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Invasion Mechanisms of Gram-Positive Pathogenic Cocci

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Summary

Gram-positive cocci are important human pathogens. Streptococci and staphylococci in particular are a major threat to human health, since they cause a variety of serious invasive infections. Their invasion into normally sterile sites of the host depends on elaborated bacterial mechanisms that involve adhesion to the host tissue, its degradation, internalisation by host cells, and passage through epithelia and endothelia. Interactions of bacterial surface proteins with proteins of the host's extracellular matrix as well as with cell surface receptors are crucial factors in these processes and some of the key mechanisms are similar in many pathogenic Gram-positive cocci. Therapies that interfere with these mechanisms may become efficient alternatives to today's antibiotic treatments.

Introduction

Within the Gram-positive cocci streptococci and staphylococci have an eminent role as clinically important pathogens. The term streptococci denotes a rather heterogeneous genus that comprises important human pathogens like *Streptococcus pyogenes* also referred to as group A streptococci (GAS), group C streptococci (GCS) and group G streptococci (GGS), *S. agalactiae* (group B streptococci, GBS), as well as *S. pneumoniae* (pneumococci). Although related, these species differ greatly in their spectrum of diseases. GAS, but also GCS and GGS, cause pharyngitis and scarlet fever as well as skin infections like erysepelas and impetigo. Moreover, they can lead to severe invasive infections like bacteremia, cellulites, and necrotizing fasciitis with high mortality due to streptococcal toxic shock syndrome. Both

pneumococci and GBS are major causes of pneumonia, meningitis, and sepsis, but remarkably, the molecular mechanisms of their pathogenesis are very different. *Staphylococcus aureus*, is a leading nosocomial pathogen, which causes skin infections (i.e. impetigo) and serious invasive diseases like endocarditis, osteomyelitis, septic arthritis, and sepsis. Because of the progressing development of antibiotic resistance, *S. aureus* remains a major health concern. Initial stages of bacterial infection depend on adhesion of the pathogen to the host tissue. To colonise the site of infection, bacteria have evolved an arsenal of surface components, named adhesins, that establish the contact to the host's extracellular matrix or to host cells (1-4). Although both staphylococci and streptococci are considered as extracellular pathogens, many of them have a remarkable capability to invade eukaryotic cells (2, 5-7). This has crucial influence not only on bacterial persistence, but also on the progression of infection into deeper tissues, and is controlled by adhesins or more specialized invasins that actively promote the internalisation by host cells. Bacterial spreading requires a certain mobility of the bacteria in the tissue. Lytic enzymes allow the bacteria to break mechanical barriers, improving the conditions for their spread. The following chapters will give insight in the existing mechanisms, highlight similarities, and point at some interesting differences in the invasion strategies of the different Gram-positive pathogenic cocci.

Bacterial adhesion to the host tissue

Streptococci and staphylococci employ whole sets of adhesins to establish contact with the host tissue (for review see: (1-4)). These sets are believed to have deciding influence on the tissue tropism of infection and may control the invasiveness of particular streptococcal strains. Gram-positive cell walls can carry chain-like glycerol phosphate polymers that are covalently coupled to glycolipids of the plasma membrane as lipoteichoic acid (LTA) or to the peptidoglycan as wall teichoic acid (WTA). LTA and WTA are thought to fulfill the function of an adhesin, but have low cell type selectivity (8-13). The extracellular protein fibronectin is a ligand for streptococcal LTA (14). Efficient adhesion of GAS to host cells, however, is thought to depend on additional high-avidity interactions (10, 15). The higher cell type specificity of the additional adhesins may greatly influence the course of infection, not least because their interactions promote internalisation of the bacteria by eukaryotic cells.

Both streptococci and staphylococci have evolved numerous adhesins that bind extracellular matrix proteins and plasma proteins (1). Among these are important anti-phagocytic factors of

the GAS, GCS, and GGS, namely M- and M-like proteins and the hyaluronic acid (HA) capsule. M proteins are multifunctional virulence factors (16-19) and were the first reported adhesins of GAS (20, 21). More than one hundred different M-serotypes are known, that differ greatly in the N-terminal sequence of the mature M protein. This serotype variability led to differences in the adhesin and invasin properties of M-proteins, which have recently been summarized in (22). Certain M- and M-like proteins as well as the hyaluronic acid capsule bind GAS and GGS to collagen (23, 24), a very abundant component of the host's extracellular matrix. Staphylococci do not possess M proteins, but some strains of *S. aureus* express the collagen binding protein CNA that facilitates the colonization of mouse joints, suggesting a role of the adhesin in septic arthritis (25). Like streptococcal adhesins, staphylococcal adhesins can either be anchored to the staphylococcal cell wall, utilizing a sortase motif LPXTG or a transmembrane region (MSCRAMMs, microbial surface components recognizing adhesive matrix molecules). Others are secreted as anchorless proteins (SERAMs, secretable expanded repertoire adhesive molecules) that bind back to the bacterial surface. An important host protein targeted by a variety of streptococcal and staphylococcal adhesins is fibronectin (Table 1). Fibronectin binding, has a key role in cell adhesion and internalisation of both streptococci and staphylococci and is the prime example for exploitation of host extracellular proteins by Gram-positive cocci. Among the fibronectin binding adhesins are some streptococcal M proteins of serotype 1. Most of the M proteins, however, have not been shown to bind fibronectin. Using its RGD-motif for integrin-mediated cell binding (26) fibronectin acts as a bridging molecule that connects bacterial adhesins with integrins on the surface of eukaryotic cells (27-33). Other extracellular proteins that bare a RGD-motif and for which both streptococcal as well as staphylococcal adhesins exist are laminin and fibrinogen. *S. aureus*, moreover, binds to vitronectin and thrombospondin, which also possess a RGD-motif (26). The influences of fibrinogen-binding adhesins on internalisation by eukaryotic cells and on invasiveness of infections, however, are the ones that have been studied to a larger extent and therefore are a topic of this article.

Degradation of extracellular proteins facilitates invasive infections

The constitutive organic components of the extracellular matrix are collagen fibers and lattices that confer tensile strength to the tissue, but also glycosaminoglycans and proteoglycans that fill the interstitial spaces. The latter are or consist of highly charged polysaccharides that create the turgor of the matrix, which is counteracting compressive

forces. The extracellular matrix is a physical barrier to the bacteria. In an injured tissue the coagulation system forms thrombi that may be a further obstacle. Therefore, proteolysis and other degradative processes are required for bacterial dissemination. Gram positive cocci possess a variety of lytic enzymes like hyaluronidase, lipases, and proteases thought to be used for tissue degradation. The best studied bacterial strategy to adopt a broad-spectrum proteolytic activity, capable of facilitating the migration through extracellular matrix is the capture and activation of human plasmin(ogen). The importance of surface bound plasmin(ogen) for streptococci is suggested by the number of factors that they have developed for its capture and the number of different mechanisms they utilize for activation of the zymogen (34). One of the plasmin(ogen) receptors of GAS is the M-like protein PAM (35). Other plasmin(ogen) receptors are secreted and bound back to the bacterial surface in hitherto unknown ways. Surface bound GAPDH and α -enolase are such receptors and were found on GAS (36, 37) but also on *S. pneumoniae* (38, 39). The α -enolase of *S. pneumoniae* has been implicated in plasmin-dependent penetration of biological membranes during invasive infections (40).

The physiological function of plasmin is to resolve thrombi by degrading fibrin. In the healthy tissue the zymogen (plasminogen) is proteolytically activated by tissue type plasminogen activators or urokinase. The activity of the generated plasmin is under tight control of plasmin inhibitors like α_2 -antiplasmin (41). Some streptococci are able to exploit the host's plasminogen activators (35, 42, 43), others express the bacterial activator streptokinase that forces the unprocessed catalytic domain of plasminogen in a proteolytically active conformation (41). Binding to plasminogen receptors may in addition prevent the inhibition of Plasmin(ogen) by host inhibitors as seen for α_2 -antiplasmin and the broad-spectrum protease inhibitor α_2 -macroglobulin (44, 45).

The ability of GAS strains to bind plasminogen was found to be associated with skin infections in human (46, 47). Since activation of plasminogen by streptokinase is species specific (48, 49), contribution of group A streptococcal streptokinase to virulence was investigated and could be demonstrated in transgenic mice that express the human ortholog. Skin infections with GAS led to a markedly increased mortality in these mice. An increase in the number of bacterial colonies that were found in the spleen suggests that an enhanced bacterial dissemination may have contributed to the fatal outcome of infection in these animals (50).

Internalisation of Gram-positive cocci by non-phagocytic host cells

The capability of GAS to promote internalisation by human non-phagocytic cells at frequencies equal or greater than classical intracellular pathogens such as *Listeria* or *Salmonella* is an observation of the mid 90s (51, 52). First evidence for the fact that intracellular habitation of GAS has *in vivo* relevance came from examination of tonsils that were excised from patients with recurrent tonsillitis. The presence of viable intracellular streptococci in these tonsils suggested that the internalisation by host cells created a reservoir of bacteria, which is responsible for the recurrence of the infection (53, 54). Such internalisation by non-phagocytic host cells has also been reported for GBS, GCS, GGS, *S. suis*, as well as *S. pneumoniae* and oral streptococci of the *viridans* group, but also for staphylococci (55-60). Internalisation by host cells has important implications in the pathogenesis of streptococcal and staphylococcal infections. Intracellular habitation of the bacteria is believed to offer protection against antibiotic treatment and immune defence, which contributes directly to bacterial survival. Infection of deeper tissues requires passage through protective epithelia or endothelia, which can be achieved by transcytosis, paracellular translocation, or cell damage that breaches the affected continuous cell layer. Internalisation by host cells is a first step in transcytosis and may thus contribute to the development of invasive disease. But in some cases cellular habitation may as well be detrimental to bacterial spreading.

Morphologies and mechanisms of internalisation

Visualising internalisation of bacteria by means of scanning electron microscopy (SEM) has become a powerful tool for the investigation of the involved mechanisms (Fig. 1). Strain-dependent differences in the morphologies of internalisation have been observed with streptococci. Two of the distinct morphologies that occurred resembled those of classical intracellular bacteria (61) (Fig. 1 A and B). Some isolates exhibited the classical membrane-ruffling pattern (trigger mechanism) when internalised by epithelial cells (Fig. 1 A), whereas other isolates showed a well defined zipper-like mechanism (Fig. 1 B). In addition, a novel, third internalisation pattern was found to dominate in many GAS strains (Fig. 1 C). Large invaginations, which appeared like a “hole” in the host cell membrane, developed during the internalisation process (62, 63). Interestingly, different internalisation patterns were also seen with group C and group G streptococci (56) as well as with non-encapsulated strains of *S. suis* (64). High resolution scanning electron microscopic studies that unravel the internalisation mechanism(s) exploited by *S. aureus* (65) are rare. In most of the studies transmission

electron microscopy of ultrathin sections has been performed to visualise the intracellular status of the staphylococci (66-69). Their internalisation resembles the zipper-like mechanism, in which the host cell membrane is zippered around the bacterium while entering the host cell (Fig. 1 D). Morphologically the process is not associated with such drastic cytoskeletal rearrangements as the trigger mechanism and the port of entry (the “zipper”) disappears within minutes.

Internalisation mediated by streptococcal and staphylococcal fibronectin binding proteins

Fibronectin-mediated internalisation by eukaryotic cells is a major mechanism common to streptococci and staphylococci. A key player in GAS is the streptococcal fibronectin-binding protein SfbI or its allelic variant protein F1. SfbI, is detectable in about 70%-85% of all GAS isolates (70-73). The interaction between SfbI and fibronectin is well studied and the current structural model is comprehensively reviewed in (74). SfbI recruits fibronectin for adherence to human epithelial cells (75) and Langerhans cells (76). Efficient internalisation by epithelial cells depends on the two fibronectin binding regions of SfbI. Interaction between the N-terminal fibrin binding region of fibronectin and the fibronectin binding repeats of SfbI co-operatively activates the adjacent spacer-region. The subsequent interaction between the activated spacer region and the collagen binding region of fibronectin was found to be a triggering step for efficient cell internalisation (33). Inhibition experiments with peptides and antibodies demonstrated that the cell binding RGD-motif (77) of fibronectin is employed for interaction with host cell integrins like $\alpha 5 \beta 1$ (30, 63, 78, 79), which is the initial step in cell internalisation processes. Immunofluorescence labelling of the $\beta 1$ -subunit underneath adherent streptococci (80) demonstrated that integrin clustering is triggering their uptake as it has been observed for other bacteria, i.e. *Neisseria gonorrhoeae* (81), *Yersinia* species (82) and last but not least *S. aureus* (31). Investigation of the cellular processes occurring during internalisation revealed that streptococci exploit existing signalling pathways of the host cells. Signal transduction of integrins is conducted by specialized adhesive structures termed focal complexes (FCs) and focal adhesions (FAs) that contain integrin clusters and numerous signalling and structural components that serve as linking or docking proteins for cytoskeletal components (83, 84). Internalisation of GAS requires presence of focal adhesion kinase (FAK) and Src kinases (Src, Yes and Fyn, together referred to as “SYF”) at the entry port (80). The Src kinases serve as co-operators for FAK, regulating the integrin-mediated signalling (85). In addition actin, tyrosine-phosphorylated proteins, paxillin, vinculin and talin are recruited to the entry port (80). During the early entry steps the small GTPases Rac and

Cdc42 are involved, while Rho is not involved (80). Based on their findings the following model of SfbI-mediated internalisation was suggested. Upon clustering of integrins by fibronectin-bound SfbI, two independent signalling pathways are activated. The first is recruitment and tyrosine-phosphorylation of FAK and paxillin with participation of the Src-kinases “SYF”. The second pathway is recruitment and activation of Rac1 together with Cdc42. Then the two pathways converge to trigger actin rearrangements that lead to bacterial uptake by a zipper-like mechanism.

In vitro studies have demonstrated that *S. aureus* can be internalised by a variety of non-phagocytic cells including epithelial and endothelial cells, fibroblasts, osteoblasts, keratinocytes, and kidney cells (69, 86-89). The underlying molecular events have been studied in numerous investigations in the past years, but despite the great variety of adhesins of *S. aureus* the fibronectin-binding proteins FnBP-A and FnBP-B were identified as key players in the *in vitro* internalisation of staphylococci (69, 90). Here again fibronectin acts as a bridging molecule, as during internalisation of streptococci. The *in vivo* relevance of fibronectin-binding has been addressed in the mouse model. *S. aureus* was detected intracellularly in the mammary gland of mice whereas a FnBP-A/FnBP-B double mutant was not internalised by the cells of the gland (91). In experiments with a rat heart valve system heterologous expression of FnBP-A was sufficient to confer an intracellular phenotype on non-pathogenic *Lactobacillus lactis* (68). As observed with streptococci, binding of *S. aureus* to fibronectin and the subsequent binding to $\alpha 5\beta 1$ integrins leads to a clustering of integrins that triggers the signalling pathways in the host cell. The signalling is similar to that caused by streptococci and accompanied by reorganization of the host actin cytoskeleton and *S. aureus* is internalised by a zipper-like mechanism (86). The internalisation process requires integrin-associated cytoplasmic proteins that link the clustered integrins with actin filaments. Protein tyrosine kinases, especially of the Src family, have been shown to be essential for integrin-mediated internalisation of staphylococci (86, 92, 93). In addition, the focal adhesion kinase (FAK) plays a dominant role in the internalisation process (65). Internalisation by host cells is very common among staphylococcal isolates. Interestingly, the only few exceptions have been found among MRSA (methicillin resistant) staphylococci that express a plasmin-sensitive protein (PIs), which seems to exert an anti-adhesive and anti-internalisation effect (69, 94).

Fibronectin-mediated internalisation by caveosomes

Microscopical approaches have shed light on a newly discovered mode of internalisation (95). Depending on the strain, SfbI-carrying streptococci, induce the formation of large invaginations while entering the host cell (63). The co-operative binding of fibronectin to SfbI, subsequent binding of fibronectin to $\alpha 5\beta 1$ integrins, and integrin clustering again were a prerequisite for efficient internalisation. Visualisation of the large invaginations at the site of streptococcal entry revealed the presence of numerous small invaginations (70-90 nm in diameter) on the surrounding eukaryotic cell surface, which were identified as caveolae of the examined HEP-2 and HUVE cells. Internalisation progresses by cumulative recruitment of caveolae to the entry port. Fusion of the caveolae forms the large invaginations. Complexes of recombinant SfbI and fibronectin were found to be sufficient for triggering the formation of the invaginations and the aggregation of caveolae. Further analysis revealed the formation of membrane-bound intracellular compartments that contained streptococci. Their nature of these cellular compartments was studied in detail. Interestingly, besides caveolin-1, their membranes did neither contain any marker enzymes of the classical endocytic pathway of eukaryotic cells, like EEA1, Rab5, Rab7, or LAMP1, nor did they contain golgi or ER markers. Analogous observations during uptake of the Simian Virus 40 were reported by Pelkmans *et al.* (96), who named the new cellular compartment “caveosomes”. In agreement with the lack of LAMP1, that is a marker for lysosomes and late endosomes, no lysosome fusion was detectable after infection with the SfbI carrying isolates, but with a SfbI-negative GAS strain (95). Interestingly, the formation of invaginations was also observed with *S. aureus*, which also employs $\alpha 5\beta 1$ integrin for its internalisation (65). It will be interesting to see whether caveosome formation occurs in this species as well and whether FnBP-A and FnBP-B have a similar function as SfbI. Such fibronectin binding proteins may allow internalisation while avoiding fusion with the degrading lysosomes in order to persist inside the host cell or even to transcytose.

M protein-mediated internalisation of streptococci

The work of Cleary *et al.*, (97) indicated contribution of genes controlled by the positive transcription regulator Mga (multigen regulator) in internalisation of GAS by epithelial cells. An insertion mutation in *mga* decreased the production of M1 protein and HA capsule as well as the internalisation. Strains of the M1 serotype, after binding to fibronectin, internalise into human lung epithelial cells. The significantly reduced internalisation of a M1-negative mutant identified the M-protein as the invasin, that is also able to exploit laminin for internalisation

(62). In case of M1 mediated internalisation the integrin-linked kinase (ILK) has been described as the link between integrins and the uptake by epithelial cells (98). Like the SfbI mediated uptake in endothelial cells, the M1 dependent entry in HeLa cells resembled a zipper-like mechanism with considerable cytoskeletal rearrangements (99). For the M1 strain but also for an M5 strain (63) early and intimate contact of streptococci with host cell microvilli and association of streptococci with polymerised actin at the site of entry was observed. At later stages M1 bacteria reside inside membrane-bound vacuoles which are positive for LAMP-1 protein, giving strong evidence for fusion with lysosomes during the intracellular trafficking (99). In addition M3, M4, M6 and M12 serotypes have been studied in respect to HEP-2 cell internalisation and all serotypes were found to be internalised avidly. M18 serotype strains which exhibit a large hyaluronic acid capsule were less efficient in internalisation because the capsule interfered with the initial cell adhesion process in this system (100).

Penetration of cellular barriers and bacterial dissemination

The strategies by which streptococci transmigrate through cell layers are diverse. It is thought that GAS, which are resistant to killing by PMNs, may exploit the ability of the neutrophils to transmigrate vascular endothelia for their spread via the blood stream (101). Work of Cywes *et al.* (102) suggests that GAS, which colonize the stratum corneae of the skin, invade deeper layers by paracellular translocation. Binding of the HA capsule of GAS to the hyaluronic acid receptor CD44 on human keratinocytes (103) induces cytoskeletal changes, that cause disruption of intracellular junctions and allow the passage into subjacent tissue (102, 104). In contrast, non-encapsulated strains were reduced in their translocation capacity and were found in vacuoles inside the host cells. The HA capsule was suggested to have two functions during paracellular translocation; disruption of the intercellular junctions by binding to CD44 and at the same time preventing streptococci from being internalised and trapped inside cellular vacuoles (102). The data demonstrate that adhesion to and internalisation by the host cells may in certain cases counteract bacterial dissemination. Further support comes from experiments with streptococci that constitutively overexpress SfbI. These bacteria turned out to be less virulent in an *in vivo* model. Experiments involving plasma-fibronectin-null mice suggested that SfbI-mediated internalisation by host cells limits streptococcal dissemination (105). At high partial pressures of O₂ expression of SfbI is increased as compared to a CO₂-rich environment (106). This suggests that SfbI-expression is favoured at superficial sites of

infection, when it is required for initial host adhesion and colonization. The result of Nyberg *et al.* (105) imply that down regulation of SfbI expression under low oxygen/high CO₂ conditions (106) or its degradation by the cysteine protease SpeB (107) or by other proteases contribute to bacterial dissemination and thus, invasive infection. Expression of M protein is regulated inversely to the one of SfbI and may favour the more CO₂-rich environment of deeper tissue sites. In invasive infections where protection against the immune system becomes more important, production of anti-phagocytic M protein is of advantage (106). In this context it will be particularly interesting to see whether M protein-mediated internalisation is hampering or facilitating streptococcal dissemination *in vivo*.

Transmigration of the microvascular endothelium of the blood brain barrier is considered as a crucial step in the pathogenesis of meningitis caused by GBS. It has been observed that GBS cross the blood brain barrier by transcytosis or injury of the cell layer (108). A study that uses a cell culture model of the microvascular endothelium in combination with a mouse model of bacteremia indicates that efficient adhesion to and internalisation by the cells is crucial for the transmigration of GBS through the endothelium. Impaired anchoring of LTA to the bacterial cell wall due to an inactivated glycosyltransferase gene *iagA*, had decreased their efficiency in internalisation and their virulence in a mouse model of meningitis (109). Very recently paracellular translocation of GBS through epithelial barriers has been reported. Soriani and colleagues used differentiated cervical and intestinal epithelial cells grown on transwell inserts (110). Although non-encapsulated strains of GBS were more efficient in adhesion and internalisation, their translocation capacity was similar to encapsulated ones. Transmission electron microscopy revealed an intimate association between GBS and the intercellular junctions and visualised the capability to cross the cell layer (110).

Cells of the immune system

As described above streptococci and staphylococci have developed molecular interactions that promote their uptake by non-phagocytic cells. Professional phagocytes present an eminent threat for the bacteria, well equipped with mechanisms to phagocytise, kill, and lyse the microorganisms. Resident macrophages that are situated in the infected tissue and polymorphonuclear neutrophils (PMNs) migrating from the blood to the site of infection constitute the first line of cellular defence. Recent data however strongly suggest that streptococci react on phagocytic uptake and express factors that allow their survival in the

hostile environment of the phagosome or even an escape from this compartment (Fig. 3). Considerable resistance of GAS strains to the intracellular killing mechanisms of PMNs has been observed (111). Such strains have the ability to escape from the phagocytic vacuole into the cytoplasm of the PMN (101). Furthermore, the streptococci internalised by the PMN exhibited a strongly increased virulence that was accompanied by an increased production of an important anti-phagocytic factor, the hyaluronic acid capsule. Staali and colleagues described the M protein of emm-type 1 together with the M-like protein H as crucial factors for intracellular survival in PMNs (112). A subsequent work suggests that M protein and protein H impair the fusion of azurophilic granules with the phagosome, an important step in the maturation of bactericidal phagosomes, thereby attenuating bacterial killing and degradation and thus, facilitating streptococcal intracellular survival (113). Further evidence for the streptococcal attempts to survive PMN phagocytosis comes from a genome wide transcription analysis, which demonstrates that GAS react upon contact with PMNs by up-regulation of a wide set of genes that are likely to contribute to streptococcal survival in PMNs and virulence (114, 115). The ability of GAS to accelerate neutrophil apoptosis (115) may allow the bacteria to escape from the phagocyte in a highly virulent state. A further, intriguing hypothesis is based on the capability of PMNs to cross vascular tissue. Streptococci internalised by PMNs may exploit them as a vehicle for their dissemination over the blood stream (101).

The importance of macrophages in clearance of GAS infection was demonstrated in a mouse model. Specific depletion of macrophages led to increased lethality. Viable macrophages took up streptococci readily by phagocytosis. Intraphagosomal killing appeared to start with detachment of capsular material. Lysosome fusion and degradation of the bacteria was observed, which explains the efficient eradication of the streptococci that was observed *in vivo*. The results suggest that macrophages are major players in preventing the long term persistence of streptococci and infection (116). Mechanisms of GAS, leading to survival in macrophages and furthermore streptococcal strategies to exploit internalisation into macrophages are not yet known, but examination of human biopsies from patients with soft tissue infections suggest that host phagocytic cells, in particular macrophages, were a reservoir for intracellular GAS during infection even after prolonged intravenous treatment with antibiotics (117).

Conclusions

Adhesion to the host tissue, its degradation, internalisation by the eukaryotic host cells and the capability of bacteria to penetrate both, epithelia and endothelia have substantial impact on the invasive character of Gram-positive cocci. The strategies applied by the different bacterial species streptococci and staphylococci show considerable similarities. Like streptokinase of GAS, staphylokinase of *S. aureus* (41), is a powerful tool for the activation of plasminogen. Some subspecies of streptococci like *S. pneumoniae* rely on other factors like their α -enolase to exploit the broad band activity of this plasma protease. Internalisation of Gram-positive cocci that appears to be a double-edged sword during infections, is strongly influenced by fibronectin binding proteins. Since it contributes to bacterial persistence, interfering therapies could be useful for the treatment for chronic infections. However, there are indications that interfering with internalisation may in some cases increase the risk of bacterial dissemination. To treat infections with Gram-positive cocci by controlling their adhesion and internalisation we will need comprehensive information about the involved bacterial and host factors, deeper insight into the tissue-specific mechanisms of pathogenesis and into the consequences of interfering treatments. It is very likely, that such therapeutic interventions will have to be well tailored to the causative bacterial strain and to the site(s) of infection.

Figures

Figure 1: Comparison of the entry mechanisms of *Streptococcus pyogenes* and *Staphylococcus aureus*. Streptococci carrying SfbI/F1 induce the formation of large invaginations through aggregation of host caveolae without any detectable cytoskeletal rearrangements (A). SfbI/F1 negative streptococcal isolates internalise in host cells either via an early intimate contact to microvilli resulting in a zipper-like internalisation mechanism (B) or the formation of membrane-ruffles is induced resulting in a trigger-like uptake-mechanism (C). *S. aureus* internalisation into HeLa cells is characterized by the formation of large membrane-ruffles thus following the trigger-like internalisation process (D). Bars represent 1 µm.

Figure 2: Internalisation of Gram-positive cocci by host cells. Schematic diagram of internalisation and intracellular trafficking of SfbI mediated (A), M-protein mediated (B) internalisation of *Streptococcus pyogenes* and FnBP's-mediated (C) internalisation of *Staphylococcus aureus* by epithelial host cells. In all the depicted internalisation processes fibronectin acts as a bridging molecule to bind the Gram-positive cocci to $\alpha 5 \beta 1$ -integrin. SfbI-protein triggers an aggregation of caveolae underneath attached streptococci, which results in the formation of invaginations resulting in internalisation. By co-opting this host cell endocytotic pathway, SfbI-carrying streptococci prevent fusion with lysosomes, because the intracellular streptococci reside inside caveosomes (A). In the case of M-protein and FnBP's mediated internalisation *S. pyogenes* (B) and *S. aureus* (C) after $\alpha 5 \beta 1$ -integrin clustering trigger an internalisation which follows the classical endocytotic pathway. Consequently such bacteria end up in phagolysosomes.

Figure 3: Possible fates of GAS in polymorphonuclear neutrophils. Streptococci that are phagocytised by PMNs may prolong their survival by impeding the phagosome maturation (112, 113). Also, escape from the phagosome into the neutrophil's cytosol has been reported that may allow persistence in the phagocyte (111). Contact with GAS accelerates apoptosis of PMNs which may allow the escape of internalised bacteria from the neutrophil (115).

Table 1: Fibronectin-binding Adhesins of Pathogenic Streptococci and *S. aureus*.

Bacteria	Adhesin	Comment	References
GAS	Fba, FbaB		(118, 119)
	FBP54	anchorless	(120)
	Lipoteichoic acid (LTA)		(8, 10, 121)
	M and M-like proteins		(122, 123)
	SfbI, SfbII (Protein F, Protein F2)		(30, 75)
	Serum opacity factor (Sof)		(71, 124, 125)
GGG/GCS	GfbA		(126)
GBS	FbsA		(127, 128)
<i>S. pneumoniae</i>	PavA	anchorless	(129)
<i>S. aureus</i>	FnBP-A, FnBP-B	MSCRAMM ^{*)}	(90, 130)
	Eap	SERAM ^{*)}	(131)
	Emp	SERAM	(132)

^{*)} MSCRAMM (microbial surface components recognizing adhesive matrix molecule); SERAM (secretable expanded repertoire adhesive molecule)

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