that the GBS PilA-BBB interaction is an important molecular event that contributes to disease progression and a detrimental outcome for the host. In addition to PilA binding collagen, other GBS factors interact with various ECM proteins and constituents to promote bacterial-BBB interactions. Recently, the GBS surface-anchored alpha C protein (APC) was shown to interact directly with glucosaminoglycans (GAGs) on brain endothelium,

and promote the establishment of GBS meningitis (Chang *et al.*, 2011). Impaired host GAG expression diminished GBS penetration in the CNS in both murine and *Drosophila* models of GBS infection. GBS interactions with other ECM components also have been described. GBS mutants lacking the cell wall-anchored fibrinogen-binding protein FbsA (Schubert *et al.*, 2004), and the laminin-binding protein Lmb (Spellerberg *et al.*, 1999), have reduced ability to adhere to or invade HBMECs *in vitro* (Tenenbaum *et al.*, 2005, 2007). Many GBS strains harbour another fibrinogen-binding protein, FbsB (Gutekunst *et al.*, 2004), which is secreted and structurally unrelated to FbsA. Interestingly, the expression level of FbsA and FbsB in ST-17 strains correlated to an increased fibrinogen-binding capacity that may contribute to the hypervirulence of this lineage (Al Safadi *et al.*, 2011). The GBS genome encodes homologues to fibronectin-binding proteins that contribute to adherence, invasion and meningeal inflammation in other streptococcal pathogens (Pracht *et al.*, 2005). Whether or not the proteins function in a similar way in GBS remains to be determined.

Fibrinogen is present in the CNS following BBB disruption and vascular damage. Furthermore, the interaction of fibrinogen with integrins and non-integrin receptors expressed on cells of the haematopoietic, immune and nervous systems can induce signalling pathways that regulate inflammation and neurodegenerative functions involved in CNS disease. Interestingly, recent studies suggest that adherence to fibrinogen may be a general property of GBS (Dramsai *et al.*, 2012; Seo *et al.*, 2012) to promote bloodstream survival and host cell interactions. An important determinant recently implicated in fibrinogen binding and BBB interaction are the GBS serine rich repeat (Srr) glycoproteins (van Sorge *et al.*, 2009; Seo *et al.*, 2012). Srr proteins have a highly conserved domain organization, including a long and specialized signal sequence, two extensive Srr regions that undergo glycosylation, and a typical LP(X)TG cell wall anchoring motif. GBS strains carry one of two *srr* gene alleles, designated *srr1* (Samen *et al.*, 2007) and *srr2* (Seifert *et al.*,

2006), which are similar in architecture but show only limited homology (<20% identity). Expression of the Srr-2 protein seems to be restricted to serotype III and ST-17 strains (Seifert *et al.*, 2006). Targeted mutagenesis of a GBS $\Delta srr1$ mutant resulted in a marked reduction in HBMEC adherence and invasion (van Sorge *et al.*, 2009). The *srr1* genes in GBS serotypes Ia, Ib and V, as well as *srr2* in the serotype III ST-17 clone, each contributed to HBMEC invasion *in vitro*, and Srr-1 promoted BBB penetration and the development of GBS meningitis in a mouse model of haematogenous meningitis (van Sorge *et al.*, 2009). Srr-1 contributes to GBS attachment to HBMECs via the direct interaction of its binding region (BR) with human fibrinogen (Seo *et al.*, 2012). Studies using recombinant Srr1-BR established a direct protein interaction with the amino acid sequence 283–410 of the fibrinogen A α chain. Structural predictions indicated that the conformation of Srr1-BR resembles that of other related bacterial proteins that bind to fibrinogen through a 'dock, lock and latch' (DLL) mechanism (Ponnuraj *et al.*, 2003). The DLL mechanism results when fibrinogen engages a binding cleft between two domains, N2 and N3. At the ligand 'dock', the flexible C-terminal extension of the N3 domain (the 'latch') changes conformation, so that it 'locks' the ligand in place, and forms a β -strand complex with the N2 domain. Deletion of the predicted latch domain of Srr1-BR abolished the interaction of Srr1-BR with fibrinogen. In addition, a mutant GBS strain lacking the Srr-1 latch domain exhibited reduced binding to HBMECs, and was significantly attenuated in an *in vivo* model of meningitis (Seo *et al.*, 2012). Further studies are required to determine if similar mechanisms for fibrinogen binding and disease progression occur in Srr-2-encoding strains.

Intercellular invasion (paracytosis)

The host integrins, ECM components and glycosaminoglycans involved in GBS–BBB interactions all preferentially localize to the basolateral surface of polarized endothelium. Thus, GBS may interact with these factors

once present in the CNS to amplify the host response and disease progression. Alternatively, early molecular interactions of GBS with the BBB and subsequent barrier disruption may alter cellular polarity. It has been demonstrated that GBS is capable of intercellular transit across an epithelial cell barrier, where the bacterium co-localized with junctional protein complexes (Soriani *et al.*, 2006). Recent data also indicate that GBS infection disrupts tight junctional complexes in brain endothelium (Kim *et al.*, 2012). An overall reduction in the distribution of the primary BBB tight junction protein, zona occludin (ZO)-1, was observed by immunofluorescence during GBS infection. Further evidence demonstrated a decrease in protein levels of ZO-1 and additional tight junction protein, occludin, following GBS infection compared with the uninfected control (Kim *et al.*, 2012). Whether these interactions act to disrupt tight junctional complexes in brain endothelium and result in a non-polarized distribution of proteins on the BBB plasma membrane, and/or promote GBS intercellular transit across the BBB, remains to be investigated.

Host factors involved in arachidonic acid metabolism also contribute to penetration of the BBB by GBS (Maruvada *et al.*, 2011). Pharmacological inhibition and gene deletion demonstrated that host cytosolic phospholipase A $_2\alpha$ (cPLA $_2\alpha$) contributes to type III GBS invasion of HBMEC monolayers and penetration into the brain *in vivo*. The mechanism probably involves lipoxxygenated metabolites of arachidonic acid, specifically cysteinyl leukotrienes released by cPLA $_2\alpha$ as well as protein kinase C α (PKC α). GBS penetration into the CNS in cPLA $_2\alpha^{-/-}$ mice was significantly lower than the penetration of wild-type mice. However, the magnitudes of bacteraemia were similar between cPLA $_2\alpha^{-/-}$ and wild-type mice, suggesting that decreased penetration was not the result of decreased levels of blood-borne bacteria. Interestingly, cPLA $_2\alpha$ deletion did not affect GBS penetration into non-brain organs, such as the kidneys and spleen, as similar numbers of bacterial counts were recovered from cPLA $_2\alpha^{-/-}$ and wild-type mice (Maruvada

et al., 2011). The basis for this selective role of host cPLA₂ α in GBS neurotropism is unknown.

GBS disruption of the BBB

The host inflammatory response to GBS contributes significantly to the pathogenesis of meningitis and CNS injury. A vascular distribution of cortical lesions in neonatal rats with GBS meningitis indicates that disturbances of cerebral blood flow contribute to neuronal damage (Kim *et al.*, 1995). Inflammation of individual brain vessels can lead to focal lesions, whereas diffuse alterations of cerebral blood flow cause generalized hypoxic/ischaemic injury and cerebral oedema (Kim *et al.*, 1995). GBS induces nitric oxide (NO) in brain endothelial cells (Glibetic *et al.*, 2001) and in microglial cells, resulting in neuronal destruction (Lehnardt *et al.*, 2006). Furthermore, arteriolar dysfunction is associated with the presence of oxygen free radicals thought to be a by-product of infiltrating neutrophils (McKnight *et al.*, 1992). Intraventricular inoculation of newborn piglets with GBS results in an early sharp rise in CSF tumour necrosis factor- α (TNF- α) levels, followed shortly by prostaglandin release and subarachnoid inflammation (Ling *et al.*, 1995). In the neonatal rat model of meningitis, TNF- α production by astrocytes, microglia and infiltrating leucocytes contributes to apoptosis of hippocampal neurons (Bogdan *et al.*, 1997) and further increases in BBB permeability (Kim *et al.*, 1997). Recent studies have verified the levels of cytokine/chemokine, myeloperoxidase (MPO) activity, oxidative stress and disruption of the BBB in the hippocampus and cortex of neonate Wistar rats, following GBS meningitis (Barichello *et al.*, 2011). In the neonate brain, the hippocampus, mainly, produced higher levels of cytokine/chemokine in the early phase of infection, while MPO activity remained elevated at 4 days post-infection in both brain structures (Barichello *et al.*, 2011). Interestingly, in the neonatal rat, simultaneous intracisternal administration of dexamethasone with GBS challenge markedly reduced the magnitude of subarachnoid inflammation, vasculopathy and neuronal injury (Kim *et al.*, 1995).

It is clear that the GBS β -haemolysin/cytolysin (β -h/c) toxin contributes much to the observed disease pathology. Haemolysin expression has been shown to directly damage brain endothelial cells (Nizet *et al.*, 1997), leptomeninges (meningioma cells) and astrocytes (Alkuwaity *et al.*, 2012) and primary neurons (Reiss *et al.*, 2011). Infection with wild-type GBS and β -h/c⁺ cell-free extracts induced cell death, whereas challenge with β -h/c-deficient (β -h/c⁻) mutant strains and β -h/c⁻ extracts did not. Notably, astrocytes were more sensitive to the cytotoxic effects of infection than meningioma cells (Alkuwaity *et al.*, 2012). In neurons, cell-free extracts of GBS β -h/c toxin induced apoptosis in a time- and concentration-dependent fashion; electron microscopy of the neurons showed condensation, shrinkage and partial fragmentation of cells and nuclei as well as damage to mitochondria (Reiss *et al.*, 2011). In these studies, GBS β -h/c-induced cell death could not be prevented by caspase inhibitors, nor was caspase activity detected in neurons, consistent with observations in other cell types including macrophages. Haemolysin expression has also been shown to promote the development of meningitis *in vivo* (Doran *et al.*, 2003; Lembo *et al.*, 2010). In a murine model of haematogenous meningitis, mice infected with β -h/c⁻ mutants exhibited lower mortality and decreased brain bacterial counts compared with mice infected with the corresponding wild-type GBS strains (Doran *et al.*, 2003). Similarly, mutants that lack the negative repressor of β -h/c, CovR (for control of virulence), exhibited high levels of toxin expression and an increased ability to penetrate the BBB *in vivo* (Lembo *et al.*, 2010). Multiple studies have demonstrated that the lipid dipalmitoylphosphatidylcholine (DPPC) provides protection against β -h/c-mediated injury in various host cells (Nizet *et al.*, 1996; Doran *et al.*, 2002b; Hensler *et al.*, 2008; Alkuwaity *et al.*, 2012). DPPC might preserve the host cell membrane by providing phospholipid replacement during pore formation and/or by direct neutralization by binding to toxin itself. The therapeutic potential of surfactant phospholipids in GBS meningitis requires further study.

9.3 GBS Activation of a CNS Inflammatory Response

The first comprehensive microarray analysis of the BBB endothelium transcriptional response to a pathogen was examined during GBS infection, revealing the induction of a specific set of 80 genes, which function together to orchestrate neutrophil recruitment, activation and enhanced survival (Doran *et al.*, 2003). The most highly induced genes, interleukin (IL)-8, CXCL1 and CXCL2, all belong to the CXC chemokine family, which acts mainly on cells of neutrophil lineage. IL-8 is the most potent chemotactic factor for neutrophils because it has a high affinity for both of the chemokine receptors (CXCR1 and CXCR2) expressed on neutrophils, and it further stimulates neutrophil respiratory burst, degranulation and adherence to endothelial cells. These chemokines have been isolated from the CSF of patients with bacterial meningitis, and IL-8 may be an important biomarker to differentiate acute bacterial meningitis from aseptic meningitis (Pinto Junior *et al.*, 2011). Other GBS-induced HBMEC genes related specifically to CNS neutrophil recruitment were ICAM-1, which when upregulated leads to the enhanced adhesion of neutrophils to the brain endothelium, and granulocyte-macrophage colony-stimulating factor (GM-CSF), which increases neutrophil migration across brain endothelium. Absent during GBS infection of HBMECs was the induction of strong pro-inflammatory cytokines, such as TNF- α or IL-1. These data suggest that the BBB represents much more than a physical barrier to GBS, and also performs a sentinel function by recognizing the threat of infection and initiating a CNS-protective innate immune response. In the case of blood-borne bacteria, a specific BMEC gene expression programme for neutrophil recruitment and activation is generated, with the absence of the concurrent production of broader spectrum cytokines (e.g. TNF- α , IL-1) that could provoke a wider or unchecked pattern of inflammatory activation potentially harmful to critical CNS structures. However, the timing and magnitude of the neutrophil recruitment response is critical for the outcome of infection. Continued exposure and invasion of the

pathogen may result in over-activation of BBB endothelium, leading to increased inflammation that may compromise BBB integrity or cause neuronal damage.

Several GBS factors have been implicated in promoting BBB activation. Infection of HBMECs with a GBS strain lacking β -h/c toxin markedly reduced expression of genes involved in the immune response, while an unencapsulated strain generally induced similar or greater expression levels for the same subset of genes (Doran *et al.*, 2003). Neutrophil migration across polar HBMEC monolayers was stimulated by GBS and its β -h/c through a process involving IL-8 and ICAM-1. Furthermore, cell-free bacterial supernatants containing β -h/c activity induced IL-8 release, thus identifying this toxin as a principal provocative factor for BBB activation (Doran *et al.*, 2003). In more recent studies, additional microarray experiments have demonstrated that the similar gene profile in HBMECs is effected by CovR regulation, which can result in high β -h/c expression (Lembo *et al.*, 2010) and PilA expression (Banerjee *et al.*, 2011). Infection of HBMECs *in vitro* with multiple PilA-deficient GBS strains resulted in less IL-8 protein secretion compared with the respective wild-type parental strains, and treatment of HBMECs with recombinant PilA protein induced IL-8 transcription, suggesting that PilA is both necessary and sufficient to activate the BBB response (Banerjee *et al.*, 2011). Infection *in vivo* with the PilA-deficient strain resulted in delayed mortality, decreased neutrophil infiltration and bacterial CNS dissemination, and less expression of KC, the murine homologue of IL-8 (Banerjee *et al.*, 2011). These results indicate that GBS PilA directly promotes IL-8 secretion and functional neutrophil signalling pathways *in vivo*, resulting in neutrophil recruitment during active GBS infection, which may function in tandem or concurrently with the β -h/c toxin to promote disease progression. These findings also demonstrate an association between leucocyte trafficking and BBB permeability and increased GBS penetration of the CNS, suggesting that polymorphonuclear leucocyte (PMN)-mediated damage of the BBB has a significant role in the pathogenesis of GBS meningitis.

9.4 Conclusions

Advances in microbial genetics, tissue culture systems and small-animal challenge models have enhanced our understanding of the molecular pathogenesis of GBS meningitis and the host response to this potentially life-threatening infection. New model systems using zebrafish (Patterson *et al.*, 2012) and *Drosophila* (Baron *et al.*, 2009; Chang *et al.*, 2011) promise the contribution of host genetics to enrich our understanding of host-GBS interactions. Comparative genomic and systems-level bioinformatics studies have revealed strain evolution associated with hypervirulence and CNS disease potential, including specific candidate gene and regulatory systems that promote bloodstream survival, HBMEC interactions and activation of host inflammatory responses. Genomics has led to the development of reverse and structural vaccinology technologies for vaccine discovery, including a type 2a pilus (BP-2a)-based GBS experimental vaccine (Nuccitelli *et al.*, 2011). In addition, genomics has led to the discovery of component proteins and virulence factors as potential vaccine targets. Enhanced understanding of the molecular basis of GBS meningitis may highlight novel bacterial and host molecules as therapeutic or immuno-prophylactic targets against this dangerous infectious disease condition of the neonate.

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