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MicroReview

Molecular pathogenesis of neonatal group B streptococcal infection: no longer in its infancy

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Summary

The process of human infection by group B *Streptococcus* (GBS) is complex and multifactorial. While this bacterium has adapted well to asymptomatic colonization of adult humans, it remains a potentially devastating pathogen to susceptible infants. Advances in molecular techniques and refinement of *in vitro* and *in vivo* model systems have elucidated key elements of the pathogenic process, from initial attachment to the maternal vaginal epithelium to penetration of the newborn blood–brain barrier. Sequencing of two complete GBS genomes has provided additional context for interpretation of experimental data and comparison to other well-studied pathogens. Here we review recent discoveries regarding GBS virulence mechanisms, many of which are revealed or magnified by the unique circumstances of the birthing process and the deficiencies of neonatal immune defence. Appreciation of the formidable array of GBS virulence factors underscores why this bacterium remains at the forefront of neonatal pathogens.

Introduction

Group B *Streptococcus* (GBS) is a Gram-positive encapsulated bacterium possessing an array of immune resistance phenotypes and secreted toxins that render it capable of producing serious disease in susceptible hosts, in particular the human neonate (Nizet *et al.*, 2000). The pathogenesis of neonatal GBS infection begins with the asymptomatic colonization of the female genital tract. Approximately 20–30% of healthy women are colonized rectovaginally with GBS, and 50–70% of infants born to these women will themselves become colonized with the bacterium (Baker and Edwards, 2001). Neonatal GBS infection comes in two forms: early onset and late onset. Early-onset infections are classified epidemiologically through 7 days of age, but have a median onset of only 6–8 h of life, with pneumonia and respiratory failure complicated by bloodstream infection and septicaemia. These 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 triggering premature labour. In contrast, late-onset GBS infection occurs in infants up to 7 months of age, with gradual symptoms related to bacteraemia, no lung involvement and a high incidence (≈ 50%) of meningitis (Baker and Edwards, 2001).

Several GBS virulence determinants contribute to neonatal disease at critical junctures of the infectious process (Fig. 1). Our review of the most recent discoveries in GBS pathogenesis will be grouped into thematic categories: adherence to epithelial surfaces, penetration of host cellular barriers, avoidance of immunologic clearance mechanisms and inflammatory activation. This discussion will highlight a new realization of the multifunctional nature of many key GBS virulence determinants.

Adherence to epithelial surfaces

Group B *Streptococcus* adhere to a variety of human cells including vaginal epithelium, placental membranes, respiratory tract epithelium and blood–brain barrier endothelium. Maximal adherence occurs at the acidic pH of vaginal mucosa (Tamura *et al.*, 1994), allowing GBS to occupy a niche that places infants at risk of vertical transmission. A low-affinity GBS interaction with epithelial cells is mediated by its amphiphilic cell wall-associated lipotechoic acid, while higher affinity interactions with host cells are mediated by a series of size-variable, pronase-sensitive, hydrophobic GBS surface proteins (Wibawan *et al.*, 1992). Recent investigations have revealed that these high-affinity protein-mediated interactions with epithelium generally proceed through an intermediary: GBS effectively binds the extracellular matrix components fibronectin,

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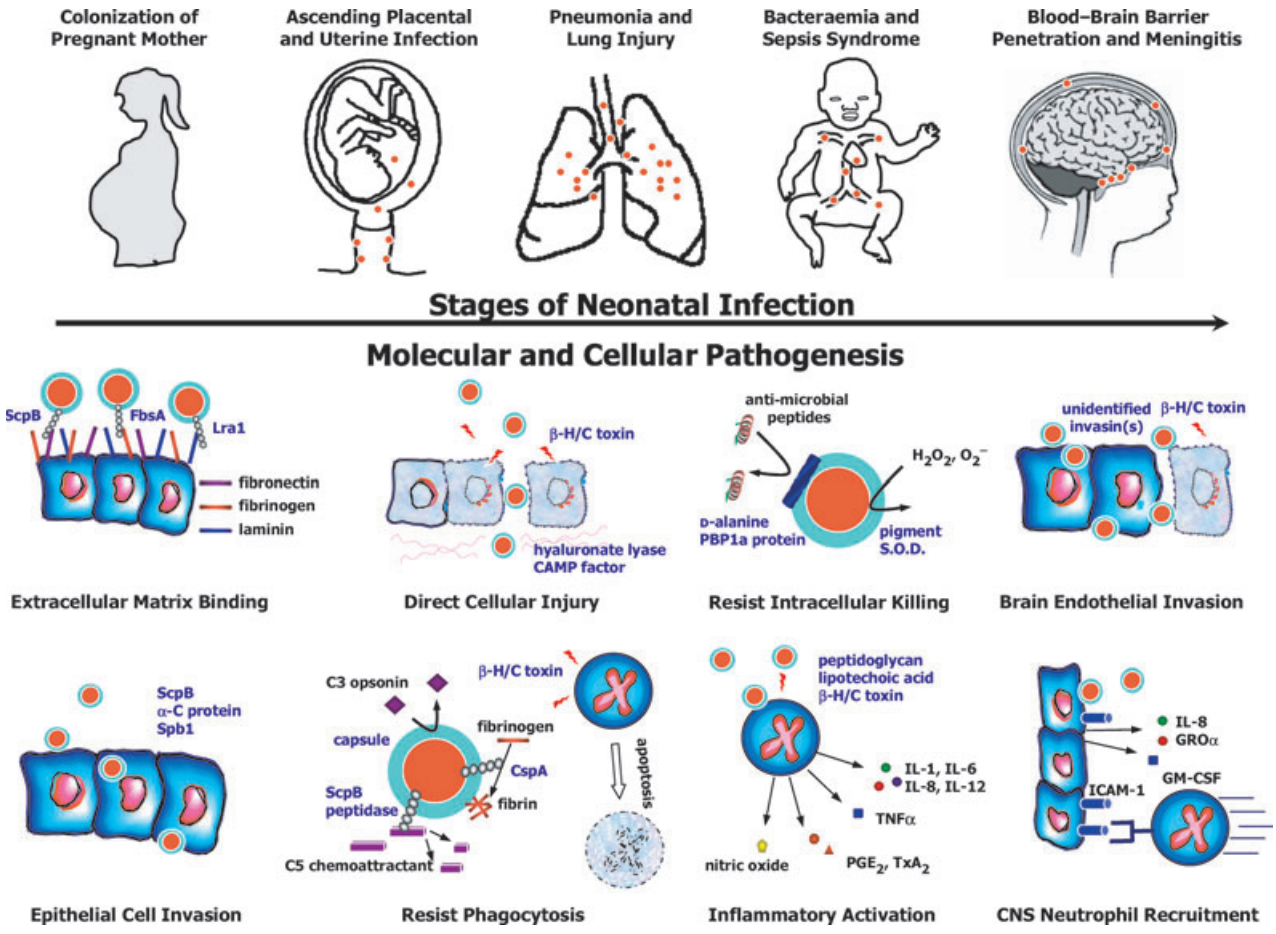

Fig. 1. Stages in the molecular and cellular pathogenesis of neonatal group B *Streptococcal* (GBS) infection. β-H/C, beta-haemolysin/cytolysin; S.O.D., superoxide dismutase; IL, interleukin; TNFα, tumour necrosis factor-alpha; PGE_2, prostaglandin E_2; TxA2, thromboxane A_2; GROα, growth-related oncogene-alpha; ICAM-1, intercellular adhesion molecule 1; GM-CSF, granulocyte-macrophage colony-stimulating factor.

fibrinogen and laminin. These proteins are known to interact with host cell-anchored proteins such as integrins and have been demonstrated to mediate adherence of related Gram-positive pathogens (Schwartz-Linek *et al*., 2004).

Unusually well adapted, GBS binds to immobilized fibronectin to facilitate mucosal colonization (Tamura and Rubens, 1995), but not to soluble fibronectin that may serve as an opsonin for phagocyte recognition (Butler *et al*., 1987). Recently, a genome-wide phage display technique was used to identify GBS genes that mediate the selective fibronectin adherence (Beckmann *et al*., 2002). An unexpected discovery in this screen was a fibronectin-binding property associated with the surface-anchored GBS C5a peptidase, ScpB. The dual functionality of ScpB was confirmed by decreased fibronectin binding of isogenic *scp*B mutants and the direct interaction of recombinant ScpB with solid-phase fibronectin (Beckmann *et al*., 2002; Cheng *et al*., 2002). Similar targeted mutagenesis studies demonstrate that adherence of GBS to laminin involves a homologue of the Lra1 adhesin family (Spellerberg *et al*., 1999), while attachment of GBS to fibrinogen is mediated by repetitive motifs within surface-anchored protein FbsA (Schubert *et al*., 2002). The transcriptional regulator RogB positively regulates the ability of GBS to bind fibrinogen and fibronectin by increasing expression of downstream genes with extracellular matrix binding motifs as well as *fbs*A (Gutekunst *et al*., 2003). GBS fibronectin binding is also dependent on cellular glutamine transport encoded by the *gln*PQ operon (Tamura *et al*., 2002). Finally, the surface protein Rib confers protective immunity and is expressed by most invasive GBS isolates (Stalhammar-Carlemalm *et al*., 1993); Rib is closely related to the R28 protein of group A *Streptococcus* (GAS) that promotes epithelial cell binding (Stalhammar-Carlemalm *et al*., 1999).

Penetration of host cellular barriers

While attachment mechanisms allow GBS to compete with other microflora for a niche on the gastrointestinal

and vaginal mucosa, the ability of the organism to penetrate host cellular barriers is a first distinguishing feature of its pathogenicity. In tissue culture, GBS are able to invade chorionic epithelial cells but not amniotic cells (Winram *et al*., 1998). Nevertheless, GBS can traverse placental membranes and weaken their tensile strength, a process that is speculated to involve local generation of oxygen radicals and prostaglandin E2 (Bennett *et al*., 1987). As a result of these processes, GBS may access the fetus within the amniotic cavity, induce placental membrane rupture or trigger premature delivery. After aspiration of infected amniotic or vaginal fluid, the newborn lung is the initial focus of GBS infection. From there, the organism rapidly gains access to the bloodstream and is circulated through other organs and tissues. GBS disruption of the lung barrier to infection appears to be a combination of three processes: intracellular invasion, direct cytolytic injury and damage induced by the inflammatory response of the newborn host.

Intracellular invasion of both alveolar epithelial and pulmonary endothelial cells by GBS was first noted in newborn macaques after intramniotic challenge (Rubens *et al*., 1991), and later confirmed in human tissue culture lines derived from both cellular barriers (Rubens *et al*., 1992; Gibson *et al*., 1993). GBS cellular invasion occurs when the organism triggers its own endocytotic uptake and enters the cell within a membrane-bound vacuole, a process that requires microfilament components of the host cytoskeleton and is now appreciated to involve host signalling pathways mediated by PI 3-kinase (Tyrrell *et al*., 2002).

Cellular invasion is correlated to GBS virulence potential, as clinical isolates from infants with bloodstream infections invade epithelial cells better than strains from the vaginal mucosa of asymptomatic women (Valentin-Weigand and Chhatwal, 1995). Genetic phenotyping of type III GBS strains identified a particular restriction digest pattern (RDP III-3) characteristic of the vast majority of isolates from invasive neonatal infection (Takahashi *et al*., 1998). Subsequent subtractive hybridization studies identified a gene unique to RDP III-3 strains encoding the surface-anchored protein, Spb1, required for maximal epithelial cell invasion (Adderson *et al*., 2003). Similarly, elimination of the genes encoding the fibronectin-binding C5a peptidase ScpB or the alpha C surface protein each significantly reduced GBS epithelial cell invasion (Bolduc *et al*., 2002; Cheng *et al*., 2002).

Early-onset GBS pneumonia is characterized by widespread damage to lung epithelium and endothelium, with haemorrhage, proteinaceous fluid and neutrophils entering the alveolar airspaces. The loss of barrier integrity allows GBS direct entry to the circulation, and appears to result largely from the actions of the GBS β-haemolysin/cytolysin (β-H/C). Mutagenesis and heterologous expression studies have identified a single open reading frame, *cyl*E, as necessary and sufficient for GBS β-H/C expression (Pritzlaff *et al*., 2001). This pore-forming toxin lyses lung epithelial and endothelial cells and compromises their barrier function (Nizet *et al*., 1996; Gibson *et al*., 1999). At subcytolytic doses, the GBS β-H/C promotes GBS intracellular invasion and triggers the release of interleukin-8 (IL-8), the principal chemoattractant for human neutrophils (Doran *et al*., 2002). The cytolytic, pro-invasive and proinflammatory effects of the GBS are all neutralized by dipalmotyl phosphatidylcholine (DPPC), the major phospholipid constituent of human lung surfactant (Doran *et al*., 2002; Nizet *et al*., 1996). This finding may in part explain the greatly elevated risk of premature, surfactant-deficient neonates to suffer severe GBS lung injury and invasive disease.

Because GBS is the leading cause of bacterial meningitis in human newborns, it is evident that a further propensity exists for the bacterium to breech the specialized endothelium comprising the human blood–brain barrier. GBS have been shown to invade and trancytose polar monolayers of human brain microvascular endothelial cells, with serotype III strains doing so most efficiently (Nizet *et al*., 1997). As seen with epithelial cell barriers, the GBS β-H/C is directly cytolytic for human brain endothelial cells and β-H/C knockout mutants show decreased blood–brain barrier penetration and lethality in a mouse model of haematogenous meningitis (Doran *et al*., 2003).

New structure function discoveries promise to shed new light on two other GBS virulence determinants suggested to play a role in penetration of host cellular barriers. Bloodstream isolates of GBS secrete high levels of an enzyme that degrades hyaluronic acid, the main polysaccharide component of host connective tissue (Kjems *et al*., 1980; Pritchard *et al*., 1994). Further analysis of the hyaluronate lyase will now be possible with solution of its crystal structure and the elucidation of the mechanism of its catalytic process (Mello *et al*., 2002). CAMP factor is a GBS extracellular protein that is toxic when injected intravenously in rabbits (Skalka and Smola, 1981). Recent electron microscopy and chemical cross-linking studies have shown that CAMP factor oligomerizes in the target membrane to form discrete pores and trigger cell lysis (Lang and Palmer, 2003).

Avoidance of immunologic clearance

Once GBS injures or penetrates cellular barriers to reach the bloodstream or deeper tissues, an immunologic response is called upon to clear the organism. Central to this response are host phagocytic cells including neutrophils and macrophages. Moreover, the effective uptake and killing of GBS by these cells requires opsonization of the bacterium by specific antibodies or serum comple-

ment. Neonates are particularly prone to GBS invasive disease because of quantitative or qualitative deficiencies in phagocytic cell function, specific anti-GBS immunoglobulin, or the classic and alternate complement pathways. In addition to these newborn host susceptibilities, GBS possess a number of virulence determinants that seek to thwart each of the key components of effective opsonophagocytic killing.

It is in the realm of immune evasion that the single best studied of all GBS virulence factors, its surface polysaccharide capsule, assumes it prominence. Almost all GBS associated with human disease are encapsulated, belonging to one of the nine recognized type-specific capsule serotypes: Ia, Ib and II through VIII. The serotype-specific epitopes of each polysaccharide are created by different arrangements of four component sugars (glucose, galactose, *N*-acetylglucosamine and sialic acid) into a unique repeating unit, but unfailingly these structures contain a terminal sialic acid (Neu5Ac) bound to galactose in an $\alpha2\rightarrow3$-linkage. GBS capsule biosynthesis is encoded in the single long transcript of a 16-gene operon now fully sequenced in type Ia, III and V strains. Recently, elegant experiments have shown that the heterologous expression of a single polymerase gene (*cps*H) from this operon can cause a type GBS Ia strain to express type III capsule epitopes, and vice versa (Chaffin *et al*., 2000).

The GBS terminal $\alpha2\rightarrow3$ Neu5Ac capsular component is identical to a sugar epitope widely displayed on the surface of all mammalian cells (Angata and Varki, 2002). Furthermore, the terminal $\alpha2\rightarrow3$-linked Neu5Ac is overexpressed in humans which in evolution have lost the genes to produce the alternative sialic acid Neu5Gc. One can hypothesize that GBS is a particularly vexsome human pathogen because its sialylated surface capsule has undergone selection to resemble host 'self' and avoid immune recognition. In fact, compared with wild-type strains, isogenic capsule-deficient mutants of GBS elicit greater degrees of proinflammatory cytokine release from human brain endothelial cells (Doran *et al*., 2003) and produce greater brain inflammation when injected intracisternally in newborn piglets (Ling *et al*., 1995). These observations probably reflect a combination of (i) decreased immune recognition as a result of GBS capsule molecular mimicry of host epitopes and (ii) increased access of host pattern recognition molecules (e.g. Toll-like receptors) to the cell wall components lipotechoic acid and peptidoglycan hidden beneath the physical 'cloak' of the polysaccharide capsule.

The sialylated GBS surface capsule protects GBS by interference with opsonophagocytosis. Deposition of complement C3 on the bacterial surface, with subsequent cleavage and degradation to opsonically active fragment C3b, is pivotal to host defence against invasive bacterial infection. Isogenic GBS mutants lacking capsule or simply the terminal $\alpha2\rightarrow3$ sialic acid bind far greater amounts of C3b, are more susceptible to killing by human neutrophils and exhibit 100-fold greater LD50 values than wild-type in the neonatal rat model (Wessels *et al*., 1989; Marques *et al*., 1992). A new discovery finds complement binding to be critical for the humoral immune response to GBS capsular polysaccharide. C3–/– mice failed to uptake GBS capsular polysaccharide into marginal zone B cells or dendritic cells and consequently produced low levels of specific anti-capsular IgM and IgG antibodies (Pozdnyakova *et al*., 2003).

Other multifunctional GBS determinants have recently been identified to contribute to the organism's ability to circumvent host clearance mechanisms. GBS mutants lacking the fibrinogen-binding protein FbsA are cleared rapidly in human blood (Schubert *et al*., 2002). The beta component of the surface C protein binds IgA in a non-immune fashion through its Fc domain, potentially sequestering this important host mucosal defence molecule (Jerlstrom *et al*., 1996). GBS strains expressing the alpha C protein component appear more resistant to phagocytic killing than strains lacking this surface epitope (Madoff *et al*., 1991), and deletion of tandem repeats within the alpha C sequence generates antigenic variability that allows the bacterium to avoid specific antibody-based opsonophagocytic clearance (Madoff *et al*., 1996). The fibronectin-binding ScpB possesses a peptidase domain that specifically cleaves human complement component C5a, ablating its neutrophil chemoattractant property. GBS expression of ScpB reduces the acute neutrophil response to sites of infection in C5a knockout mice reconstituted with human C5a (Bohnsack *et al*., 1997). A related GBS cell surface protease named CspA targets host fibrinogen, producing adherent fibrin-like cleavage products that coat the bacterial surface and interfere with opsonophagocytic clearance (Harris *et al*., 2003).

The application of signature-tagged mutagenesis for *in vivo* screening in a GBS neonatal rat sepsis model has helped identify unexpected virulence genes encoding factors that help the bacterium survive immune clearance (Jones *et al*., 2000). For example, *pon*A, which codes for an extracytoplasmic penicillin-binding protein (PBP1a), promotes resistance to phagocytic killing independent of capsule (Jones *et al*., 2003). GBS mutants with deletion of PBP1a are less virulent after both lung and systemic challenge, which is correlated to an increased susceptibility to cationic anti-microbial peptides (defensins, cathelicidins) produced by host epithelial cells and phagocytes (Hamilton *et al*., 2004). Another way GBS avoids antimicrobial peptide clearance is through the D-alanylation of lipotechoic acid in the bacterial cell wall. This process is carried out by gene products of the *dlt* operon. A GBS *dlt*A mutant exhibited decreased negative surface charge

that impeded cationic host defence peptides from reaching their cell membrane target of action (Poyart *et al*., 2003).

Sometimes the best defence is a good offence, and it appears that in certain circumstances GBS can kill a host phagocyte before being killed themselves. The GBS β-H/C is directly cytolytic to macrophages and neutrophils (Liu *et al*., 2004). As a consequence, β-H/C-negative mutants are more rapidly killed by these cells during *in vitro* phagocytic assays and more quickly cleared from the bloodstream in animal infection models. GBS can trigger macrophage apoptosis through β-H/C-mediated pore formation and calcium influx or by β-H/C independent processes that may include modification of phosphatidylserine metabolism (Buratta *et al*., 2002; Ulett *et al*., 2003).

Curiously, while streptococci are commonly thought of as 'extracellular pathogens', GBS has been shown to survive for prolonged periods within the phagolysosome of macrophages and to be >10-fold more resistant to killing by hydrogen peroxide killing than catalase-positive *Staphylococcus aureus* (Wilson and Weaver, 1985). One GBS defence against oxidative stress is the enzyme superoxide dismutase (SodA), as evidenced by the fact that a *sod*A mutant is highly susceptible to macrophage killing and survives poorly *in vivo* (Poyart *et al*., 2001). Another factor allowing GBS to survive inside phagocytes is the production of an orange carotenoid pigment, a property unique among haemolytic streptococci and genetically linked to the *cyl* operon encoding the GBS β-H/C. The free radical scavenging properties of the carotenoid neutralize hydrogen peroxide and singlet oxygen, therefore providing a shield against the key elements of phagocyte oxidative burst killing (Liu *et al*., 2004).

Activation of inflammatory responses

Resisting phagocytic clearance in the bloodstream, GBS may disseminate to reach end organs such as bones, joints and, most ominously, the central nervous system (CNS). The host inflammatory response to invasive infection mounts, and development of the sepsis syndrome and multiorgan dysfunction often ensues. Peptidoglycan and other GBS components associated with the cell wall, not including the surface polysaccharide capsule, appear to be the most provocative agents in triggering host cytokine cascades, in particular the proximal mediators tumour necrosis factor-alpha (TNFα) and interleukin-1 (IL-1). GBS induction of NF-κB signalling and TNFα release from human monocytes *in vitro* requires CD14 and the receptors for complement components 3 and 4 (Medvedev *et al*., 1998). Recently, the importance of complement components in amplifying GBS TNFα induction was corroborated when reduced levels of the cytokine were observed in the blood of C3 or C3 receptor-deficient mice stimulated with GBS (Levy *et al*., 2003). Knockout mouse studies indicate GBS cell wall peptidoglycan-induced activation of p38 and NF-κB depends on the cytoplasmic TLR adaptor protein MyD88, but does not proceed via the well-studied TLR2 and/or TLR4 (Henneke *et al*., 2002). However, an additional, as-yet-undiscovered secreted GBS factor appears to activate phagocytes via TLR2 and TLR6 (Henneke *et al*., 2001). GBS β-H/C activity is correlated directly to systemic hypotension and liver necrosis in a rabbit model of septicaemia (Ring *et al*., 2002). Recently it was discovered that GBS β-H/C and cell wall components act synergistically to induce macrophage production of inducible nitric oxide synthase (iNOS) and generation of nitric oxide (NO) (Ring *et al*., 2000), a potent factor in the sepsis cascade.

One location in which an overexuberant host inflammatory response to GBS may be particularly unwelcome is the confines of the brain and CNS. In the infant rat model, early GBS meningitis is characterized by acute neutrophilic inflammation in the subarachnoid space and ventricles, vasculopathy and neuronal injury. The initiation of the CNS inflammatory response is triggered by the blood–brain barrier endothelium, which activates a specific pattern of gene transcription for neutrophil recruitment, including production of chemokines (e.g. IL-8, Groα), endothelial receptors (ICAM-1) and neutrophil activators (GM-CSF) (Doran *et al*., 2003). The principal provocative factor for the blood–brain barrier inflammatory gene response is the GBS β-H/C, and this toxin has also been shown to contribute to the development of meningitis (Doran *et al*., 2003) and neuronal apopotosis (J. Weber, pers. comm.) *in vivo*.

Opening the post-genomic era of GBS research

The year 2002 witnessed the long anticipated publication of two complete GBS genomes, one from serotype III, strain NEM316 by the Institut Pasteur (Glaser *et al*., 2002) and a second from serotype V, strain 2603 V/R by The Institute for Genomic Research (Tettelin *et al*., 2002). These ≈ 2.2 megabase sequences each contain >2100 predicted protein-encoding open reading frames. Revealed in these analyses are several new candidate GBS pathogenicity factors identified (i) as orthologues to known virulence determinants in the other more extensively studied streptococcal pathogens, e.g. GAS and *Streptococcus pneumoniae* (SPN), and/or (ii) by their possession of the classic C-terminal LP(X)TG sorting signal of Gram-positive cell wall-anchored proteins. Examples would include predicted GBS surface proteins with significant homology to the fibronectin-binding protein PFPB of GAS or the adhesin PspC of SPN. Other interesting targets for future analyses of GBS regulation of virulence

include over 100 candidate transcriptional regulators, including at least 17 two-component systems (sensor histidine kinase + response regulator) and paralogues of the Rgg, RofA and Nra global transcriptional regulators of GAS (Glaser *et al*., 2002). Overall, slightly over half of the genes in the GBS genomes could be considered to share orthologues with either GAS or SPN.

Some interesting observations have arisen from initial analysis of the completed GBS genome sequences. Comparative hybridization experiments using whole genome microarrays were performed between the sequence serotype V strain and 19 other GBS strains of varying serotypes, revealing a surprising amount of genetic heterogeneity even among strains of the same serotype (Tettelin *et al*., 2002). Many of the predicted virulence genes present in GBS but not in GAS or SPN were found associated with mobile genetic elements such as bacteriophages or transposon insertion sequences, suggesting potential horizontal acquisition of virulence factors by GBS from other bacterial species (Tettelin *et al*., 2002). Indeed, most of the known and putative GBS virulence genes are found clustered within 14 chromosomal 'islands' of unique GBS sequence. How might GBS have acquired so many novel genetic islands? In addition to phage transduction and conjugation, orthologues of several genes necessary for competence in SPN have been identified in the GBS genome (Glaser *et al*., 2002). Although GBS has not been considered to be naturally transformable, these discoveries should stimulate further investigation of the potential for GBS competence induction or fuel speculation of its existence in an evolutionary ancestor of the species.

Summary and perspectives

Group B *Streptococcus* remains a formidable human pathogen that strikes at our most vulnerable target, the newborn infant. And just as expansion of efforts to identify GBS carriage among pregnant women and offer intrapar-

Table 1. Key virulence factors of group B *Streptococcus*.

Virulence factor	Genetic basis	Chemical nature	Molecular or cellular action(s)	Proposed contribution(s) to disease pathogenesis
Exopolysaccharide surface capsule	*cps*A-L, *neu*A-D	High-molecular-weight polymer with terminal sialic acid residues	Impairs complement C3 deposition and activation Decreases immune recognition, perhaps through molecular mimicry of host sialic acid epitopes	Blocks opsonophagocytic clearance Delays neutrophil recruitment
β-Haemolysin/cytolysin	*cyl*E	CylE protein (79 kD)	Forms pores in cell membranes Induces apoptosis Promotes cellular invasion Triggers iNOS, cytokine release	Direct tissue injury Penetration of epithelial barriers Induction of sepsis syndrome Phagocytic resistance
+ linked pigment	*cyl* locus	Carotenoid	Antioxidant effect blocks H_2O_2, singlet oxygen	Impairment of oxidative burst killing
Hyaluronate lyase	*hyl*B	HylB enzyme (110 kD)	Cleaves hyaluronan and chondroitin sulphate	Spread through host tissues Impairment of leukocyte trafficking
C5a peptidase	*scp*B	ScpB protein (120 kD)	Cleaves human C5a Binds fibronectin	Inhibit PMN recruitments Extracelluar matrix attachment Epithelial adherence and invasion
CAMP factor	*cf*b	CAMP protein (24 kD)	CAMP reaction (co-haemolysin) Binds to Fc portion of IgG, IgM	Direct tissue injury Impairment of antibody function
Lipotechoic acid	Complex	Amphiphilic glycerol phosphate polymer of complex lipids and short-chain fatty acids	Binds host cell surfaces Binds host pattern recognition receptors (TLRs) Alanylation inhibits host anti-microbial peptides	Epithelial cell attachment Activation of the sepsis syndrome Resistance to neutrophil killing
C protein (alpha and beta components)	*bca* (alpha) *cba* (beta)	Alpha: protein with mutiple identical tandem repeats (14–145 kD); beta: 84–94 kD variants	Binds cervical epithelial cells Blocks intracellular killing by neutrophils non-immune binding of IgA	Epithelial cell adherence Epithelial cell invasion Resistance to phagocytic clearance
Serine protease	*csp*A	CspA protein (142 kD)	Cleaves fibrinogen to fibrin-like fragments	Resistance to phagocytic clearance? Promotes tissue spread
Fibrinogen receptor	*fbs*A	FbsA protein (44.2 kD)	Binds fibrinogen through repetitive structure motifs	Extracellular matrix attachment Epithelial adherence Resistance to opsonophagocytic killing

tum antibiotic prophylaxis has reduced the attack rate of early-onset neonatal infection, GBS has emerged as equally important pathogen in other patient populations such as the elderly, pregnant women, diabetics and the immunocompromised. As this review has highlighted, the unique pathogenesis of GBS infection is the by-product of a diverse array of colonization and survival factors. The recent availability of well-characterized isogenic mutants lacking individual virulence determinants has allowed precise hypothesis testing *in vitro* and *in vivo*, often demonstrating a variety of virulence roles for a single GBS factor. For example, the GBS β-H/C toxin may contribute to disease pathogenesis through direct tissue injury, promotion of intracellular invasion, triggering of apoptosis, resistance to phagocytosis and activation of host inflammatory pathways.

The multifunctional nature of several GBS virulence factors (summarized in Table 1) poses a particular challenge to the underdeveloped defences of the newborn. Increased knowledge of the molecular basis of GBS pathogenesis will help clarified the ontogeny of effective innate immunity in the early stages of human life, and provide novel targets for chemotherapy or immunoprophylaxis of GBS infections. Efficacious vaccines based on protein-conjugated GBS capsular polysaccharides are poised for phase III clinical trials, and recent studies have highlighted the potential for conserved GBS surface proteins (e.g. ScpB, Sip, BSP) to serve as serotype-independent vaccine. The next generation of GBS research, accelerated by the power of genomics, promises to enhance the status of this complex pathogen as a model organism for molecular microbiology and immunology investigations.

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