Structural Characterization of *Streptococcus pneumoniae* Serotype 9A Capsule Polysaccharide Reveals Role of Glycosyl 6-O-Acetyltransferase *wcjE* in Serotype 9V Capsule Biosynthesis and Immunogenicity*

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**Background:** The identity and immunogenicity of *wcjE*-mediated capsule features in *Streptococcus pneumoniae* serotype 9V are unclear.

**Results:** Isogenic serotype 9A lacks 6-O-acetylation of βManNAc present in the 9V capsule.

**Conclusion:** *wcjE* mediates βManNAc-6-O-acetylation, a modification that is preferentially targeted by anti-serotype 9V antibodies.

**Significance:** Elucidating the role of the widely conserved capsule biosynthesis gene *wcjE* aids in understanding pneumococcal-host interactions.

The putative capsule O-acetyltransferase gene *wcjE* is highly conserved across various *Streptococcus pneumoniae* serotypes, but the role of the gene in capsule biosynthesis and bacterial fitness remains largely unclear. Isolates expressing pneumococcal serotype 9A arise from precursors expressing *wcjE*-associated serotype 9V through loss-of-function mutation to *wcjE*. To define the biosynthetic role of 9V *wcjE*, we characterized the structure and serological properties of serotype 9V and 9A capsule polysaccharide (PS). NMR data revealed that both 9V and 9A PS are composed of an identical pentasaccharide repeat unit, as reported previously. However, in sharp contrast to previous studies on 9A PS being devoid of any O-acetylation, we identified O-acetylation of α-glucuronic acid and α-glucose in 9A PS. In addition, 9V PS also contained −CH2 O-acetylation of β-N-acetylmannosamine, a modification that disappeared following *in vitro* recombinatorial deletion of *wcjE*. We also show that serotyping sera and monoclonal antibodies specific for 9V and 9A bound capsule PS in an O-acetate-dependent manner. Furthermore, IgG and to a lesser extent IgM from human donors immunized with serotype 9V PS displayed stronger binding to 9V compared with 9A PS. We conclude that serotype 9V *wcjE* mediates 6-O-acetylation of β-N-acetylmannosamine. This PS modification can be selectively targeted by antibodies in immunized individuals, identifying a potential selective advantage for *wcjE* inactivation and serotype 9A emergence.

The Gram-positive diplococcus *Streptococcus pneumoniae* is an obligate colonizer of the human nasopharynx that can subsequently spread to normally sterile sites (*e.g.* blood, middle ear, etc.) to cause a wide spectrum of diseases. Pneumococcal survival in hosts is facilitated by expression of a polysaccharide (PS)2 capsule that prevents recognition and clearance of the bacterium by the host immune system. To date, 93 capsule types (commonly referred to as serotypes) have been identified according to their reactivity with reference antisera (*e.g.* group antisera, factor sera, etc.) or, more recently, according to their reactivity with monoclonal antibodies (mAb) (1–3). Hosts can produce serotype-specific antibodies that can mediate bacterial clearance and prevent colonization and disease. Thus, expression of heterogeneous capsule PS may help *S. pneumoniae* avoid humoral immunity in host.

Genes in the capsule synthesis (*cps*) locus of *S. pneumoniae* mediate synthesis of the repeating unit of the capsule PS, as well as export and polymerization of the repeating units. The *cps* locus has been sequenced for all known serotypes (1, 4, 5). Comparative analysis of *cps* genes and capsule structures has led to the assignment of biosynthetic roles to many *S. pneumoniae cps* gene products (6, 7). However, because of the incomplete description of various capsule structures, the role of many *cps* genes remains undefined. One example of a highly conserved *cps* gene with undefined enzymatic activity is *wcjE*, which can be found in the *cps* loci of 14 serotypes: 9V, 11A, 11D, 11F, 15F, 20, 31, 33A, 35A, 35C, 42, 43, 47A, and 47F (5) (9V, 11A and 20 are included in the current 23-valent pneumococcal capsule PS vaccine, PPV-23 (8)). The gene encodes a putative transmembrane O-acetyltransferase homologous to Pfam family PF01757, which includes peptidoglycan and lipopolysaccharide O-acetyltransferases (9–12). Accordingly, molecular, serological, and...
structural analyses revealed that wcjE in 11A, 11F, and possibly 11D mediates \(-\text{CH}_2\) O-acetylation of \(\beta\)-galactose (Gal) of the PS capsule (1, 13). However, 9V PS has no \(\beta\)Gal in its capsule PS, and the enzymatic target of the wcjE gene product in serotype 9V and all other wcjE-associated serotypes is unclear.

Serotype 9A arises from serotype 9V following loss-of-function mutations to wcjE (14), providing a suitable model for studying the role of 9V wcjE in capsule synthesis and pneumococcal fitness. Serotypes 9V and 9A cross-react with the factor serum 9d, but only serotype 9V reacts with factor serum 9g and the monoclonal antibody Hyp9VG2 (14), suggesting that the cell wall PS content of these samples was determined to be low as judged by NMR data (phosphocholine peak at 3.22 ppm, see supplemental Fig. S1). To de-\(\alpha\)-acetylated samples, PS was incubated in 0.2 \(\text{m}\) sodium hydroxide for 3 h at room temperature. Equal mass of purified PS was incubated in water as “native PS” controls.

**NMR Spectroscopy**—NMR spectra were obtained on a Bruker Avance II (700 MHz \(^1\text{H}\) ) spectrometer equipped with a cryo-probe, processed with NMRPIPE (18), and analyzed with NMRVIEW (19) or ACD/NMR Processor Academic Edition (Advanced Chemistry Development, Inc., Toronto, Canada). The \(^1\text{H}\) one-dimensional and decoupled \(^1\text{H}-^1\text{C}\) two-dimensional heteronuclear multiple quantum coherence (HMQC) data were collected at 45 °C. Chemical shifts were assigned according to the previously reported assignments of 9V capsule PS (15). The molar ratio of \(\beta\)ManNAc-6-O-acetate was determined by integrating the \(^1\text{H}\) peaks between 4.40 and 4.55 ppm and subtracting the estimated integral of \(\beta\)Glc H1, as determined by the \(\beta\)ManNAc:\(\beta\)Glc ratio in de-\(\alpha\)-acetylated samples. Molar ratios of all other \(\alpha\)-acetate substitutions were calculated by integrating resolved \(^1\text{H}\) signals corresponding to \(\alpha\)-acetylated residues (see below) compared with the integration of all \(\beta\)-ManNAc anomeric signals between 4.85 and 5.00 ppm.

**Antibodies and Serum Samples**—The serotyping factor sera 9d (cross-reacts with 9A and 9V), 9g (reacts only with 9V), and group 9 antiserum (cross-reacts with 9A, 9V, 9N, and 9L) were purchased from SSI (Copenhagen, Denmark). Monoclonal antibodies (mAb) were produced against 9V PS as described (20). Hybridoma culture supernatants were used as a source for the 9V-specific mAb Hyp9VG2 (14) and 9A/9V cross-reactive mAb Hyp9VM7 (see below). Pool 32 is a pool of equal volume of sera from 100 anonymous elderly donors vaccinated with PPV-23. The remaining serum samples used in this study are from healthy single donors that received PPV-23 vaccination and are described elsewhere (21).

**Flow Cytometric Serotyping Assay (FCSA)**—Bacteria concentrations were normalized according to \(A_{630}\) and incubated for 10 min at 4 °C with 1:50,000 dilution of factor sera 9d or 9g, 1:200 dilution of Hyp9VG2 culture supernatant, or 1:50 dilution of Hyp9VM7 culture supernatant. As a negative control, bacteria were incubated with serotype 11A specific Hyp11AG2 and Hyp11AM1 mAb. Surface-bound antibody was detected with goat anti-rabbit Ig conjugated to fluorescein isothiocyanate, rabbit anti-mouse IgG-conjugated phycoerythrin, or rabbit anti-mouse IgM phycoerythrin-Cy7-conjugated antibodies (Southern Biotechnology Associates, Inc., Birmingham, AL). Stained bacteria were analyzed using a FACSCalibur (BD Biosciences). Data analysis was performed with FCS Express (De Novo Software, Los Angeles, CA).
**RESULTS**

**NMR Characterization Reveals O-Acetate Substitutions in 9A PS**—The **wcjD** gene conserved in the 9A and 9V cps loci encodes a putative O-acetyltransferase (S), which led us to hypothesize that O-acetate (OAc) substitutions are probably present in both 9A and 9V capsule PS. To examine this hypothesis, we purified capsule PS from strain SSISP 9V/4 (herein referred to as 9V PS) and from strain MNZ869 (herein referred to as 9A PS) and obtained $^1$H NMR data for both PS samples before and after de-O-acetylation (do) by mild alkali hydrolysis (Fig. 2 and supplemental Fig. S1). The $^1$H NMR spectrum of native 9AdO PS was indistinguishable from the spectrum of 9VdO PS (15), we show that native 9A PS also contains OAc/NAc ratio of $\sim$1:1, whereas 9A PS contains an OAc/NAc ratio of $\sim$1:1. It is very clear that 9A PS contained OAc substitutions, albeit at a lower rate relative to 9V PS.

**TABLE 1**

| O-Acetylation of corresponding sites in Fig. 1 | Previous study (15) | Current study |
|-----------------------------------------------|---------------------|---------------|
| 9V                                            | 9A                  | 9V            |
| R$^1$                                         | 0.04$^a$            | 0.004         |
| R$^4$                                         | 0.03                | 0.001         |
| R$^5$                                         | 0.25                | 0.55          |
| R$^7$                                         | 0.17                | 0.25          |
| R$^8$                                         | 0.06                | 0.09          |
| R$^9$                                         | 0.55                | 1.04          |
| Sum$^b$                                       | 1.1                 | 1.97:1        |
| OAc:NAc$^c$                                   | 11:1:1              | 1.03:1        |

*Values are expressed as molar equivalents.

$^a$ Sum of all O-acetylation sites according to integration of resolved peaks in $^1$H NMR spectra.

$^b$ Molar ratio of O-acetal to N-acetae according to integration of acetate proton signals in $^1$H NMR spectra.

In contrast to previous studies, which have shown that 9A PS is identical to 9VdO PS (15), we show that native 9A PS also contains hydrolysis-sensitive signals in the anameric region (Fig. 2A) and between 2.14 and 2.16 ppm (Fig. 2B). By integrating the signals between 2.14 and 2.18 ppm (OAc) and signals between 2.02 and 2.06 ppm (NAc), we estimate that 9V PS contains OAc/NAc ratio of $\sim$2:1, whereas 9A PS contains an OAc/NAc ratio of $\sim$1:1. It is very clear that 9A PS contained OAc substitutions, albeit at a lower rate relative to 9V PS.

**9A PS Lacks the $-$CH$_2$ O-Acetylation of $\beta$ManNAc Present in 9V PS—**$^1$H-13C HMQC data have been obtained for 9A and 9V PS samples to unambiguously identify the position of the O-acetate substitutions. Guided by previously reported chemical shift assignments (15), we were able to assign most signals in the 9VdO spectra (supplemental Table S1 and supplemental Fig. S2) confirming that the glycosidic backbone of the PS samples analyzed in this study is also composed of the $\sim$1-4$\alpha$Glc(1-4)$\alpha$-GlcUA(1-3)$\alpha$Gal(1-3)$\beta$ManNAc(1-4)$\beta$Glc(1-pentasaccharide repeat unit (Fig. 1).

Next, we examined the presence or absence of key hydrolysisis-sensitive peaks previously assigned to 9V O-acetate substitutions (15). As shown in Fig. 3A, HMQC spectra of 9V and 9A PS samples both show cross-peaks at 4.77/75.46 and 5.37/77.61 ppm ($^1$H/13C). These signals correspond to H2 and H3 groups of $\alpha$GlcUA, respectively, in the presence of geminal O-acetylation. Accordingly, we observed hydrolysis-sensitive anameric...
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signals shared by 9V and 9A PS (Fig. 3B) corresponding to αGlcUA-2-OAc, αGlcUA-3-OAc, and αGal adjacent to αGlcUA-3-OAc (1H/13C: 5.34/94.68, 5.13/97.08, and 5.33/102.44 ppm, respectively). Together, these findings indicate O-acetylation of αGlcUA in both 9A and 9V PS. Some hydrolysis-sensitive signals shared by the 9A and 9V 1H NMR spectra were not readily detectable in the HMQC spectra. As shown in the 1H spectra of both 9V and 9A (and not 9VdO or 9AdO), signals assigned to the anomeric protons of αGlc-2-OAc, αGlc-3-OAc, and βManNAc-4-OAc (15) are observed at 5.54, 5.50, and 4.95 ppm, respectively (Fig. 2, A and C).

A major difference observed in the 9V and 9A HMQC spectra is the presence of a strong pair of 1H signals at 4.46 and 4.27 ppm (13C = 66.11 ppm) in 9V PS but not in 9A and 9VdO PS samples (Fig. 3C). These signals correspond to the –CH2 protons of βManNAc with geminal O-acetylation (15). In addition, the loss of signals at 3.46/79.13 ppm and the doublet signals at 3.89, 3.91/63.13 and 3.79, 3.80/63.12 ppm (data not shown), assigned to the H5 and H6 chemical shifts of βManNAc in 9A and 9VdO, indicates the presence of βManNAc-6-OAc in 9V but not 9A PS. To quantify the degree of O-acetylation at each identified site on 9V and 9A PS, we followed previous strategies (15). We determined the molar ratios of most O-acetate substitutions to be comparable between 9V and 9A (Fig. 1B). Total O-acetylation rates obtained with this strategy (i.e. 1.96 and 0.92 for 9V and 9A PS, respectively) were comparable with molar content calculated using acetate signals (1.97 versus 1.03, see above). Therefore, we propose revised structures for 9A and 9V PS as shown in Fig. 1 and Table 1.

βManNAc-6 O-Acetylation Is Mediated by wcjE—We recently demonstrated that genetic inactivation of wcjE alone was enough to effect seroswitching of serotype 9V to 9A (14). Because βManNAc –CH2 O-acetylation was associated with the 9V capsule PS structure, we hypothesized that wcjE mediates this O-acetate substitution. To test this hypothesis, we purified and analyzed the capsule PS of the isogenic strains JC01 (9V) and JC02 (9A). One-dimensional 1H NMR spectra were indistinguishable from the spectra of PS purified from clinical counterparts (supplemental Figs. S1 and S3A). Notably, both spectra show signals at 5.55, 5.50, 5.39, 4.96, and 4.80 ppm, indicating the presence of αGlc-2-OAc, αGlc-3-OAc, αGlcUA-3-OAc, βManNAc-4-OAc, and αGlcUA-2-OAc, respectively. Only the 1H spectrum of JC01, however, displayed strong signals at 4.47 and 4.28 ppm. Accordingly, the acetate region of JC01 contained three distinct peaks between 2.15 and 2.18 ppm, although the spectrum for JC02 only contained two discernable signals in this region (supplemental Fig. S3B). In the 1H-13C HMQC spectra, cross-peaks corresponding to H2 and H3 O-acetylation of αGlcUA are observed at 4.77/75.46 and 5.37/77.61 ppm for both JC01 and JC02 (data not shown). However, only JC01 shows strong signals corresponding to H6 O-acetylation of βManNAc at 4.46/66.14 and 4.27/66.15 ppm (supplemental Fig. S3C). Thus, we conclude that inactivation of wcjE activity results in the loss of βManNAc-6-O-acetylation characteristic of serotype 9V.

9A- and 9V-specific Factor Sera and mAbs Do Not Bind to O-Acetylated PS—O-Acetylation of 9A PS contradicts the widely accepted structure of this PS (15, 22). We investigated the possibility that bacteria expressing two different capsule types (i.e. 9A with or without O-acetylation) could be serologically identical by testing factor sera (Fs) and mAb binding to bacteria expressing 9V and 9A PS by FCSA and purified preparations of these PS by ELISA. As expected, FCSA revealed that Fs 9d antibodies and Hyp9VM7 mAb bound only 9V-coated plates (Fig. 4A). None of these antibodies bound to bacteria expressing 9V and 9A PS by FCSA and purified preparations of these PS (Fig. 4B and supplemental Fig. S4). Plates coated with 9AdO or 9VdO PS, however, were not bound by any of these serotyping antibodies. We also examined the binding of antibodies in serogroup 9 (Sg9) antiserum, which, in addition to 9A and 9V PS, bind the non-O-acetylated PS of serotypes 9N and 9L. Binding of Sg9 antiserum antibodies was indistinguishable between 9VdO and 9AdO (Fig. 4B), although ~10-fold reduced concentrations of antiserum achieved comparable signals in 9A and 9V PS coated plates (supplemental Fig. S4).
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![Graph A](image1)

**FIGURE 4. Serotyping antibody binding to 9A and 9V PS is O-acetate-dependent.** A, binding of polyclonal antibodies in factor sera 9d and 9g (left two columns) and the monoclonal antibodies Hyp9VM7 and Hyp9VG2 (right two columns) to bacterial strains expressing serotype 9V (SSISP 9V/4, top row) and serotype 9A (MNZ869, bottom row) was examined by FCSA. Each histogram box contains the geometric mean fluorescence intensities of negative controls (top) and test samples (bottom). B, relative binding of antibodies to ELISA wells coated with 9V PS (light gray bars), 9VdO PS (dark gray bars), and 9AdO (white bars). Antibodies are indicated at the bottom of the graph as follows: Sg9, antiserum specific for serogroup 9 at a 1:1000 dilution; Fs9d, factor serum 9d at a 1:1000 dilution; Fs9g, factor serum 9g at a 1:1000 dilution; 9VM7, factor serum 9d at a 1:1000 dilution; 9VM7 and 9VdG are tissue culture supernatants of monoclonal antibodies Hyp9VM7 and Hyp9VG2. C, inhibition of factor serum 9d antibodies binding to 9A PS-coated plates was examined by inhibition ELISA and is expressed as a percentage of the A_{0.5} of negative control (i.e. wells that did not receive inhibitor PS). Values in B and C are averages of duplicate runs.

To further confirm OAc-mediated binding by typing serum, we tested the inhibition of antibody binding to 9A-coated plates using native and de-O-acetylated 9V and 9A PS. Native 9V and 9A PS inhibited factor serum 9d antibodies (Fig. 4C) and Hyp9VM7 mAb (data not shown) binding in a dose-dependent manner. Neither de-O-acetylated PS sample was able to inhibit antibody binding. We concluded that PS O-acetylation mediates 9A and 9V epitopes targeted by typing antibodies.

**Serum from Human Donors Immunized with 9V PS Display O-Acetate-mediated Binding to Capsule PS**—We observed that serotyping antibodies can selectively bind 9V PS in an O-acetate-dependent manner, suggesting that human antibodies may also selectively bind wcjE-mediated epitopes. We examined the binding of IgG, IgA, and IgM antibodies in serum of human donors vaccinated with 9V PS to purified 9V, 9A, 9AdO, and 9VdO PS (Fig. 5). IgG antibodies in a human serum pool (P32) yielded stronger signals in 9V PS-coated plates, followed by 9A PS and then 9VdO and 9AdO plates (the latter two yielded a comparable signal). A similar trend was observed when examining IgG of five single donors, where almost all serum (n = 4) samples displayed greater than 2-fold increased IgG signal to 9V PS plates compared with 9A (Fig. 5A). In all samples, IgG signal was the least in 9VdO and 9AdO PS plates. When examining IgM signals, 9V PS-associated signals was clearly higher for only P32, s43, and s44, although the difference between 9V PS-coated plates and plates coated with other PS preparations was not as marked as that observed for IgG (Fig. 5B). IgA binding was low for all samples, and no PS preparation was clearly associated with a greater signal compared with other PS (Fig. 5C).

The stronger IgG signal in 9V- and 9A-coated plates is not likely due to an increased amount of native PS coated on ELISA wells relative to the de-O-acetylated sample, as some serum samples (e.g. s32 and s44) displayed equal or greater IgM and IgA signals in 9VdO- and 9AdO-coated plates (Fig. 5 and supplemental Fig. S5). Relative binding of IgM and IgA among all plates remained indistinguishable even after absorption with purified serotype 22F PS and cell wall PS (data not shown), supporting that antibody binding to capsule PS is mediating A_{0.5} signal and that all PS preparations comparably adhered to ELISA wells.

**DISCUSSION**

Previous studies comparing 9V and 9A capsule properties were performed under the supposition that 9A PS contained no O-acetylation and thus used de-O-acetylated 9V PS samples as a surrogate for 9A PS (15, 23, 24). In contrast to the widely accepted view, we present compelling NMR evidence that 9A PS is extensively O-acetylated on its αGlcUA and to a much smaller extent on its αGlc and βManNAc moieties. Why previous structures of 9A PS were devoid of O-acetylation (15) remains unclear, although it is possible that the relatively labile O-acetate groups may have become fully hydrolyzed in prior purification or handling procedures, e.g. phenol-mediated bacteria lysis, enzymatic removal of nucleic and amino acids during purification, etc. This possibility is supported by the detection
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of higher 9V PS O-acetylation rates in this study compared with prior reports, despite using similar strategies to quantify these rates (15). Our revised structure for 9A PS (Fig. 1) is supported by the fact that O-acetate integrity was required to mediate characteristic binding by both factor serum 9d and the mAb against runs. P32 is a pool of 100 serum samples from individuals immunized with 9V PS, s32, s43, s44, s48, and s53 are serum samples from single healthy donors immunized with 9V PS.

The presence of O-acetylation on 9A PS is also consistent with genetic studies showing that both 9A and 9V cps loci contain identical alleles of the putative O-acetyltransferase gene wcjD (5, 14). wcjD encodes a soluble O-acetyltransferase homologous to Pfam family PF00132 (7), which includes the putative 11A-associated αGlc-2,3-O-acetyltransferase wcwT and βGal-4-O-acetyltransferase wcwC (13). Given that the O-acetylation sites are shared by both 9V and 9A PS, we propose that wcjD mediates O-acetylation of the 2- and 3-positions of αGlcUA and, to a smaller extent, O-acetylation of the structurally similar 2- and 3-positions of αGlc. We cannot readily explain the mechanism of βManNAc-4-OAc substitution found in 9A PS. It is possible that wcjD or a conserved O-acetyltransferase encoded outside the cps locus mediates this O-acetate substitution. To investigate these possibilities and fully define the role of wcjD, further biochemical studies are necessary.

We identified βManNAc-6-O-acetylation in 9V but not 9A PS. Furthermore, this O-acetate substitution is lost following recombinatorial deletion of wcjE in strain JC02, highlighting the enzymatic target of the putative transmembrane O-acetyltransferase encoded by wcjE. The putative 9V wcjE gene product shares 93 and 91% amino acid sequence identity with the wcjE gene products of serogroup 11 and serotype 15F, respectively. Consistent with enzyme homology, the 6-βManNAc(1–4)βGlc target residues of serotype 9V wcjE resembles the 6-βGal(1–4)βGlc target of wcjE in 11A, 11F (Fig. 6) (13), and 11D. The reported PS structure of serotype 15F capsule contains 2 m equivalents of unplaced O-acetylated groups (25). As 15F capsule contains βGal(1–4)βGlc identical to 11A, we hypothesize that 15F wcjE also mediates βGal 6-O-acetylation, although further work is necessary to examine this proposal. Taken together, the wcjE gene product of these serotypes may mediate hexopyranose-6-O-acetylation.

The cps loci of the remaining nine serotypes that contain wcjE (i.e. serotypes 20, 31, 33A, 35A, 35C, 42, 43, 47A, and 47F) conserve a galactofuranosyl (Gal) mutase gene (glf) and galactofuranosyltransferase gene (wciB), the genes putatively necessary to integrate βGal into the capsule repeat unit (6, 7). The fully elucidated PS structure of 35A contains βGal-2-OAc and βGal-5,6-OAc (Fig. 6) (26), in congruence with the 35A cps locus containing two putative O-acetyltransferase genes, wcjE and wciG (5). The reported capsule PS structure of the wcjE-associated serotype 20 also contains βGal-5,6-OAc (26). The serotype 33F cps locus contains a frameshifted pseudogene of wcjE and a putatively intact allele of wciG (5). Although 33F cps locus contains glf and wciB, and 33F PS contains two potential βGal enzymatic targets, 33F PS has βGal-2-OAc but not βGal-5,6-OAc (27). Thus, we propose that in glf/wciB-associated serotypes, wcjE may mediate βGal-5,6-OAc substitution.

Although wcjE is conserved across multiple pneumococcal serogroups, the gene appears to be biochemically expendable as reflected by the unconventional emergence of wcjE-null serovarants. Serotypes 9A and 11E arise from strains expressing serotypes 9V and 11A, respectively, following loss-of-function mutations to wcjE (1, 14). Given the complex interaction of cps genes, it is commonly thought that pneumococcal serotypes evolve once and that all the cps loci of a clinically relevant serotype can be genetically traced back to a single founding event. However, each clinical isolate expressing serotypes 9A and 11E contain unique and unrelated mutations to wcjE, revealing the recurrent and independent evolution of these serotypes (1, 14).

Serotypes 9A and 11E are significantly more likely to occur among strains isolated from bacteremia cases than asymptomatic nasopharyngeal colonization (28, 29), suggesting a link

3 M. H. Nahm, unpublished data.
between wcjE inactivation and bacterial survival during invasive disease. The host factor selecting for wcjE loss-of-function mutations may be antibodies to the capsule. We observed that human subjects immunized with 9V PS produce IgM and IgG antibodies that bind more strongly to 9V PS than 9A PS, in agreement with the observation that human antibodies can selectively target serogroup 9 epitopes mediated by O-acetylation (23). Our observations are also consistent with previous reports that highlight the important role of O-acetylation in shaping the humoral response to PS antigens (13, 30–32). As the IgG isotype is predominant in blood, 9V-specific antibodies that target /H9252 ManNAc-6-OAc-associated epitopes may provide a selective advantage for serotype 9A and explain why this serotype is strongly associated with bacteraemia isolates. Studies are underway to determine whether antibodies that strongly bind to 9V PS also mediate preferential opsonophagocytosis of strains expressing serotype 9V over those expressing 9A.

Actively circulating wcjE-null variants are expected to share a common loss-of-function mutation, so the lack of two isogenic wcjE-null 9A or 11E isolates (1, 14) suggests wcjE facilitates host-to-host transmission of 9V and 11A. A wcjE-associated benefit in relation to transmission is a possible reason why this gene is conserved across various serotypes. Perhaps wcjE-mediated –CH₂ O-acetylation of capsule PS prevents recognition by host lectins encountered during nasopharyngeal colonization or promotes better adhesion to the mucosal surface. Another possibility is that wcjE-mediated extracellular O-acetylation may target other PS targets in addition to capsule, which in turn provides an advantage over wcjE-null counterparts. Fully elucidating the enzymatic targets of wcjE may lead to the discovery of key host-microbe interactions that mediate serotype-associated prevalence and pathology.

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