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The Adhesion and Invasion Mechanisms of Streptococci

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Abstract

Streptococci are common human pathogens, colonizing multiple parts of the human body such as the upper respiratory tract, urethra, gastrointestinal tract, and oral cavity. Since they cause a variety of serious infections including heart diseases, meningitis, and oral diseases, streptococci are considered to play an important role in human diseases. Two critical steps in the pathogenesis of streptococcal infection are the adhesion to and invasion of host cells. This invasion is a strategy of streptococci to evade the host immune response and antibiotic therapy, as well as to penetrate to deeper tissues. To establish interaction between bacteria and host cells, adhesion is the initial step. To effectively adhere to host cells, streptococci express multiple adhesins, and the expression of different adhesins may lead to distinct mechanisms of subsequent invasion. The binding of streptococcal molecules to host proteins triggers downstream signal transduction in the host cells, leading to the uptake of bacteria. In this review, we present the adhesion and invasion mechanisms of different streptococci and the interaction with host cells leading to internalization.

Introduction

The *Streptococcus* genus is made up of common gram-positive bacteria, and includes two major pathogenic species, *Streptococcus pyogenes* (also known as group A streptococcus, GAS) and *Streptococcus pneumoniae*, and other invasive pathogens such as *Streptococcus agalactiae* (group B Streptococcus, GBS), *Streptococcus suis,* and *Streptococcus mitis*. GAS was reported to cause more than 500,000 deaths worldwide anually (Carapetis et al. 2005). GAS-induced disease involves the invasion of different types of eukaryotic cells via distinct mechanisms. M protein and streptococcal fibronectin binding protein I (SfbI) are two main virulence factors that are critical to invasion (Cunningham 2000; Molinari et al. 2000; Cywes and Wessels 2001; Rohde et al. 2003; Amelung et al. 2011; Siemens et al. 2011). Streptococci use these virulence factors to evade antibiotic treatment and host immune responses such as phagocytotic clearance (Kwinn and Nizet 2007; Nitsche-Schmitz et al. 2007; Nizet 2007; Smeesters et al. 2010; Barnett et al. 2013), and can cause a wide spectrum of human infections including superficial skin infections and life-threatening diseases (Cunningham 2000; Olsen and Musser 2010; Liang et al. 2012). Additionally, GAS infection can lead to post-infection sequelae, such as post-streptococcal glomerulonephritis, rheumatic fever, and rheumatic heart disease (Luca-Harari et al. 2009; Jackson et al. 2011; Steer et al. 2012; Walker et al. 2014). The human upper respiratory tract can be asymptomatically colonized by the Gram-positive and opportunistic pathogen *S. pneumoniae*. Although colonization by *S. pneumoniae* can occur at any stage in life, infants and elderly are the most vulnerable groups (van der Poll and Opal 2009; Shak et al. 2013; Valles et al. 2016). The morbidity from *S. pneumoniae* infections remains high worldwide, in spite of the available treatments

(Alonsodevelasco et al. 1995; van der Poll and Opal 2009). Many virulence factors contribute to its pathogenicity, including: (i) toxins, such as pneumolysin; (ii) adhesins, such as pneumococcal surface protein A (PspA), choline-binding protein A (CbpA; also known as PspC), and pneumococcal adherence and virulence protein A (PavA); (iii) extracellular enzymes, such as surface neuraminidase (Nan) A, NanB, NanC, and pneumococcal phospholipase A2 (PLA2); (iv) the pneumococcal capsule; and (v) pili. (Werdan et al. 2014; Iovino et al. 2016; Weiser et al. 2018; Yau et al. 2018). Using these virulence factors, *S. pneumoniae* is able to colonize host cells and translocate to deeper tissues, causing human diseases ranging from meningitis and sepsis to otitis media and pneumonia, or even invading the heart through the coronary circulation (Tonnaer et al. 2006; Brown et al. 2014; Wunderink and Waterer 2014; Gilley et al. 2016; Valles et al. 2016). Another Gram-positive and opportunistic pathogen, which can colonize the human genitourinary and gastrointestinal tract, is *S. agalactiae* (Verani et al. 2010). Numerous virulence factors of *S. agalactiae* have been identified to date, including pili, fibrinogen binding protein, Alpha C protein (ACP), serine rich repeat proteins (Srr), capsular polysaccharides (CPS), factor H-binding protein, superoxide dismutase (SodA), Streptococcal C5a peptidase of GBS (ScpB) and CAMP factor (LaPenta et al. 1997; Schubert et al. 2004; Rajagopal 2009; Landwehr-Kenzel and Henneke 2014). *S. agalactiae* plays an important role in the development of neonatal septicemia and meningitis, as well as invasive infections in immunocompromised adults and elderly (Verani et al. 2010; Pimentel et al. 2016). Maternal *S. agalactiae* infections can lead to neonatal diseases, by attaching to maternal vaginal epithelial cells via host extracellular matrix (ECM) molecules, then penetrating the uterine compartment and moving

to the neonatal lung, followed by its entry into the blood system, and eventually causing pneumonia, sepsis, and meningitis in the newborn (Rajagopal 2009). Pneumonia and sepsis often occur within the first 7 days after birth while meningitis occurs during the period from the 7th day after birth to 2 or 3 months of age (Verani et al. 2010; Melin and Efstratiou 2013). Pneumonia developing in the first week after birth often leads to respiratory failure and worsens to septic shock rapidly. Neonatal meningitis will often result in severe sequelae such as deafness, visual impairment, cognitive impairment, and seizures (Schuchat 1998; Maisey et al. 2008; Libster et al. 2012; Melin and Efstratiou 2013). Based on its capsular polysaccharide immunologic reactivity, GBS has been classified into ten serotypes (Ia, Ib, II, III, IV, V, VI, VII, VIII, IX) (Rosa-Fraile et al. 2014). Serotypes Ia, Ib, III, and V are estimated to cause 85–90% of all GBS diseases (Hickman et al. 1999; Rajagopal 2009), while serotype III is responsible for causing meningitis (Edmond et al. 2012). Although it is known that GBS needs to penetrate the blood-brain barrier (BBB) to cause meningitis, the mechanism behind this is not clearly known. *S. suis* is a major swine pathogen colonizing the digestive and respiratory tracts of pigs. It can cause several diseases in pigs including septicemia, arthritis, endocarditis, pneumonia, and meningitis (Gottschalk et al. 2010; Goyette-Desjardins et al. 2014). It is also reported that *S. suis* can be transmitted from pigs to humans via contact, and is associated with streptococcal toxic shock syndrome (STSS), septicemia, arthritis, and meningitis (Tang et al. 2006; Feng et al. 2010). The virulence factors of *S. suis* include CPS, muraminidase-released protein, extracellular factor, suilysin (SLY), and hyaluronic acid lyase (Vecht et al. 1991; Okwumabua et al. 1999; Gottschalk and Segura 2000; Haas et al. 2015; Segura et al. 2017). At least 35 serotypes of *S. suis* have been identified based

on different CPS antigens (Okura et al. 2016), among which serotype 2 is the major serotype isolated from humans infected by *S. suis* (Gottschalk et al. 2007; Mai et al. 2008). This pathogen can cross epithelial barriers and enter the bloodstream, causing localized and/or systemic infections, or causing meningitis by crossing the BBB (Haas and Grenier 2018; Zheng et al. 2018). *S. suis* is increasingly gaining attention for its prevalence worldwide as an emerging zoonotic pathogen. In 1998 and 2005, the epidemics occurring in China caused mortality up to 56% and 18.6%, respectively (Tang et al. 2006; Feng et al. 2010). In addition, *S. suis* infections in Northern Vietnam are thought to be a major cause of adult bacterial meningitis (Nguyen et al. 2008; Wertheim et al. 2009). In Thailand, *S. suis* is also thought to be an emerging human pathogen (Kerdsin et al. 2009), as by the end of 2013, the number of human infection cases of *S. suis* had risen to 1642 (Goyette-Desjardins et al. 2014). Although the high pathogenicity of *S. suis* has resulted in an increasing number of studies about the mechanisms by which *S. suis* causes infection, it is still not fully clear. *S. mitis* is one of the commensal and relatively benign bacteria colonizing the skin, genitourinary tract, and gastrointestinal tract, and it can be found on almost all surfaces of the oral cavity, such as teeth, tongue, and mucosal surfaces (Carrascosa et al. 1994; Pearce et al. 1995; Aas et al. 2005). Although the mechanism of pathogenesis is still not fully clear, *S. mitis* can cause multiple human diseases (Doern and Burnham 2010), including vasculitis and endocarditis, or even sacroiliitis (Mitchell 2011; Al-Farsi et al. 2018; Basaranoglu et al. 2018). In the Mitis group of streptococci, *S. mitis* and *S. pneumoniae* are closely related, and both have evolved to express a similar collection of virulence factors (Kilian et al. 2008). Johnston et al. showed that in five *S. mitis* isolates, 72-83% of the

virulence genes of *S. pneumoniae* are present, among which *sodA* is present in all five strains (Johnston et al. 2010). Though originating from a common ancestor, compared to *S. pneumoniae, S. mitis* is more capable of adapting to a commensal lifestyle, which may be explained by a loss of virulence factors in *S. mitis* (Kilian et al. 2008; Rukke et al. 2014). In this review, we focus on the molecules involved in host invasion by different streptococcus bacteria strains, the interaction of bacterial adhesins or invasins with their respective receptors, and how they mediate streptococcal invasion. For host invasion by GAS, the most common streptococcus bacteria strain, we discuss the two main invasion pathways, which are mediated by SfbI and M protein.

GAS invasion

It has been demonstrated that highly pathogenic GAS strains can cause invasive disease in humans (Watanabe et al. 2016). GAS invasion is highly dependent on invasins exposed on the bacterium surface such as M protein and SfbI, which trigger two main uptake mechanisms: (i) The binding of M protein to α5β1 integrins facilitates host cytoskeleton rearrangement to cause membrane ruffling, and (ii) the binding of SfbI protein to host cells activates a caveolae-mediated internalization process, both of which eventually lead to the uptake of GAS (Dombek et al. 1999; Molinari et al. 2000; Rohde et al. 2003) (Figure 1). M protein is considered one of the most robust virulence factors of GAS strains (Cunningham 2000). It is present in the GAS cell wall, binds to host cell surface proteins and contributes to the interaction between the bacterium and host cells (Fischetti 2016). Subtyped by the M protein gene (*emm*), different M strains possess different pathogenicity, and M1 and M3 strains are most prevalent in

invasive diseases (Bessen et al. 1996; Sellers et al. 1996; Kiska et al. 1997; Ikebe et al. 2015; Stevens and Bryant 2016; Watanabe et al. 2016). Another molecule

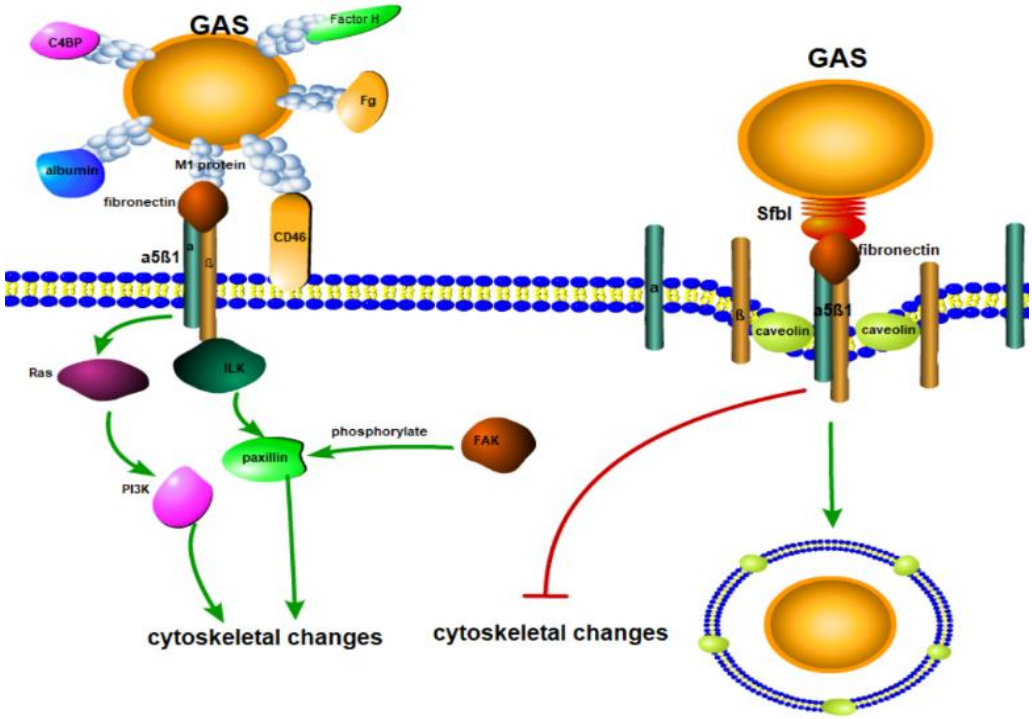

Figure 1. Two distinct mechanisms of GAS invasion. M protein and SfbI protein are two major invasins of GAS. M1 protein, a subtype of M protein, can bind with fibronectin (Fn). Subsequently, Fn binds to α5β1 integrins, which triggers the intracellular signaling. Activated Ras is able to recruit and activate phosphatidylinositol 3-kinase (PI 3-K), leading to the cytoskeletal changes. Alternatively, integrin-linked kinase (ILK) can bind with β integrin, and transmit signals to paxillin, which provides docking sites for actin binding protein. Also, paxillin can be phosphorylated by focal adhesion kinase (FAK), leading to more docking sites. Additionally, M1 protein can bind to other molecules to facilitate invasion, such as albumin, CD46, C4BP, factor H and fibrinogen (Fg). During SfbI-mediated invasion, the binding of SfbI protein to fibronectin leads to the exposure of the RGD region of fibronectin, which facilitates the binding of fibronectin to α5β1 integrins. Then, the intracellular signaling leading to cytoskeleton rearrangement is inhibited, followed by integrin clustering and caveolae aggregation, which contributes to caveolae-mediated invasion.

that plays an important role in the adhesion to epithelial cells is SfbI, which is known to promote the uptake of streptococci by eukaryotic cells (Hanski and Caparon 1992; Molinari et al. 1997). Rohde et al. (Rohde et al. 2003) demonstrated that invasion by some GAS strains was dependent on SfbI, a fibronectin-binding protein, which was essential in the caveolae-mediated endocytic process. Here we review the two invasins involved in the process of GAS invasion.

M protein

M protein is considered the major virulence molecule on the surface of *S. pyogenes* (Maxted 1956). It is of great importance to *S. pyogenes* as M protein-knockout mutants with all surface and secretory molecules but lacking M protein can not survive in human blood containing phagocytes (Perezcasal et al. 1992). The M protein forms a dimeric coiled-coil α-helix, consisting of a highly variable terminal region (Brandt et al. 1997; McNamara et al. 2008). It possesses a highly conserved C-terminal domain and a hypervariable N-terminal domain. The N-terminal region extends into the extracellular space and the C-terminal region is anchored in the cell surface (Fischetti 1989, 1991). More than 200 different serotypes of M protein such as M6, M12, and M18 have been identified, classified by distinct N-terminal regions determined by their *emm* gene. Each host individual is likely to be infected by different group A streptococcal types in different stages of their lifetime (Lancefield 1962; Beall et al. 1996). Among all serotypes of M protein, M1 protein is considered as the primary invasin of the highly invasive strain 90-226, as it contributes to about 90% of measured cell invasion by streptococci (Cue et al. 1998). Another experiment that supports this view

confirmed that human endothelial cells can phagocytose M1 wild-type GAS but not an M1 knockout mutant (Ochel et al. 2014). M1 protein is involved in cellular invasion by *S. pyogenes*, and a highly invasive M1 strain was reported to rely heavily on the expression of M1 (Cue and Cleary 1997; Jadoun et al. 1997). M1 protein is able to interact with several cellular receptors, including membrane cofactor protein (CD46), a molecule on the surface of most human cells (Liszewski et al. 1991). CD46 was demonstrated to effectively promote epithelial cell invasion by M1 and M3 strains (Rezcallah et al. 2005). In their study, deletion of the CD46 cytoplasmic domain significantly reduced the ability to invade epithelial cells by streptococci (Rezcallah et al. 2005). A recent study showed that CD46 was able to allow GGS_124 penetrate into deep tissues of mice, causing a higher mortality rate (Yoshida et al. 2016). These results suggest that CD46 plays an important role in streptococcal invasion. Other types of cell surface receptors involved in bacterial invasion, are integrins. This was shown by the fact that uptake of the M1 90-226 strain by epithelial cells can be blocked by antibodies against α5β1 integrin (Cue et al. 1998; Dombek et al. 1999). M1 protein is unable to bind to integrins directly, but can bind to the ECM protein fibronectin (Fn), and Fn possesses the ability to bind to integrins (Hynes 1992; Cue et al. 1998). After binding to integrins, M1 protein interacts with Ras, a small G protein, to activate and recruit phosphatidylinositol 3-kinase (PI3K), which in turn induces cytoskeletal changes that are required for GAS invasion (Cantrell 2001; Chan et al. 2002; Purushothaman et al. 2003). This pathway also involves integrin-linked kinase (ILK), paxillin, and focal adhesion kinase (FAK). By providing several docking sites for actin binding protein and signaling molecules, paxillin forms an essential adaptor (Turner 2000). ILK is able to interact with paxillin via a paxillin

binding subdomain and can bind to the cytoplasmic region of β integrins by its C-terminus (Nikolopoulos and Turner 2001). FAK can phosphorylate paxillin and creates additional docking sites for cytoskeleton rearrangement (Vindis et al. 2004). The binding of M1 protein to Fn initiates FAK-induced phosphorylation (Wang et al. 2007) and eventually triggered the formation of membrane protrusions involved in phagocytosis of the streptococcal chain, leading to a zipper-like invasion by GAS (Ochel et al. 2014). M protein also binds to other host proteins to mediate the interaction between GAS and host cells, including C4b binding protein (C4BP), factor H, fibrinogen (Fg), and albumin (Ryc et al. 1989; Akesson et al. 1994; Johnsson et al. 1996). Thus, M protein significantly enhances the virulence of GAS by binding or recruiting host proteins.

M3 strains of GAS have been isolated from invasive streptococcal disease patients and also contribute to invasive streptococcal disease (Terao et al. 2002; Sumby et al. 2006). M3 strains have a weak ability to invade HEp-2 cells, which could be explained by their lack M1 protein and SfbI. In contrast to this, M3 strains express the Fn-binding protein of group A streptococci type B (*fbaB*) gene (Terao et al. 2002), which encodes FbaB protein that was demonstrated to facilitate invasion of endothelial cells (Nerlich et al. 2009). The FbaB-mediated invasion consists of four steps: (i) membrane protrusions form at the invasion site; (ii) F-actin accumulates in the vicinity of invading streptococci and Ras-related C3 botulinum toxin substrate 1 (Rac1), a small GTPase, accumulates and becomes activated; (iii) the phagosomal membrane acquires endosomal marker proteins; and (iv) the bacteria are transported to the terminal lysosomal compartment (Nerlich et al. 2009; Amelung et al. 2011).

SfbI invasion

Fibronectin (Fn) possesses a string of five Fn type I (F1) modules within the N-terminal domain (Desimone et al. 1992), which are functionally recognized by SfbI protein. SfbI consists of three major regions: (i) a C-terminal region that contains several fibronectin-binding repeats (FBR region), (ii) a region containing a number of proline-rich repeats (PRR region), and (iii) an N-terminal region with several aromatic amino acid residues (AroD region) (Talay et al. 1994; Towers et al. 2003; Towers et al. 2004). Molinari et al. (Molinari et al. 1997) demonstrated that the fibronectin-binding domains of SfbI protein play a role in mediation of eukaryotic cell invasion by *S. pyogenes*. SfbI-dependent invasion involves the use of host cell caveolae (Rohde et al. 2003). With shapes ranging from almost flat to cup-like depressed, to flask-like in some cases (Schlormann et al. 2010), caveolae are invaginations that are present in eukaryotic plasma membranes. They are defined as 60-80nm wide pits that exert several biological functions, including mediating the uptake of bacteria (Parton and del Pozo 2013). One caveola consists of many caveolin 1 (CAV1) proteins, which form the primary structure of caveolae (Pelkmans and Zerial 2005; Parton and del Pozo 2013). SfbI protein binds to a fibrin-binding fragment of fibronectin by two distinct domains, and eukaryotic cell invasion by streptococci is effectively increased by the cooperation of these two regions (Talay et al. 2000). The SfbI protein binds to fibronectin via a tandem β-zipper cooperation, in which the fibronectin-binding region (FnBR) forms a sequence that is anti-parallel to the C-terminal sequence of each F1 module (Schwarz-Linek et al. 2003; Bingham et al. 2008). The binding of SfbI protein to fibronectin involves opening of the RGD region via changes in the

fibronectin quaternary structure. This facilitates the binding of the RGD region to α5β1 integrins on the surface of host cells and inhibits intracellular signaling by rearranging the cytoskeleton. Both competitive RGD peptides and antibodies against the β-subunit of the integrin can block the interaction between the RGD-region of fibronectin and integrins on host cell surfaces, inhibiting the invasion of streptococci significantly (Jadoun et al. 1998; Ozeri et al. 1998; Molinari et al. 2000; Ozeri et al. 2001). The tripeptide RGD region in fibronectin and other extracellular ligands, which enables recognition by integins, is not found in SfbI, so the binding of SfbI protein to fibronectin plays an important role in the process of streptococcal invasion. Binding of SfbI to fibronectin leads to subsequent integrin clustering and caveolae aggregation, formation of large encapsulating invaginations, and eventually promotes the uptake of streptococci. Once inside the host cells, the streptococci stay in a membrane-bound compartment in the cytoplasm, termed caveosomes. Contributing to its survival in cytoplasm, these SfbI-expressing streptococci possess a unique ability to bypass the lysosomal degradation machinery of the host cells (Rohde et al. 2003). It was also demonstrated that the uptake efficiency is affected by the amount of bound fibronectin on the bacterial surface (Ozeri et al. 1998). This led to the hypothesis that a threshold of fibronectin bound to integrins is necessary to trigger signaling. Schwarz-Linek et al. discovered that one SfbI protein can bind up to five fibronectin molecules (Schwarz-Linek et al. 2003), which means one streptococcus can bind to a great number of integrins on a host cell, which benefits its invasion. Ezrin, another host protein involved in SfbI-mediated invasion (Tsukita et al. 1997; Rox et al. 2017), consists of a C-terminal domain, an N-terminal domain, a poly-proline region, and an α-helical domain (Tsukita et

al. 1997). During the uptake process of bacteria, Ezrin plays an effective role in the signal transduction of host protein kinases and cell receptors, such as PI3K, which acts as an important factor in the invasion pathway (Hirao et al. 1996; Schoenwaelder and Burridge 1999; Bretscher et al. 2002).

S. pneumoniae invasion

In most cases, *S. pneumoniae* primarily adheres to and invades upper respiratory tract cells and subsequently spreads to other tissues through the bloodstream, which can be promoted by sialic acid (Kadioglu et al. 2008; Henriques-Normark and Tuomanen 2013; Iovino et al. 2013; Hatcher et al. 2016). Murine lung invasion by *S. pneumoniae* has been shown to be enhanced by administering sialic acid (Trappetti et al. 2009). After initial adhesion, *S. pneumoniae* can invade cells of the respiratory tract via at least two pathways: (i) *S. pneumoniae* binds to platelet-activating factor receptor (PAFR) on the surface of respiratory epithelial activated by cytokines through phosphorylcholine (ChoP) moieties, and is subsequently engulfed by host cells via the PAFR recycling pathway (Cundell et al. 1995). (ii) Another pathway similar to this is triggered by the binding of the polymeric immunoglobulin receptor (PIGR) to CbpA, followed by pneumococcal translocation through epithelium (Zhang et al. 2000). Binding of platelet-activating factor (PAF) to PAFR facilitates the signal transduction of host cells (Shukla 1992), and binding of *S. pneumoniae* to PAFR promotes the adhesion to and internalization by host endothelial cells (Cundell et al. 1995; Ring et al. 1998; Radin et al. 2005). *S. pneumoniae* has been shown bind to PIGR present on host nasopharyngeal epithelial cells, which enhanced the translocation through epithelium. As antibody against PIGR effectively inhibits the adherence, PIGR is

thought to participate in the adhesion process, (Zhang et al. 2000). The two pathways both lead to the release of pneumococci into the interstitium. Consistent with the experiments of Iovino et al., neither of these two pathways involves the disruption of tight junctions between host cells (Iovino et al. 2013). PIGR and CbpA are also involved in brain invasion. A recent study demonstrated that brain invasion by *S. pneumoniae* can be mediated by a pilus-related adhesin (RrgA) that is able to interact with PIGR and platelet endothelial cell adhesion molecule (PECAM-1). Pneumococcal entry into the brain of mice was significantly blocked by antibodies against these two proteins (Iovino et al. 2017). In addition, CbpA can bind to the laminin receptor (LR), which facilitates streptococci crossing the BBB, eventually leading to meningitis (Orihuela et al. 2009). Agarwal et al. (Agarwal et al. 2013) identified another invasion pathway mediated by the host complement protein C1q. C1q acts as a bridge molecule between pneumococci and host cells, by binding to pneumococcal exposed surface proteins via its globular heads and by binding host cell receptors via its N-terminal stalk region, thereby significantly promoting the uptake of *S. pneumoniae* by host epithelial and endothelial cells. Additionally, van Ginkel et al. (van Ginkel et al. 2003) showed that *S. pneumoniae* is able to spread to olfactory nerves from nasopharyngeal epithelium, providing a novel mechanism for *S. pneumonia* invasion of the brain via a non-hematologic route. Streptococcal invasion of the heart also involves the binding of LR and PAFR with their ligands, as demonstrated by the reduction of microlesion formation in $PAFR^{-/-}$ mice or by using antibody against LR. (Brown et al. 2014) (Figure 2)

S. agalactiae invasion

To activate downstream signaling and eventually invade host cells, *S. agalactiae* interacts with proteins on the host cell surface (Beckmann et al. 2002; Cheng et al. 2002; Bolduc and Madoff 2007; De Gaetano et al. 2018). *S. agalactiae* can bind to vitronectin (Vtn), an ECM protein, via its plasminogen binding surface Protein (PbsP) present in the cell wall, and Vtn acts as a bridge molecule between *S. agalactiae* and host cells (De Gaetano et al. 2018). Vtn plays a role in the invasion process of invasive pathogens, and Vtn-mediated binding can initiate integrin-dependent internalization of bacteria (Grashoff et al. 2004; Singh et al. 2010). Vtn is present in extracellular fluids and endogenous secretions, is expressed on the surface of different cell types, and can be upregulated upon injury and inflammation (Hallstrom et al. 2016; Aulakh 2018). PbsP, which is anchored in the cell wall, possesses a plasminogen-binding region and one or two streptococcal surface repeat regions, which contribute to GBS virulence and

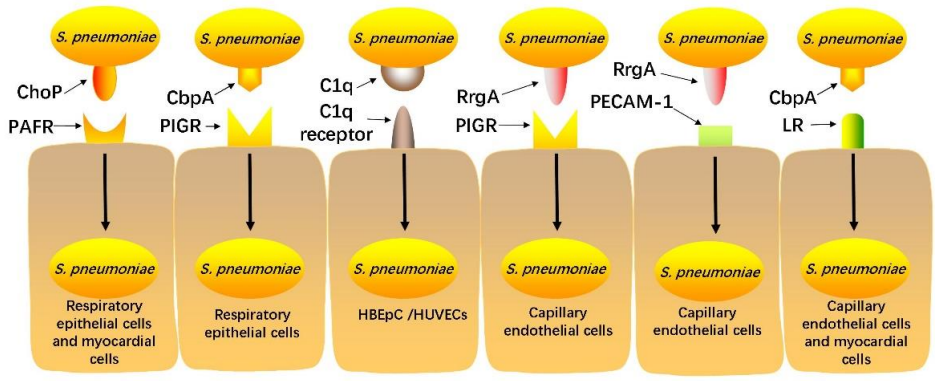

Figure 2. Different molecular mechanisms by which *S. pneumoniae* invades host cells. The invasion of *S. pneumoniae* is dependent on the binding of bacterial surface proteins with host receptors. Different ligand-receptor interactions are involved in different host cell types.

are essential in plasmin-dependent invasion (Bumbaca et al. 2004; Buscetta et al. 2016). Binding of ligands to host-cell receptors also activates Ras, which subsequently triggers the recruitment of PI3K to the membrane of host cells (Reuther and Der 2000). Activated PI3K interacts with GTPases, both of which can be activated by the binding of *S. agalactiae* directly, and are responsible for actin rearrangement (Burnham et al. 2007a; Burnham et al. 2007b). Akt, a serine kinase that can modulate downstream effector molecules is also modulated by PI3K, which eventually affects actin rearrangement (Stokoe 2005; Burnham et al. 2007a); an indispensable step in cell invasion by GBS (Boone and Tyrrell 2012).

ACP, a cell wall anchored protein, is also involved in GBS invasion. The N-terminal domain of ACP has been classified into two structural regions (D1 and D2). D1 includes a β sandwich while D2 consists of 3 antiparallel α helix coils (Auperin et al. 2005). An important function in cell invasion is exerted by the N-terminal domain of ACP, since the soluble form of the N-terminal domain significantly inhibits GBS internalization (Bolduc et al. 2002). Subsequent studies revealed that both D1 and D2 are involved in the invasion process. GBS affinity to α1β1 integrin and its ability to invade cervical epithelial cells was decreased by converting a KTD motif within D1 to KTA (Bolduc and Madoff 2007). Moreover, when a mutation was induced in a glycosaminoglycan-binding site within D2, invasion was blocked (Baron et al. 2007). These results suggest both D1 and D2 can mediate GBS entry, but the mechanisms may be distinct.

Additional studies have shown that fibrinogen-binding proteins (Fbs) were relevant to cell invasion by GBS, including FbsB (Schubert et al. 2004), the Srr

that can mediate the uptake of GAS by microvascular endothelial cells (Seo et al. 2012; Seo et al. 2013), and FbsC (Buscetta et al. 2014). A novel molecule named fibronectin-binding protein A (SfbA) has been identified as well, which contributes to GBS invasion of brain endothelial cells and meningitis (Mu et al. 2014). This view is supported by a subsequent study that demonstrated that the ability to breach the BBB and to cause meningitis was reduced when *sfbA* mutant strains were used to infect mice (Stoner et al. 2015).

A significant number of studies report that β-hemolysin/cytolysin (β-H/C) triggers cytolysis of host cells, thereby promoting GBS invasion of epithelial and endothelial barriers, including the BBB, as well as endothelial and epithelial cells of the host lung (Nizet et al. 1996; Nizet et al. 1997; Gibson et al. 1999; Doran et al. 2002; Doran et al. 2003; Liu et al. 2004). β-H/C-deficient GBS mutants showed attenuated virulence to cause infections such as meningitis, sepsis, and pneumonia (Doran et al. 2002; Doran et al. 2003; Hensler et al. 2005). Additionally, several other life-threatening diseases can be induced by β-H/C, including cardiac impairment and liver failure (Ring et al. 2002; Hensler et al. 2008), underscoring the importance of β-H/C in GBS-mediated disease

S. suis invasion

S. suis is able to interact with epidermal cells in wounded skin or intestinal epithelial cells (Gottschalk et al. 2010; Segura et al. 2016), and the gastrointestinal tract is a common entry site both in humans and pigs (Ferrando et al. 2015). Ferrando et al. (Ferrando et al. 2015) demonstrated that *S. suis* was able to cause the disruption of intercellular tight junctions and subsequently cross

the intestinal epithelial barrier in humans through a paracellular route. *S. suis* can eventually cause meningitis, after reaching the bloodstream and crossing the BBB, (Fulde and Valentin-Weigand 2013; Haas and Grenier 2018). To cause meningitis, crossing the BBB is a critical step for bacteria, but the mechanism is not clearly known. Several mechanisms by which *S. suis* penetrates the BBB have been shown, such as invasion of the cells making up the BBB via the endocytic pathway and disruption of intercellular tight junctions (Gottschalk et al. 2010; Fittipaldi et al. 2012; Fulde and Valentin-Weigand 2013). Kong et al. (Kong et al. 2017) recently demonstrated that *S. suis* translocation across the BBB was significantly promoted by the interaction of factor H-binding protein of *S. suis* with globotriaosylceramide of host cells (Kong et al. 2017). Another virulence factor of *S. suis* named SLY can trigger pore-formation in host cell membranes and also contributes to the invasion of *S. suis* (Seitz et al. 2013). Norton et al. showed that the invasion of epithelial cells by SLY-negative strains was inhibited (Norton et al. 1999). SLY is thought to contribute to the formation of membrane ruffles, thereby facilitating the invasion into epithelial cells by *S. suis* (Benga et al. 2004; Seitz et al. 2013). In addition to epithelial cells, SLY also plays a role in the invasion of human brain microvascular endothelial cells (HBMEC). Lv et al. (Lv et al. 2014) demonstrated that *S. suis* invasion into HBMEC can be facilitated by SLY, which can activate GTPase Ras homolog gene family member A (RhoA) and Rac1 to remodel the actin cytoskeleton. Nevertheless, since a SLY knockout mutant also disseminates in the host, SLY is not indispensable in *S. suis* infections (Lun et al. 2003). Additionally, by translocation through the blood-cerebrospinal fluid barrier, *S. suis* can invade the host brain, and this process is associated with its adherence to and entry into the choroid plexus epithelial cells and transportation

by endocytic vacuoles (Haas and Grenier 2018).

S. mitis invasion

S. mitis can interact with human gingival fibroblasts (HGFs), and its entry into HGFs is associated with both bacterial virulence factors and host proteins (Rukke et al. 2012; Di Giacomo et al. 2013; Cataldi et al. 2016; Di Giulio et al. 2018). The invasion of HGFs by *S. mitis* is mediated by Focal Adhesion Kinase (FAK), integrin β1, and the two cytoskeleton proteins vinculin and F-actin (Cataldi et al. 2016; Di Giulio et al. 2018). Chitlac-nAg silver nanoparticles can enhance the internalization of *S. mitis*, which is thought to be a strategy of bacteria to evade the toxicity of silver. Nevertheless, the effects of Chitlac-nAg on the invasion of *S. mitis* can be inhibited by saliva, and it is hypothesized that proteins within the saliva play a role in inhibiting the uptake of *S. mitis* (Di Giulio et al. 2018). Similar to *S. pneumoniae*, to which it is closely related, *S. mitis* was shown to possess a capsule, which is an important virulence factor (Kilian et al. 2008). The capsule of *S. pneumoniae* has been well studied, and is associated with numerous functions such as evasion of mucus-mediated clearance, and modulation of inflammatory responses (Bootsma et al. 2007; Nelson et al. 2007). Although it was shown that the capsule of *S. mitis* inhibits its adherence to oral epithelial cells (Rukke et al. 2016), the underlying mechanism remains unknown.

Conclusion

Streptococci can form a severe threat to human health, and novel members of streptococci are constantly isolated, such as *Streptococcus tigurinus*, causing infective endocarditis (Zbinden et al. 2012). Recent years, great progress has

been made in uncovering the molecular mechanisms of streptococcal adhesion and invasion. Streptococci are difficult to eradicate due to their ability of adherence to and invasion of eukaryotic cells. By expressing distinct adhesins and invasins in different environments, streptococcal infections can be difficult to treat. Both extracellular and intracellular molecules are involved in the adhesion and invasion, and there is a demand for a better understanding of these associated molecules. In spite of the advances in antimicrobial therapy based on the current molecular research, streptococcal infections still cause a high morbidity and mortality from diseases such as meningitis. Thus, identification and targeting of molecular patterns involved in invasive bacterial infections, and pathways by which streptococci adhere to and invade host cells, are crucial in developing novel effective therapeutic strategies.

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