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REVIEW

Streptococcus mitis: walking the line between commensalism and pathogenesis

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SUMMARY

Streptococcus mitis **is a viridans streptococcus and a normal commensal of the human oropharynx. However,** ***S. mitis*** **can escape from this niche and cause a variety of infectious complications including infective endocarditis, bacteraemia and septicaemia. It uses a variety of strategies to effectively colonize the human oropharynx. These include expression of adhesins, immunoglobulin A proteases and toxins, and modulation of the host immune system. These various colonization factors allow** ***S. mitis*** **to compete for space and nutrients in the face of its more pathogenic oropharyngeal microbial neighbours. However, it is likely that in vulnerable immune-compromised patients** ***S. mitis*** **will use the same colonization and immune modulation factors as virulence factors promoting its opportunistic pathogenesis. The recent publication of a complete genome sequence for** ***S. mitis*** **strain B6 will allow researchers to thoroughly investigate which genes are involved in** ***S. mitis*** **host colonization and pathogenesis. Moreover, it will help to give insight into where** ***S. mitis*** **fits in the complicated oral microbiome. This review will discuss the current knowledge of** ***S. mitis*** **factors involved in host colonization, their potential role in virulence and what needs to be done to fully understand how a an oral commensal successfully transitions to a virulent pathogen.**

INTRODUCTION

Streptococcus mitis is an interesting organism. It has generally been considered a relatively benign oral streptococcus and member of the oral commensal flora. Nevertheless, *S. mitis* can cause a range of invasive disease in humans and it is emerging as a cause of bloodstream infections in neutropenic and immune-compromised patients, and in patients undergoing cytotoxic anti-cancer chemotherapy (Marron *et al.*, 2000; Ahmed *et al.*, 2003; Hoshino *et al.*, 2005; Husain *et al.*, 2005; Han *et al.*, 2006; Kohno *et al.*, 2009). Among the oral streptococci, *S. mitis* is a leading cause of infective endocarditis and bacteraemia. In studies by the Health Protection Agency (HPA) in the UK the rate of bacteraemia caused by *S. mitis* exceeded that of group A or Group B streptococci (HPA, 2009). In these successive studies *S. mitis* bacteraemia increased from 1.9 cases per 100,000 in the period 2002–2004 to 2.4 cases per 100,000 in 2008 (HPA, 2004, 2009). More alarmingly, the *S. mitis* strains isolated from bacteraemic patients in these studies were routinely resistant to commonly used antibiotics (HPA, 2009). These results echo previous observations in the USA, which demonstrated high rates of antimicrobial resistance in *S. mitis* isolates (Doern *et al.*, 1996). Patients tend to become infected with their own commensal strains and the mortality rate from *S. mitis* bacteraemia ranges from 6 to 30% (Bochud *et al.*, 1997). So is *S. mitis* a commensal or a

pathogen? A recent analysis of the genome of *S. mitis* strain B6 sought to identify any genetic factors that may distinguish it from its more pathogenic relative, *Streptococcus pneumoniae* (Denapaite *et al.*, 2010). However, the majority of identified virulence factors present in the *S. pneumoniae* genome are also present in the *S. mitis* genome.

To make a significant impact on the treatment of *S. mitis* infections there needs to be a greater understanding of how *S. mitis* colonizes the human oropharynx. A thorough examination of how *S. mitis* interacts with other oral commensal organisms and the host immune system will hopefully help to identify how it makes the transition from a commensal to a pathogenic state. This review serves to briefly examine the state of *S. mitis* research and will hopefully act as a call to arms to streptococcal researchers to answer the question of how a commensal organism with few identified toxins and virulence factors can be a successful pathogen in the human host.

THE GENOME OF *S. MITIS*

The physiological similarity of *S. mitis* and its close relative *S. pneumoniae* has long been established. However, only very recently have several articles investigated the genetic basis for this by analysing the sequence and structure of the *S. mitis* genome (Siboo *et al.*, 2003; Kilian *et al.*, 2008; Romero *et al.*, 2009; Denapaite *et al.*, 2010; Johnston *et al.*, 2010). These illuminating studies have provided an insight into the diverse genome structures of different *S. mitis* isolates in comparison with the relatively tight clonality of *S. pneumoniae* isolates sequenced to date. The *S. mitis* genomes sequenced to date have a GC content of 40%, are between 1.8 and 2.1 Mb in length and encode roughly 2100 genes. This is similar to the sequenced *S. pneumoniae* genomes. Denapaite *et al.* (2010) published the first completed *S. mitis* genome sequence of strain B6. The sequence of B6 was compared with the unfinished genome sequence of *S. mitis* strain NCTC 12261 and sequences of *S. pneumoniae* strains and what was particularly striking was the cross-relationship of gene distribution relative to the origin of replication of the *S. pneumoniae* R6 genome to that of *S. mitis* B6. Altering the layout of genes with respect to the position of the origin of replication can change temporal expression of those genes and could alter the response of an organism to external environments and stimuli. This may provide some explanation as to why *S. pneumoniae* is a successful pathogen of the respiratory tract whereas *S. mitis* is rarely found causing lung disease. However, the distribution of genes found in strain B6 has yet to be verified in other *S. mitis strains*. Future bioinformatic analysis of different sequenced *S. mitis* strains will confirm whether this is the case. Alternatively, an altered repertoire of virulence genes in *S. mitis* compared with *S. pneumoniae* may account for its reduced pathogenicity in the human host. It is possible that the full complement of virulence genes is needed to initiate disease because small changes in virulence gene content in *S. pneumoniae* can lead to marked differences in pathogenicity.

Another notable feature of the *S. mitis* B6 genome is that it contains homologues of many of the previously identified *S. pneumoniae* virulence factors (Denapaite *et al.*, 2010). Several autolysins, choline-binding proteins and cell-wall-anchored adhesins are present. Future work will hopefully clarify why the presence of these genes does not equip *S. mitis* with the necessary virulence factor arsenal to overwhelm the human immune system as successfully as *S. pneumoniae*. It is possible that the observed absence of genes involved in the synthesis of a polysaccharide capsule in strain B6 is the critical distinguishing factor between the two organisms. This would presumably render *S. mitis* extremely vulnerable to neutrophil killing. Some strains of *S. mitis* contain a capsule locus but expression of capsule genes or their distribution in *S. mitis* isolates has not been thoroughly investigated (Kilian *et al.*, 2008). Johnston *et al.* (2010) performed a microarray analysis of several strains of *S. mitis* and detected the presence of capsule loci of non-pneumococcal serotypes. A thorough analysis of the gene sequence and expression profile of the putative capsule loci of *S. mitis* clinical strains is imperative. This will help to determine whether the capsule may facilitate the evasion by *S. mitis* of the host immune system as is seen with the *S. pneumoniae* capsule. Also absent from B6 are the choline-binding proteins PspA, PspC, PcpA, a hyaluronidase and a pneumolysin. Conversely, studies screening clinical *S. mitis* isolates for virulence factors have identified a significant carriage rate for pneumolysin homologues (Jefferies *et al.*, 2007; Farrand *et al.*, 2008). The seemingly conflicting data between sequenced strains and patho-

gens found in the clinic provides a strong argument for further sequencing of *S. mitis* genomes. It is important to note that four further *S. mitis* genome sequences are in progress. Three of these strains; SK597, SK564 and SK321, are identified as oral strains not associated with pathogenic disease. A thorough comparison of all isolates will hopefully shed light on the genetic criteria that discriminate between pathogenic and commensal *S. mitis* strains.

THE COMMENSAL LIFESTYLE OF *S. MITIS*

Streptococcus mitis is a pioneer colonizer of the neonatal human oropharynx and is a numerically significant commensal throughout life (Li *et al.*, 2004; Kirchherr *et al.*, 2005, 2007; Bek-Thomsen *et al.*, 2008). Interestingly, the origin of *S. mitis* remains to be determined. It has been proposed that commensal bacteria are transferred from the primary care-giver, the external environment, and from other areas of the respiratory tract (Hohwy *et al.*, 2001). Successful colonization of the oropharynx is dependent on a number of different factors. Initially, *S. mitis* needs to express a number of adhesins that promote primary attachment to host tissues (Kawashima *et al.*, 2003; Plummer & Douglas, 2006). Analysis of its genome sequence confirmed that *S. mitis* B6 encodes numerous cell-wall-anchored and choline-binding proteins that are putative adhesins (Denapaite *et al.*, 2010). Few, if any, of these proteins have been experimentally confirmed to promote attachment to host oropharyngeal cells though several are homologues of known pneumococcal adhesins.

Following primary attachment, *S. mitis* needs to employ several strategies to circumvent the innate and acquired immune systems of the host. Research indicates that neonatal saliva contains secretory immunoglobulin A (sIgA) antibodies that react with *S. mitis* (Kirchherr *et al.*, 2007). However, these antibodies do not completely block adherence and subsequent colonization. In an interesting study by Wirth *et al.* (2008) colonization of the oropharynx of human infants appeared to induce strain-specific sIgA, which appeared to clear the IgA targeted strain. However, *S. mitis* exhibits clonal and antigenic diversity resulting in frequent turnover of different strains of *S. mitis* in the oropharynx (Kirchherr *et al.*, 2005, 2007). This results in an observed constant succession of different clonal variants of *S. mitis* in the human oropharynx, presumably facilitated by genetic recombination of *S. mitis* strains with their nearest viridans streptococcal neighbours (Johnsborg *et al.*, 2008). In addition to this, *S. mitis* can produce an IgA1 protease. This therefore provides *S. mitis* with two strong defences against the host adaptive immune system.

It is not known exactly how *S. mitis* interacts with the host innate immune system. However, *S. mitis* has been shown to exert a strong immunomodulatory effect on human cells. Incubation of gingival epithelial cells with *S. mitis* induces the expression of human β-defensin 2 (hBD-2; Eberhard *et al.*, 2009) a host antimicrobial peptide that can kill oral pathogens. In contrast, *S. mitis* is remarkably tolerant to hBD-2 and other antimicrobial peptides (Nishimura *et al.*, 2004; Ouhara *et al.*, 2005) (Fig. 1A). In addition to this, *S. mitis* can also modulate the expression of the proinflammatory chemokine interleukin-8 (IL-8; Eberhard *et al.*, 2009) (Fig. 1C). The tissue destruction associated with periodontal disease is largely mediated by the host inflammatory response to infection by oral pathogens. On its own, *S. mitis* does not promote IL-8 expression. However, co-incubation of *S. mitis* with the oral pathogens *Fusobacterium nucleatum* or *Aggregatibacter actinomycetemcomitans* dampens the production of IL-8 triggered in response to the pathogens (Zhang *et al.*, 2008; Sliepen *et al.*, 2009). Taken together these data indicate that *S. mitis*, as a beneficial commensal, can supplement host immunity and promote its own survival in the crowded oral niche in the face of competition from microbial pathogens.

PHYSIOLOGY

The physiology of *S. mitis* cells closely resembles that of its nearest genetic neighbour, *S. pneumoniae*. The *S. mitis* cells are arrow-headed in shape and approximately 0.5 μm in length. The cells grow in pairs or short chains. Like *S. pneumoniae*, *S. mitis* contains phosphorylcholine residues in the teichoic acids of its cellular envelope (Bergström *et al.*, 2000). The *S. mitis* B6 genome encodes homologues of the pneumococcal genes responsible for the incorporation of choline into its teichoic acid, the *licD1* and *licD2* operons and *licD3* (Denapaite *et al.*, 2010). Choline is an amino-alcohol that serves as an anchoring receptor for the choline-binding proteins (Hakenbeck *et al.*, 2009). Choline-binding proteins

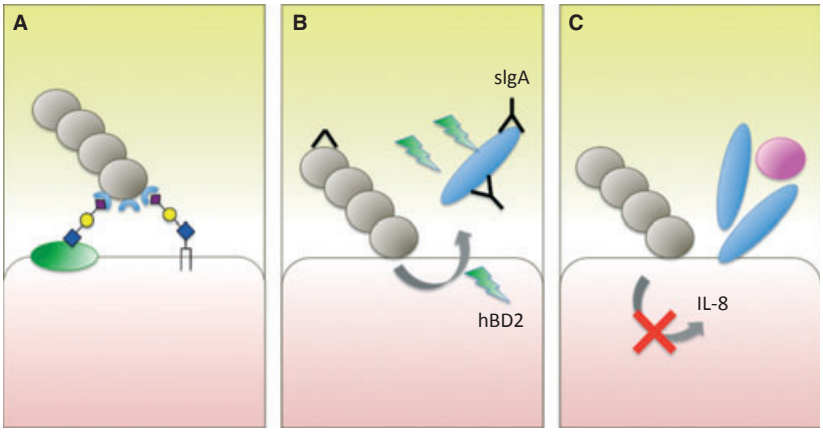

Figure 1 Colonization of the oropharynx by *Streptococcus mitis*. The *S. mitis* uses several strategies to successfully colonize the human oropharynx. (A) Initial attachment. The first step in *S. mitis* oral colonization involves direct attachment of the bacterial cells to oral epithelia. *Streptococcus mitis* has been shown to adhere to host cell glycoproteins and glycolipids. (B) Immune evasion. Following initial attachment of *S. mitis*, the host oral epithelial cells induce expression of human β-defensin-2 (hBD-2). *Streptococcus mitis* has a relatively high tolerance for hBD-2, whereas it effectively kills other oral pathogens such as *Fusobacterium*. *S. mitis* can also avoid the antibacterial effects following antibody recognition by cleaving serum immunoglobulion A (sIgA) with its sIgA protease. (C) Immune modulation. *Streptococcus mitis* modulates the host immune response to both commensals and pathogens colonizing the oropharynx by suppressing the expression of the proinflammatory chemokine interleukin-8 (IL-8). Together these three strategies ensure that *S. mitis* is a long-term resident of the oral ecosystem promoting its own survival and collaborating with the host immune system to suppress pathogen overgrowth and an excessive inflammatory immune response against the oral microbial residents.

bind to the phosphorylcholine residues present in the cell wall teichoic acids and lipoteichoic acids via a conserved choline-binding domain (Llull *et al.*, 2006). This domain is able to specifically bind to choline or its structural analogues; ethanolamine and diethylaminoethanol (Campuzano *et al.*, 2009). This apparent flexibility in affinity for amino-alcohols could explain why strains of *S. mitis* have been identified that lack choline-containing teichoic acids and rather substitute ethanolamine as the teichoic acid amino-alcohol (Bergström *et al.*, 2003). Several choline-binding proteins have been identified in *S. pneumoniae* as virulence factors and *S. mitis* encodes homologues of many of these proteins. However, the roles of these proteins in *S. mitis* virulence and host–cell interaction has not been investigated.

One of the choline-binding proteins, CbpD, is a cell wall hydrolase whose expression is activated under control of ComX, the regulator of competence gene expression (Johnsborg *et al.*, 2008). Competence for genetic transformation is thought to play a major role in gene acquisition and clonal diversity for oral streptococci. *Streptococcus mitis* strains encode competence operons homologous to that observed in *S. pneumoniae* (Bensing *et al.*, 2001a,b; Denapaite *et al.*, 2010). Although the Com operon in *S. mitis* NCTC 12261 lacks the full suite of genes required for full competence (Kilian *et al.*, 2008). Competence is activated via the two component system ComDE in response to high concentrations of the competence-stimulating peptide, the *comC* gene product. Activation of CbpD in *S. pneumoniae* promotes lysis of non-competent cells and DNA release in a process termed fratricide. The competence genes in *S. mitis* SF100 are fully functional (Bensing *et al.*, 2001a). It is likely that this process contributes to the clonal diversity in *S. mitis* observed in epidemiological studies. Competence and genetic transformation also play a large role in the acquisition of antibiotic resistance genes and so play a role in the pathogenesis of *S. mitis*. Studies have shown that *S. mitis* can both acquire resistance genes from neighbouring oral microbes and donate resistance genes to its more pathogenic relative *S. pneumoniae* (Hannan *et al.*, 2010). It is relatively straightforward to detect the transfer of antibiotic resistance genes in the laboratory. However, the likelihood remains that *S. mitis* and *S. pneumoniae* can act as reservoirs of a suite of virulence factors. Given the emergence of vaccine escape strains of *S. pneumoniae*, it is critical to analyse the potential for *S. mitis* to donate capsule genes that could potentially alter the capsule epi-

topes that are targeted by the currently used pneumococcal vaccine.

VIRULENCE FACTORS

Very few studies have looked at the role of putative *S. mitis* virulence factors in animal models of disease. As a result there is a paucity of information relating *S. mitis* genes with homology to known streptococcal virulence factors to their roles in disease pathogenesis. Most of the information on genes encoding virulence factors is derived from genetic studies sequencing the *S. mitis* genome or screening *S. mitis* isolates for *S. pneumoniae* homologues. Table 1 summarizes the known virulence genes in *S. pneumoniae* that have homologues in *S. mitis*. The following sections describe what is known about various genes and proteins that contribute to the pathogenesis of *S. mitis* in human disease.

PHAGE PROTEINS

The direct binding of *S. mitis* to human platelets contributes to the pathogenesis of *S. mitis* infective endocarditis (Mitchell *et al.*, 2007; Seo *et al.*, 2010). Platelet binding by *S. mitis* strain SF100, an endocarditis isolate, is mediated in part by two bacteriophage-encoded proteins, PblA and PblB (Bensing *et al.*, 2001a,b). Deletion of these genes results in a reduction in SF100 platelet-binding activity and virulence in a rabbit co-infection model of infective endocarditis (Mitchell *et al.*, 2007). The genes encoding these proteins reside within the temperate bacteriophage ØSM1, a member of the Siphoviridae family (Siboo *et al.*, 2003). Interestingly, the surface expression of these two platelet adhesins is dependent on the ØSM1 lytic cycle. Expression of the SM1 phage-encoded holin and lysin results in the permeabilization of a subpopulation of SF100 cells, releasing PblA and PblB into the surrounding medium (Mitchell *et al.*, 2007). Following this, both PblA and PblB attach to choline residues within the cell walls of viable bacteria where they then mediate the binding of viable bacteria to platelets. It was subsequently shown that α2-8-linked sialic acid of the platelet membrane ganglioside GD3 is the target receptor for the phage-encoded proteins PblA and PblB (Mitchell and Sullam, 2009).

Interestingly, Willner *et al.* (2010) showed that ØSM1 is prevalent in the normal human population through metagenomic screening of oropharyngeal viruses in healthy individuals. This supports previous data showing that both commensal and disease-causing *S. mitis* isolates express proteins that react with PblA and PblB antiserum on their cell surfaces (Mitchell *et al.*, 2007). Willner *et al.* (2010) were also able to show that commonly ingested substances efficiently induced ØSM1 gene expression in *S. mitis* SF100. These data together infer that *S. mitis* is already pre-coated with these phage-encoded platelet-binding virulence factors in the oropharynx before entering the bloodstream through damaged gingival or oral mucosal surfaces. It is likely that the platelet binding proteins can also promote attachment to oral mucosal surfaces but further research is needed to confirm this.

It is important to note that while PblA and PblB substantially contribute to the adherence of *S. mitis* to platelets, the other virulence factors that contribute to the adherence of *S. mitis* to platelets, as yet, remain to be fully elucidated. Very recently Seo *et al.* (2010) identified that the lysin of bacteriophage ØSM1 can also contribute to the attachment of *S. mitis* SF100 to platelets by binding directly to platelet-associated fibrinogen. The lysin specifically interacts with the D fragment of the Aα and Bβ chains and deletion of the lysin genes results in a more profound loss of virulence and platelet-binding activity that is seen in the PblA-deleted and PblB-deleted strain. This identification of a phage lysin with a direct role in the pathogenesis of a microbial organism serves as a cautionary tale for researchers wishing to use phages as antimicrobial chemotherapies because sub-lytic concentrations may occasionally have the unwanted effect of enhancing the organism's virulence.

Immunoglobulin A1 protease

Streptococcus mitis can produce an IgA1 protease, related to the IgA1 proteases of *S. oralis*. These proteases are cell-wall-anchored zinc metalloproteases that cleave peptide bonds in IgA1 (Senior & Woof, 2006). This proteolytic cleavage of IgA1 generates Fab and Fc fragments which can, in essence, decouple the recognition of *S. mitis* antigens from mechanisms for their elimination. In addition to this, any remaining bacterial bound Fab fragments could mask relevant epitopes from the immune system and pre-

Table 1 A comparison of virulence gene carriage in *Streptococcus pneumoniae* and *Streptococcus mitis*

S. pneumoniae virulence gene	Function	Present in *S. mitis*	Known function in *S. mitis*	References
Toxins and secreted enzymes				
Pneumolysin	Cholesterol dependent cytolysin	+/–	Mitilysin/Lectinolysin: Cholesterol dependent cytolysin +/– lectin binding domain	Farrand *et al.* (2008); Jefferies *et al.* (2007); Denapaite *et al.*, 2010; Johnston *et al.*, 2010
Hyaluronidase	Degrades hyaluronic acid in connective tissue	+/–	Expression and function untested	Johnston *et al.* (2010); Denapaite *et al.* (2010)
LPXTG proteins				
NanA	Neuraminidase	+/–	Neuraminidase	Johnston *et al.* (2010); Denapaite *et al.* (2010)
ZmpB	IgA protease	+/–	IgA protease	Johnston *et al.* (2010); Denapaite *et al.* (2010)
PsrP	Keratin 10 binding protein	+/–	MonX, Expression and function untested	Denapaite *et al.* (2010)
PulA	Alkaline amylopullulanase	+	Expression and function untested	Denapaite *et al.* (2010)
Choline-binding proteins				
LytA	Autolysis	+/–	Autolysis	Johnston *et al.* (2010); Denapaite *et al.* (2010)
LytB	Autolysis	+/–	Autolysis	Johnston *et al.* (2010); Denapaite *et al.* (2010)
PcpA	Host cell binding	–		Denapaite *et al.* (2010)
PspA	Host cell binding	–		Denapaite *et al.* (2010)
PspC	Host cell binding	–		Denapaite *et al.* (2010)
PcpC	Host cell binding	–		Denapaite *et al.* (2010)
Capsule locus genes	Polysaccharide capsule production, promotes Immune evasion	+/–	Expression and function untested	Johnston *et al.* (2010); Denapaite *et al.* (2010)
Com locus genes	DNA uptake	+	DNA uptake	Bensing *et al.* (2001)a; Denapaite *et al.* (2010); Johnston *et al.*, 2010
Phage adhesins				
PblA/PblB	Expression and function untested	+/–	Platelet adhesin	Mitchell *et al.* (2007); Mitchell & Sullam (2009); Romero *et al.* (2009)
Lysin	Carriage unknown	+/–	Fibrinogen adhesin	Seo *et al.* (2010)

+, gene present in all strains tested; +/–, gene present in some strains; –, gene not present in tested strains.

vent the binding of other antibody isotypes, activation of complement, and complement-mediated lysis. The functional biochemistry of the *S. mitis* IgA1 proteases has been thoroughly investigated but the direct role of these proteases in the virulence of *S. mitis* models of infection has yet to be established.

LPXTG PROTEINS

There are 18 predicted cell-wall-associated surface proteins bearing the cell wall attachment motif LPXTG encoded within the genome of *S. mitis* B6 (Denapaite *et al.*, 2010). One of these proteins, NanA2, encodes a putative sialidase homologous to sialidases of *S. pneumoniae*. Another of these cell-wall-associated proteins, MonX, is a homologue of the *S. gordonii* platelet-binding adhesin GspB and the *S. pneumoniae* keratin-binding adhesin PsrP (Takamatsu *et al.*, 2005, 2006; Shivshankar *et al.*, 2009). The gene encoding this protein is not present in the genome of *S. mitis* NCTC 12261 and its distribution frequency in clinical and commensal isolates is unknown. MonX in B6 is encoded alongside the genes encoding its partner accessory secretion sys-

tem and glycosylation enzymes. It will be important to demonstrate whether these LPXTG proteins play a role in the pathogenesis and host colonization of *S. mitis*. Future studies that examine the function of these proteins in wild-type and isogenic deletion mutant strains *in vitro* and *in vivo* will hopefully dissect their contribution to the virulence of *S. mitis*.

CYTOLYSIN

Streptococcus mitis does not elaborate a wide range of toxins but it has been shown to both encode and express a toxin similar to the *S. pneumoniae* pneumolysin and the *S. intermedius* intermedilysin (Whatmore *et al.*, 2000; Jefferies *et al.*, 2007; Farrand *et al.*, 2008; Johnston *et al.*, 2010) both cholesterol-dependent, pore-forming toxins. Jefferies *et al.* (2007) identified an *S. mitis* -specific toxin, named mitilysin that is a homologue of pneumolysin encoded by the *mly* gene. Mly is functionally similar to pneumolysin in haemolytic assays and cross-reacts with pneumolysin antibodies. Similarly, Farrand *et al.* (2008) identified another homologue, lectinolysin or LLY, which contains a lectin-binding domain that is unique among this family of toxins. This lectin-binding domain mediates specificity of the toxin for difucosylated residues within the Lewis y and Lewis b antigens. Interestingly, these toxins have not been identified within the sequenced genomes of B6 or NCTC 12261. However, homologues have been identified in clinical isolates (Neeleman *et al.*, 2004; Jefferies *et al.*, 2007). It is critical to ascertain the distribution of these genes in *S. mitis* isolates to determine the role of these toxins in *S. mitis* pathogenesis.

ASSOCIATED DISEASES

For the majority of people *S. mitis* is a commensal oral streptococcus that presents no great immunological threat. However, *S. mitis* has emerged as a significant pathogen in elderly patients, immunocompromised patients and in patients undergoing cytotoxic chemotherapy treatment for cancer. *Streptococcus mitis* is also an infrequent opportunistic pathogen of normal healthy infants and adults implicated in a wide range of diseases from dental caries, to bacterial infective endocarditis, bacteraemia, meningitis, eye infections and pneumonia. Very little is understood about how exactly *S. mitis* causes these various diseases although the direct binding of *S. mitis* cells to platelets in the human bloodstream has been directly implicated in the pathogenesis of *S. mitis* infective endocarditis (Mitchell *et al.*, 2007; Seo *et al.*, 2010). Platelet binding by *S. mitis* strain SF100 has been shown to be mediated in part by the phage-encoded adhesins PblA, PblB and ØSM1 Lysin (Bensing *et al.*, 2001a,b; Mitchell *et al.*, 2007; Mitchell & Sullam, 2009; Seo *et al.*, 2010). It is likely that other adhesins will be identified in the future as virulence factors and factors promoting both colonization and virulence. For example, the MonX protein identified in *S. mitis* strain B6 is a likely candidate adhesin that may promote both oral colonization and possibly platelet binding given its similarity with homologues to *S. gordonii* and *S. pneumoniae* (Takamatsu *et al.*, 2005, 2006; Shivshankar *et al.*, 2009; Denapaite *et al.*, 2010). Future work will hopefully confirm whether this is the case.

FUTURE PERSPECTIVES

So much about the biology of *S. mitis* remains unknown. It is vital to fully investigate the interaction of *S. mitis* with its human host and other members of the oral microbiota if we are to understand how exactly *S. mitis* makes the transition from a commensal to a pathogenic state. The full repertoire of *S. mitis* genes that contribute to the colonization of the oropharynx needs to be identified along with those genes that mediate the internalization of *S. mitis* into host oral epithelial cells (Stinson *et al.*, 2003; Rudney & Chen, 2006). The growing collection of *S. mitis* genome sequences will serve as valuable tools to aid in the dissection of molecular factors that contribute to *S. mitis* host colonization and virulence.

In addition to this, the mechanisms of how *S. mitis* dampens the inflammatory immune response when other pathogens stimulate inflammation needs to be systematically investigated. It is also possible that *S. mitis* may be resistant to sIgA deposition through the action of the IgA protease. As a result this could mediate resistance to neutrophil and other white blood cell phagocytosis or resistance to complement killing. The reason why *S. mitis* is so tolerant of antimicrobial peptides when periodontal pathogens such as *S. mutans* are not is also unknown. More work needs to be done to thoroughly investigate how

S. mitis interacts directly with the host mucosal epithelia with a view to developing therapeutic reagents that modulate or disrupt this interaction. Several animal models have been successfully used to dissect the progression of *S. mitis* infective endocarditis and some of the virulence factors involved. The mouse, rat and rabbit models of infective endocarditis and co-infection studies comparing wild-type strains vs. virulence factor knockout mutants have all yielded useful data (Lowy *et al.*, 1983; Mitchell *et al.*, 2007; Seo *et al.*, 2010). However, little is known about factors that contribute to *S. mitis* colonization in animal models. Host and pathogen factors that contribute to the virulence of *S. pneumoniae* have been investigated in mouse nasopharyngeal carriage models (Richards *et al.*, 2010). Hopefully, these models will be used in the future to thoroughly dissect factors that contribute to *S. mitis* colonization and virulence. Ideally, to combat *S. mitis* infections, one would aim to specifically decolonize *S. mitis* from the oropharynx but current evidence suggests that doing this would only facilitate expansion of more pathogenic oral microbes and promote subsequent damaging inflammation (Teughels *et al.*, 2007; Van Hoogmoed *et al.*, 2008; Sliepen *et al.*, 2009).

It is likely that the biological characteristic that make *S. mitis* a successful and beneficial commensal are also those that promote its opportunistic pathogenesis in immune-compromised patients. There is much work to be done to tease out the molecular mechanisms of *S. mitis* colonization, pathogenesis and immunobiology. However, in the long run it will contribute to our understanding of oral microbiology and oral immunology and hugely benefit vulnerable and immunocompromised patients.

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