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REVIEW

Pneumococcal modification of host sugars: a major contributor to colonization of the human airway?

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SUMMARY

***Streptococcus pneumoniae* colonization of the human airway is an essential precursor for disease; however, the mechanisms by which the bacterium establishes and maintains colonization are poorly understood. It is becoming increasingly clear that *S. pneumoniae* expresses glycosidases that can modify many glycan structures present in the human airway, including *N*-linked glycans, *O*-linked glycans, and glycosaminoglycans. Many of these glycosidases have been shown to contribute to *in vivo* colonization. Although the precise role of these glycosidases during colonization remains to be elucidated, *in vitro* assays suggest that pneumococcal modification of host sugars may contribute to colonization in a variety of ways. Experimental evidence supports a role for pneumococcal glycosidases in providing a carbon source for growth, biofilm formation, competition with other bacteria within the airway, and exposing receptors for adherence. Herein we review the ability of *S. pneumoniae* to modify host sugars and the functional effects of these modifications.**

INTRODUCTION

Despite the introduction of the pneumococcal conjugate vaccine, *Streptococcus pneumoniae* remains an important human pathogen causing an estimated 1.6 million deaths per year worldwide (World Health Organization, 2008). The majority of these deaths are the result of pneumococcal pneumonia, although *S. pneumoniae* is also an important cause of bacteremia and meningitis. Despite the importance of pneumococcal disease, the bacterium frequently colonizes the naso-oropharynx of healthy individuals. Although colonization is normally asymptomatic and cleared by the host, colonization of the upper respiratory tract is thought to be a critical step in the disease process. An increased understanding of the mechanisms by which *S. pneumoniae* establishes and maintains colonization of the host will aid our understanding of pneumococcal pathogenesis and potentially enable the development of more effective measures for disease prevention.

The human airway, including the epithelial cell surface, the mucin layer, secreted molecules, immune cells, and many of the normal bacterial flora, are heavily glycosylated. The ability of a bacterium to modify these glycan structures could contribute to the establishment and maintenance of colonization in numerous ways. Bacterial modification of host sugars could contribute to: revealing receptors for adherence, modification of host defense molecule function, progression of the bacteria through the mucin layer, interspecies competition, biofilm formation, and release of carbohydrates for growth (Fig. 1).

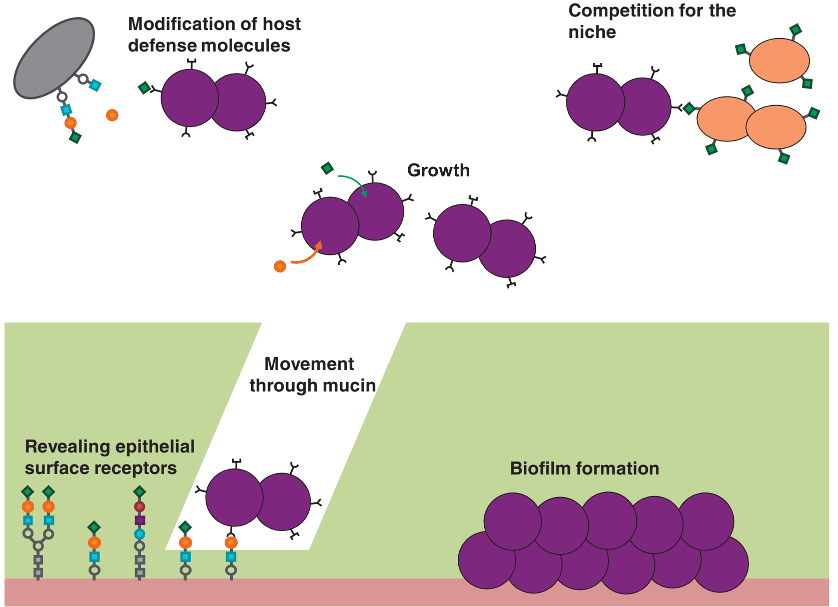

Figure 1 Potential roles of pneumococcal modification of airway glycans to bacterial colonization.

Streptococcus pneumoniae is adept at manipulating sugars. Each strain expresses one of more than 90 different carbohydrate capsules (Jin *et al.*, 2009). Furthermore, genome sequencing has revealed that sugars are fundamental to the lifestyle of *S. pneumoniae*. Thirty per cent of the transporters in *S. pneumoniae* are predicted to be sugar transporters, a greater proportion than is found in any other sequenced bacteria occupying the same niche (Tettelin *et al.*, 2001). The pneumococcus also encodes at least 10 extracellular sugar-cleaving enzymes (glycosidases) that have been shown to or could potentially modify glycan structures present in the airway (Table 1). Given the critical nature of pneumococcal colonization and the potential for the bacterium to manipulate sugars present in the airway, it seems likely that modification of host glycans contributes to the establishment and maintenance of pneumococcal colonization. Despite the potential importance of pneumococcal sugar modification, the ability of *S. pneumoniae* to modify glycan structures present in the airway and the biological contribution of this modification has, until recently, gone largely unstudied.

PNEUMOCOCCAL GLYCOSIDASES

The extracellular glycosidases expressed by *S. pneumoniae* include both exoglycosidases, which cleave terminal sugars, and endoglycosidases, which cleave internal sugar linkages. The cleavage specificity of many pneumococcal glycosidases has been defined in the context of use for biochemical carbohydrate manipulation (Table 1). Many pneumococcal glycosidases are specific for sugar structures present in the human airway, supporting the hypothesis that their role is to modify human glycan structures; for example, the β-galactosidase activity of BgaA is specific for galactose β1-4 linked to *N*-acetylglucosamine (Galβ1-4GlcNAc), commonly found in complex *N*-linked glycan structures (Zeleny *et al.*, 1997).

The majority of pneumococcal glycosidases have distinct cleavage specificities (Table 1), the exception being the expression of up to three neuraminidases that cleave terminal sialic acid (NanA, NanB, and NanC) (Camara *et al.*, 1994; Berry *et al.*, 1996; Tettelin *et al.*, 2001). It has been demonstrated that NanA and NanB possess neuraminidase activity; however, the enzymatic activity of NanC remains to be confirmed. The localization and cleavage specificity of NanA and NanB differ, suggesting that these neuraminidases may serve distinct functions (Gut *et al.*, 2008). NanA is localized to the cell surface in a sortase-dependent manner and cleaves α2-3- and α2-6-linked sialic acid. Recent structural analysis supports the hypothesis that NanA is a hydrolytic enzyme with broad specificity for different sialic acid linkages (Xu *et al.*, 2008). NanB, on the other hand, is secreted

Table 1 Summary of the characteristics of pneumococcal glycosidases

Glycosidase	Activity	Gene no[2]	Localization	Cleavage specificity	Reference[4]
StrH	*N*-acetylglucosaminidase	SP_0057	Surface exposed[1,3]	β1-2; β1-3; β1-6; β1-2	Clarke *et al.* (1995)
BgaC	β-galactosidase	SP_0060	Surface exposed	β1-3	Jeong *et al.* (2009)
SpuA	Pullulanase	SP_0268	Surface exposed[3]	α1-6	Bongaerts *et al.* (2000)
Hyl	Hyaluronate lyase	SP_0314	Surface exposed[3]	4; β13; β1	Berry *et al.* (1994)
Eng (SpGH101)	*O*-glycosidase	SP_0368	Surface exposed[3]	β13; 1; Ser/Thr	Caines *et al.* (2008)
EndoD	Endo-β-*N*-acetylglucosaminidase	SP_0498	Surface exposed[1,3]	R; α1-6; α1-3; β14; β14; Asn	Muramatsu *et al.* (2001)
BgaA	β-galactosidase	SP_0648	Surface exposed[3]	β1-4	Zahner & Hakenbeck (2000)
NanC	Neuraminidase[1]	SP_1326	Secreted[1]		
NanB	Neuraminidase	SP_1687	Secreted	α2-3	Berry *et al.* (1996)
NanA	Neuraminidase	SP_1693	Surface exposed[3]	α2-3; α2-6; α2-6	Camara *et al.* (1994)

[1]Predicted.
[2]location within the TIGR4 genome (Tettelin *et al.*, 2001).
[3]LPXTG.
[4]Reference cited is that in which the gene encoding the enzyme activity was first identified.
Symbols represent: ◆ sialic acid, ● galactose, ○ mannose, ■ *N*-acetylglucosamine, □ *N*-acetylgalactosamine, ▲ glucose, ⬘ glucuronic acid, *R* = can be further substituted.

and has strict specificity for α2-3-linked sialic acid, suggesting that the enzyme is an intramolecular trans-sialidase (Gut *et al.*, 2008). The structure of NanB is highly similar to that of NanL the intramolecular trans-sialidase of *Macrobdella decora*. The active site and key residues that define the substrate specificity of NanL are conserved within NanB. Furthermore, NanB like NanL can convert *N*-acetylneuraminic acid to 2,7-anhydro-neuraminic acid which is the product of intramolecular trans-sialidases. The role of this class of enzymes is undefined, although it has been postulated that the sialic acid could be transferred to other glycoconjugates. Together these data support distinct functions for

NanA and NanB. The role of NanC is not yet defined, but the predicted amino acid sequence shares a high level of sequence identity with NanB (46%).

To the best of our knowledge the genes encoding the pneumococcal glycosidases are present in all strains screened with the exception of *nanB* and *nanC*, suggesting that the majority of these enzymes perform functions important to pneumococcal pathogenesis. Ninety-six per cent of isolates contain *nanB* and 51% of isolates contain the *nanC* locus (Pettigrew *et al.*, 2006). Some isolates from invasive disease lack both *nanB* and *nanC*, bringing into question the requirement for either of these loci; however, we cannot exclude the possibility that some *S. pneumoniae* strains encode another neuraminidase yet to be identified (S. King, unpublished).

With the exception of NanB and NanC, the characterized glycosidases secreted by *S. pneumoniae* are known or predicted to be attached to the surface of the bacterium (Tettelin *et al.*, 2001; Jeong *et al.*, 2009), suggesting that their contribution to pneumococcal pathogenesis may be more efficient in close proximity to the bacterial cell surface.

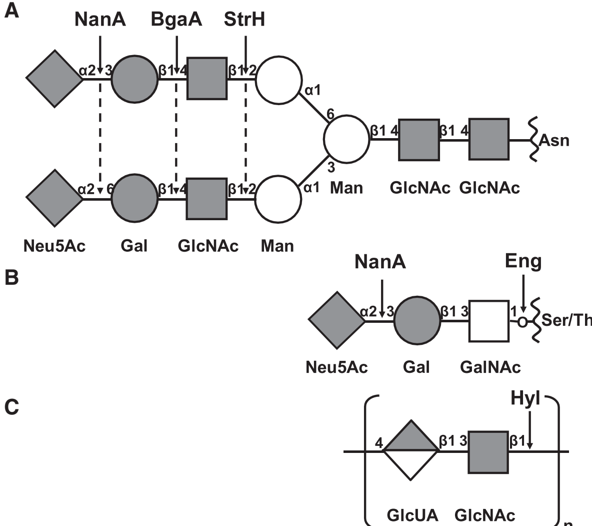

Figure 2 Schematic representation of representative glycan structures found in the airway. The structures of a complex *N*-linked glycan structure (A), a sialylated core-1 glycan structure (B), and the glycosaminoglycan hyaluronic acid (C) are shown. The sugar residues are labeled beneath their corresponding symbols (Neu5Ac, sialic acid; Gal, galactose; GlcNAc, *N*-acetylglucosamine; Man, mannose; GalNAc, *N*-acetylgalactosamine; GlcUA, glucuronic acid). Lines represent linkages between the sugar residues and numbers indicate the specific linkages. Arrows above the schematic indicate cleavage sites of the glycosidases; neuraminidase (NanA), β-galactosidase (BgaA), *N*-acetylglucosaminidase (StrH), *O*-glycosidase, (Eng), hyaluronate lyase (Hyl).

PNEUMOCOCCAL MODIFICATION OF AIRWAY GLYCANS

Glycan structures present in the human airway include *O*-linked glycans, *N*-linked glycans, and glycosaminoglycans (Fig. 2). *S. pneumoniae* has been demonstrated to modify representatives of all three classes of glycan structures through the activities of specific glycosidases (Berry *et al.*, 1994; King *et al.*, 2006; Marion *et al.*, 2009). Complex *N*-linked glycans are modified by the sequential cleavage of terminal sialic acid, galactose, and *N*-acetylglucosamine. This sequential deglycosylation results in the exposure of mannose; however, the significance of mannose exposure is unknown at this time. Sequential deglycosylation of *N*-linked glycans is performed by three surface-associated exoglycosidases, neuraminidase (NanA), β-galactosidase (BgaA), and *N*-acetylglucosaminidase (StrH). Mutation of each of the genes encoding these glycosidases prevented the exposure of mannose and the resultant phenotypes were complemented by the addition of exogenous enzyme (King *et al.*, 2006). Terminal sialic acid was also cleaved from terminal sialylated core-1 *O*-linked glycans in a NanA-dependent manner, before cleavage of the core structure [Galβ1-3*N*-acetylgalactosamine (GalNAc)] by *O*-glycosidase, Eng (Marion *et al.*, 2009). Hyaluronic acid consists of repeating disaccharide units of glucuronic acid (GlcUA) and *N*-acetylglucosamine (GlcUAβ1-3GlcNAcβ1-4). *S. pneumoniae* expresses a hyaluronate lyase that is sufficient and essential for degradation of hyaluronic acid (Berry *et al.*, 1994; Berry & Paton, 2000). Further characterization has demonstrated that the enzyme cleaves the β1-4 linkage of hyaluronic acid by β-elimination (for review see Jedrzejas, 2007). The other identified pneumococcal glycosidases are also predicted to contribute to modification of host glycan structures; for example, certain complex *O*-linked glycan structures found on mucin could be degraded by other pneumococcal glycosidases, including BgaC (Jeong *et al.*, 2009).

EFFECTS OF SUGAR MODIFICATION

Some published data support the hypothesis that glycosidases play an important role(s) during coloniza-

tion of the airway (King *et al.*, 2004; LeMessurier *et al.*, 2006; Song *et al.*, 2008a). Increased transcription of *nanA* is observed in pneumococcal variants that colonize the airway more efficiently, bacteria adherent to human airway epithelial cells, and bacteria colonizing the mouse airway. As interest increases in the function of these glycosidases, the role of an increasing number has been determined in *in vivo* colonization models. Mutants in some loci, for example *bgaA* and *strH*, have so far shown no detectable phenotype in mouse models of colonization (King *et al.*, 2006). Mutants in *nanA* show variable phenotypes in models of colonization, which may be the result of differences in methodology, including bacterial strains, animal strains and species, inoculating dose, and time-frame of experiments (King *et al.*, 2004; Orihuela *et al.*, 2004; Manco *et al.*, 2006). A mutant in the gene encoding Eng showed a significant reduction in the ability to colonize mice, supporting the hypothesis that modification of *O*-linked glycans contributes to colonization of the airway (Marion *et al.*, 2009). In contrast, a *bgaC* mutant showed increased ability to colonize the mouse nasopharynx during early stages of infection (Jeong *et al.*, 2009).

The contribution of glycosidases to colonization in model systems does not necessarily indicate that these enzymes contribute to colonization of the human airway. *S. pneumoniae* is a human-specific pathogen and glycan structures are highly diverse varying between species, individuals, and even different copies of the same molecule within the same individual. An additional challenge of using animal models is that as each glycosidase may contribute to colonization in multiple ways, elucidating the exact role of glycosidases in colonization *in vivo* will be challenging. For this reason, identifying potential specific roles of modification of host sugars has largely focused on *in vitro* assays. The roles of glycosidases in several processes thought to be critical in colonization are discussed below.

GROWTH

Streptococcus pneumoniae requires sugars for growth; however, the concentration of free sugars in the normal human airway is low (Philips *et al.*, 2003). Despite this paradox, there has been little work to determine how *S. pneumoniae* acquires sugars for growth during colonization. Given the abundance of complex glycan structures in the airway and the ability of *S. pneumoniae* to modify host sugars, it seems likely that sugars released from human glycans by pneumococcal glycosidases support the growth of the bacteria during colonization. Although it is unknown how *S. pneumoniae* grows in the airway, transcription of *nanA* and a putative sialic acid transporter is increased in the presence of human airway epithelial cells (Song *et al.*, 2008b).

In vitro studies have demonstrated that the bacterium can grow on *N*-linked glycans as a sole carbon source in a glycosidase-dependent manner. As predicted from the sequential deglycosylation studies described above, efficient growth was dependent on neuraminidase NanA, β-galactosidase BgaA, and *N*-acetylglucosaminidase StrH (Burnaugh *et al.*, 2008). Although sequential deglycosylation of *N*-linked glycans is dependent on NanA, a second pneumococcal neuraminidase with specificity for α2-3-linked sialic acid (Gut *et al.*, 2008), NanB, contributed to growth on *N*-linked glycans under different assay conditions that used defined media with *N*-linked glycans provided as a sole carbon source (King *et al.*, 2006; Burnaugh *et al.*, 2008). At the present time, the relative contributions of NanA and NanB to growth on *N*-linked glycans *in vivo* is unknown.

Given the importance of growth to pneumococcal colonization it is likely that there are multiple mechanisms to obtain sugars. It has been demonstrated that *S. pneumoniae* grows on mucin (Yesilkaya *et al.*, 2008). As mucin is decorated with both *N*-linked and *O*-linked glycans, it is unknown which sugar structure(s) *S. pneumoniae* is modifying. Growth on mucin was proposed to be *nanA*-dependent; however, while our studies confirm that *S. pneumoniae* can use mucin as a sole carbon source, we were unable to demonstrate a dependence on expression of NanA (G. Bobulsky personal communication). Further investigation is required to determine if *S. pneumoniae* can use *O*-linked glycans for growth, although, the bacterium can grow on the core-1 structure (Galβ1-3GalNAc) as a sole carbon source for growth *in vitro*; this disaccharide may be made available to the bacterium by the activity of the *O*-glycosidase Eng (C. Marion personal communication). Sugars released from other airway glycan structures known to be modified by pneumococcal glycosidases, for example hyaluronic acid, are likely also used as carbon sources.

ROLE OF GLYCANS IN BIOFILM FORMATION

It is becoming increasingly clear that biofilm formation at the mucosal surface is important for pneumococcal colonization, as it is for many bacterial pathogens (Moscoso *et al.*, 2009). Formation of a biofilm by *S. pneumoniae* is likely a multifactorial process, possibly including DNA, pneumococcal proteins, and capsular polysaccharide. Additionally, there is mounting evidence that modification and utilization of host sugars contributes to biofilm formation. Mutants in the genes encoding glycosidases NanA, NanB, BgaA, and BgaC are reduced in their ability to form an *in vitro* biofilm, as are mutants in a component of the pneumococcal galactose transporter and a component of a predicted ABC transporter within the *nanA*-locus (TIGR4 SP1682) (Munoz-Elias *et al.*, 2008; Parker *et al.*, 2009). A role for pneumococcal glycosidases was also supported by the report that NanA and NanB expression is increased in pneumococcal biofilms (Oggioni *et al.*, 2006; Trappetti *et al.*, 2009).

Recently published work suggests that free sialic acid contributes to efficient pneumococcal biofilm formation. Addition of sialic acid to an *in vitro* biofilm model significantly increased the number of adherent bacteria, whereas the addition of 26 other sugars had no effect (Trappetti *et al.*, 2009). The increased biofilm formation by sialic acid could be diminished by the addition of neuraminidase inhibitors or a sialic acid analog (Trappetti *et al.*, 2009). Approximately, a 100-fold increase in sialic acid concentration was required for efficient biofilm formation by a *nanA–nanB* locus mutant. The exact gene(s) that contributes to biofilm formation in this system remains to be defined because this locus is proposed to encode proteins for the transport and utilization of sialic acid in addition to NanA and NanB. No role was identified for *nanC*, the gene proposed to encode a third pneumococcal neuraminidase, the biological function of which remains unknown. Bacterial colonization was increased in a mouse model following addition of free sialic acid, an effect that was diminished by the addition of neuraminidase inhibitors or sialic acid analogs. The precise role of sialic acid in biofilm formation remains to be defined; however, these data suggest that sialic acid may be a signaling molecule that increases the capacity of *S. pneumoniae* to form a biofilm. Alternatively, sialic acid could be a component of the biofilm matrix.

BACTERIAL COMPETITION

In addition to the host glycan structures present in the airway, other bacterial species present in the respiratory tract are also decorated with sugars. The lipopolysaccharide of *Neisseria meningitidis* and some isolates of *Haemophilus influenzae* are decorated with sialic acid α2-3 linked to galactose (Gilbert *et al.*, 1996; Hood *et al.*, 2001). The presence of terminal sialic acid promotes survival by decreasing the bactericidal effect of complement through interaction with factor H (Ram *et al.*, 1998). *S. pneumoniae* cleaves sialic acid from the surface of these bacteria in a NanA-dependent manner (Shakhnovich *et al.*, 2002). Although it is not known if this desialylation occurs *in vivo*, these data suggest that pneumococcal neuraminidase targets a mechanism of bacterial adaptation to its host and thereby plays a role in interspecies competition in the human airway. This hypothesis is supported by several published studies reporting that *S. pneumoniae* may have inhibitory effects on *H. influenzae* in the human host. The hyaluronic acid capsule expressed by the respiratory pathogen *Streptococcus pyogenes* may also be targeted by *S. pneumoniae*. The capsule has been shown to modify the recognition of the bacteria with the host immune system and contribute to bacterial adherence (Dale *et al.*, 1996; Schrager *et al.*, 1998; Cortes & Wessels, 2009). Degradation of the hyaluronic acid capsule would potentially result in both release of sugars for growth and a reduction in the ability of *S. pyogenes* to colonize and cause disease.

BACTERIAL ADHERENCE

Despite several decades of research investigating pneumococcal adherence and the elucidation of several mechanisms, it is still not clear how *S. pneumoniae* initially adheres to the human airway epithelium [for review of adherence mechanisms see Hammerschmidt (2006)]. This maybe in part because *S. pneumoniae* uses multiple mechanisms of adherence, which may vary among strains in, their contribution to adherence, their adherence to discrete cell types, or their adherence to cells of different activation states.

Several studies suggest that receptors on airway epithelial cells are sialylated and are revealed by *S. pneumoniae* neuraminidase activity. Tong *et al.* (1999) demonstrated increased pneumococcal

binding to neuraminidase-treated chinchilla tracheas. Furthermore, *S. pneumoniae* adherence to human epithelial cells is inhibited in the presence of sialylated glycoconjugates (Barthelson *et al.*, 1998). More direct evidence that neuraminidase NanA reveals receptors for adherence was provided by studies demonstrating that a *nanA* mutant was significantly reduced in adherence to a human airway epithelial cell line (Detroit 562 ATTC CCL-138) and that the adherence of this mutant was complemented by the addition of purified neuraminidase (King *et al.*, 2006).

Initial adherence of pneumococci to resting epithelial cells is proposed to occur through binding to glycoconjugates (Hammerschmidt, 2006). This is supported by data indicating that *S. pneumoniae* adheres to a number of different glycan structures, including the GSL globoside (GalNAcβ1-3Galα1-4Galβ1-4Glc-Cer) (Cundell & Tuomanen, 1994), GalNAcβ1-4Gal found in gangliosides GM1 and GM2 (Krivan *et al.*, 1988), and the milk oligosaccharide lacto-*N*-neotetraose (LNnT) (Galβ1-4GlcNAcβ1-3Galβ1-4Glc) (Andersson *et al.*, 1988). In addition, *S. pneumoniae* adherence is inhibited by the addition of specific sugar structures including those containing Galβ1-3GlcNAc (Andersson *et al.*, 1983). It has not been determined if these interactions occur on the epithelial cell surface or what serves as the bacterial adhesin(s) to mediate binding.

Given the apparent role of NanA in adherence, the ability of *S. pneumoniae* to sequentially deglycosylate glycan structures and the evidence that initial adherence to epithelial cells involves a glycan structure, it is possible that other glycosidases may also contribute to pneumococcal adherence. Subsequent studies demonstrated that mutants in the genes encoding β-galactosidase, BgaA and *O*-glycosidase, Eng are significantly reduced in their adherence to human airway epithelial cell lines. These enzymes could further deglycosylate complex *N*-linked glycans (BgaA) and *O*-linked glycans (Eng) following the removal of terminal sialic acid; although, neither mutant could be complemented by the addition of recombinant enzyme (King *et al.*, 2006; Marion *et al.*, 2009). This result may be linked to the level of glycosidase activity under the conditions used or to the inability to add sufficient enzyme. Alternatively, these data may suggest that the role of these proteins in adherence is independent of their enzymatic activity. This hypothesis is supported by the demonstration that *S. pneumoniae* expressing enzymatically inactive BgaA is not reduced in its ability to adhere (J. Sladek personal communication). This finding also opens the intriguing possibility that pneumococcal glycosidases may perform functions independent of their enzymatic activity. This hypothesis was recently strengthened by the finding that NanA contributes to the invasion of brain endothelial cells independently of its enzymatic activity (Uchiyama *et al.*, 2009).

In contrast to the other glycosidase mutants, a *bgaC* mutant showed increased adherence to and invasion of human airway epithelial cells (Jeong *et al.*, 2009). This finding could be explained if Galβ1-3GlcNAc acts as a receptor on epithelial cells as suggested by Andersson *et al.* (1988). In this case, the absence of BgaC, which cleaves this sugar linkage, would increase adherence.

Finally the pneumococcal pullulanase, SpuA, has also been proposed to mediate adherence via binding to α-glucan molecules including glycogen (van Bueren *et al.*, 2007). The recombinantly expressed N-terminal carbohydrate binding molecule of SpuA binds to human airway epithelial cells, although, the absence of the glycoside hydrolase region in this molecule makes determination of the role of SpuA in adherence impossible at the current time.

GLYCOSIDASES OF OTHER BACTERIAL SPECIES

Evidence suggests that many of the other common respiratory pathogens like *H. influenzae* which inhabit the airway appear to lack the range of extracellular glycosidases expressed by *S. pneumoniae*. However, published studies demonstrate that related streptococci express glycosidases and that these may contribute to colonization by similar mechanisms. Although expression of glycosidases differs between streptococcal species, many species found in the oral cavity including *Streptococcus oralis*, *Streptococcus intermedius*, *Streptococcus sanguinis*, and *Streptococcus mutans* express a range of glycosidases (van der Hoeven *et al.*, 1990; Homer *et al.*, 1994). Further characterization of these glycosidases and their functions has been restricted by the limited knowledge of the genes encoding these enzymes; however, some functions have been determined. It is known that many of these glycosidases contribute to growth on mucin and other glycoconjugates, presumably by sequential

deglycosylation similar to that of *S. pneumoniae* (van der Hoeven *et al.*, 1990; Homer *et al.*, 1994; Byers *et al.*, 1999). These glycosidases may also play a role in other aspects of colonization; for example, *S. intermedius* hyaluronidase is proposed to contribute to biofilm dispersion. Other bacterial species that come into contact with a wide range of carbohydrate structures also express an array of glycosidases; for example, the genome of *Bifidobacterium longum* a gram-positive anaerobe that forms part of the normal microflora of the gastrointestinal tract, encodes more than 40 predicted glycosidases (Schell *et al.*, 2002). Together these data suggest that the functions of glycosidases described for *S. pneumoniae* have wider implications for other bacterial species.

CONCLUSION

Evidence increasingly supports the hypothesis that modification of host glycans contributes to pneumococcal pathogenesis. *S. pneumoniae* has been shown to modify *N*-linked glycans, *O*-linked glycans, and glycosaminoglycans found in the airway, through the activity of extracellular glycosidases. This review has focused on the role of glycosidases in colonization; however, there are also *in vivo* data suggesting that many of these enzymes also play a role during invasive disease (Polissi *et al.*, 1998; Hava & Camilli, 2002). *In vitro* experimental evidence suggests that the modification of host sugars can contribute to the ability of *S. pneumoniae* to grow and form biofilms in the airway, compete with other bacterial species within the same niche, and adhere to human epithelial cells. The contribution of pneumococcal glycosidases to other stages of colonization, including progression of the bacteria through the mucin layer and modification of the interaction with the host immune system, remain to be determined. Our understanding of the contribution of glycosidases to pneumococcal colonization will also be increased by investigation of the roles of other pneumococcal glycosidases, like endo-β-*N*-acetylglucosaminidase EndoD, in colonization. The next major challenge will be determining the precise role of these enzymes *in vivo*. This will be complicated by the multiple functions of these glycosidases and differences in glycosylation between species. Despite these challenges, the increasing evidence that these glycosidases play an important role in pneumococcal pathogenesis suggests that targeting these enzymes may be exploited in the development of new therapeutic or preventative measures.

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