Phase-Variable Expression of lptA Modulates the Resistance of Neisseria gonorrhoeae to Cationic Antimicrobial Peptides

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Journal Title: Antimicrobial Agents and Chemotherapy
Volume: Volume 58, Number 7
Publisher: American Society for Microbiology | 2014-07-01, Pages 4230-4233
Type of Work: Article | Final Publisher PDF
Publisher DOI: 10.1128/AAC.03108-14
Permanent URL: https://pid.emory.edu/ark:/25593/pfq8v

Final published version: http://dx.doi.org/10.1128/AAC.03108-14

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Accessed October 29, 2019 11:33 PM EDT
Phosphoethanolamine (PEA) decoration of lipid A produced by Neisseria gonorrhoeae has been linked to bacterial resistance to cationic antimicrobial peptides/proteins (CAMPs) and in vivo fitness during experimental infection. We now report that the lptA gene, which encodes the PEA transferase responsible for this decoration, is in an operon and that high-frequency mutation in a polynucleotide repeat within lptA can influence gonococcal resistance to CAMPs.

Neisseria gonorrhoeae is a strict human pathogen that has caused the sexually transmitted infection termed gonorrhea for thousands of years. Over the millennia, N. gonorrhoeae has developed multiple mechanisms to resist innate host defenses, including cationic antimicrobial peptides/proteins (CAMPs) produced by phagocytes and epithelial cells (1). Phosphoethanolamine (PEA) decoration of the lipid A possessed by N. gonorrhoeae and N. meningitidis has been shown to contribute to their resistance to CAMPs by a mechanism that likely involves a reduction in ionic interactions of CAMPs with the bacterial surface (1–6), resistance of N. gonorrhoeae to complement-mediated killing by normal human serum (3, 4), N. gonorrhoeae fitness during experimental infection in mice and humans (5, 7), and the proinflammatory potential of N. gonorrhoeae (7, 8). Most communal Neisseria do not encode LptA (8), but N. gonorrhoeae and N. meningitidis (2, 3, 8) typically contain lptA and produce multiple isoforms of lipid A that differ in PEA decoration at the 4′ and/or 1 position, though the basis of these isoforms has not been fully defined. We now provide evidence that gonococcal LptA is within an operon and that N. gonorrhoeae resistance to a model CAMP (polymyxin B; PMB) is modulated by high-frequency mutation due to a phase-variable (PV) polynucleotide stretch in the lptA coding sequence.

Organization and expression of the lptA locus in N. gonorrhoeae. Bioinformatic analysis of the DNA sequence of the N. gonorrhoeae FA 1090 chromosome (http://www.genome.ou.edu/gono.html) suggested that lptA is transcriptionally linked to two upstream genes (serC and a hypothetical gene annotated as NGO1282) and a downstream gene (nfnB) (Fig. 1). This hypothesis was confirmed by results from reverse transcription-PCR (RT-PCR) experiments (Fig. 2A) that demonstrated transcriptional linkage of lptA with the serC, hypothetical, and nfnB genes; details of the experimental procedures and a list of oligonucleotide primers are provided in the legends of Fig. 1 and 2 and in Table 1, respectively. However, primer extension analysis of total N. gonorrhoeae RNA performed as described previously (9) identified a transcriptional start point (TSP) positioned 61 nucleotides (nt) upstream of the lptA translational start codon and four nt downstream of near-consensus −10 and −35 elements (Fig. 1 and 2B). Thus, we tentatively conclude that lptA expression in N. gonorrhoeae can be initiated by two promoters upstream of serC and lptA, respectively. The mechanisms that control use of these promoters are now under investigation.

Analysis of the online FA 1090 genome sequence indicated that the lptA coding sequence contains a polynucleotide tract consist-
FIG 2 Transcription of the \( lptA \) coding sequence. (A) Transcriptional linkage between \( serC \), NGO1282, \( lptA \), and \( nfnB \). All RT-PCRs were performed on purified RNA harvested (RNasy minikit; Qiagen) from a log-phase culture of strain FA19 grown as described previously (9). First-strand cDNA was generated using SuperScript II reverse transcriptase (Invitrogen) and a gene-specific reverse primer (LptA8) that binds in the \( nfnB \) gene and primes elongation of a single-stranded cDNA toward \( serC \). PCR was then performed to confirm transcriptional linkage between pairs of genes. Sections A, B, and C of the gel are grouped by forward- and reverse-primer locations and separated by 1-kb PLUS DNA ladders (Invitrogen). Lane 1, FA19 cDNA; lane 2, \(-RT \) negative control (RT omitted); lane 3, FA19 genomic DNA positive control; lane 4, no-template negative control. Section A, \( "serC" \text{=} \( serC \_F \); NGO1282 \( \text{=} \) \( NGEG\_02068\_R \). Section B, \( "serC" \text{=} \( serC \_F \); \( "lptA" \text{=} \) \( lptA\text{rev} \). Section C. \( "lptA" \text{=} \) \( LptA6 \_R \text{=} \) \( nfnB \); \( LptA8 \text{=} \) \( lptA\text{rev} \). (B) Primer extension of the \( lptA \) transcript. Primer extension analysis was performed as described previously (9) using 20 \( \mu \)g of FA19 total RNA as the template and a radioactively \( [\gamma\text{-}^{32P}]\text{ATP} \) labeled reverse primer (LptA7 \_R) that anneals 67 bp downstream of the \( lptA \) start codon. RNA was purified and cDNA generated as described for panel A. Radioactive single-stranded cDNA products were separated on a polyacrylamide gel alongside sequencing reactions that used the same reverse primer (LptA11 \_R) that was the forward primer used for generation of the sequenced \( lptA \) promoter template. The TSP corresponds to the band labeled with an asterisk and is 4 bp downstream of a consensus \( \sigma^T \)-type \(-10 \) element. A second band appeared running approximately 4 to 5 nucleotides shorter than the proposed +1 TSP band and could be due to a degraded mRNA transcript.

TABLE 1 Oligonucleotide primers used in this study

<table>
<thead>
<tr>
<th>Primer name</th>
<th>Primer sequence</th>
<th>Purpose</th>
</tr>
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<tbody>
<tr>
<td>LptA6</td>
<td>5'-CGGTCTTTGATGATGATCAGTT-3'</td>
<td>Transcriptional linkage</td>
</tr>
<tr>
<td>LptA7</td>
<td>5'-GGCTTTTCTTTCCTGATTTCTT-3'</td>
<td>Sequencing of the poly-T tract</td>
</tr>
<tr>
<td>LptA7 _R</td>
<td>5'-AAGAAATACAGGGAAAGAAAGGC-3'</td>
<td>Primer extension</td>
</tr>
<tr>
<td>LptA8</td>
<td>5'-ACGGTTGCAATGCATACCTCCG-3'</td>
<td>Transcriptional linkage</td>
</tr>
<tr>
<td>LptA11</td>
<td>5'-CGGCTGCGAATTTTGCGTTACG-3'</td>
<td>Primer extension</td>
</tr>
<tr>
<td>LptAdelL</td>
<td>5'-TGGAGGTACATGAAATTAGAC-3'</td>
<td>Sequencing of the poly-T tract</td>
</tr>
<tr>
<td>lptAJk4</td>
<td>5'-TAAAGAATTTTTCTCAATATCCGGAT-3'</td>
<td>Sequencing of the poly-T tract</td>
</tr>
<tr>
<td>lptArev</td>
<td>5'-GGCTCGAGTCTCGCTGTTATAC-3'</td>
<td>Transcriptional linkage</td>
</tr>
<tr>
<td>LptAstart</td>
<td>5'-TCTAGAAATACGCTGTGCATG-3'</td>
<td>Sequencing of the poly-T tract</td>
</tr>
<tr>
<td>NGEG_02068_R</td>
<td>5'-GGCCGGCAAGCGATTTCCAT-3'</td>
<td>Transcriptional linkage</td>
</tr>
<tr>
<td>serC _F</td>
<td>5'-CGACTACGGACTGATTTACG-3'</td>
<td>Transcriptional linkage</td>
</tr>
</tbody>
</table>
of PMB. The PMB MIC against BB22 was 0.2 μg/ml (Fig. 3A), which is similar to the PMB MIC against an lptA deletion mutant of FA 1090 described previously (5). DNA sequence analysis of the lptA sequence of BB22 revealed that it possessed a T-9 tract that would result in premature truncation of LptA (Fig. 3). We then selected for spontaneous variants of BB22 that would grow on GCB agar containing 10 μg/ml of PMB. In four separate experiments, spontaneous PMB-resistant variants arose at a frequency of approximately 10⁻⁶; in contrast, spontaneous erythromycin-resistant mutants (selected at 1 μg/ml) were recovered at a frequency of 10⁻⁸ (data not presented). The PMB MIC against PMB-resistant mutants of BB22 was, like that seen with parental strain FA 1090, 100 μg/ml (see strain BB22R data in Fig. 3A). DNA sequencing of the lptA PV tract from sixteen randomly picked PMB-resistant revertants of BB22 showed that all possessed a wild-type T-8 tract (see BB22R in Fig. 3A) and would produce a full-length LptA (Fig. 3B). Based on this reversion frequency, we estimate that the poly-T tract in lptA phase varies at an approximate frequency of 10⁻⁵. This frequency is 2 to 3 orders of magnitude lower than that seen with other PV genes of N. gonorrhoeae, which may be due to its shorter tract (8 nt) and A/T characteristics that reduce slipped-strand mispairing events compared to the results seen with longer, G/C-rich repeats (10–12).

Conclusions. Production of PEA-decorated lipid A by N. gonorrhoeae has been linked with bacterial resistance to mediators of innate host defense, the capacity of N. gonorrhoeae to elicit a pro-inflammatory response, and in vivo fitness (3–5, 7, 8). The structurally variable lipooligosaccharide (LOS) chemotypes produced by gonococci have been linked to PV genes that encode enzymes responsible for adding carbohydrates within the branched-chain oligosaccharide region (11, 12). Our work now extends this PV expression property of LOS to the lipid A isomers and emphasizes the complexity of LOS structures that can be presented by N. gonorrhoeae. Importantly, to our knowledge, this is the first direct evidence that gonococcal resistance to CAMPs can be modulated by a PV process.

FIG 3 The lptA PV poly-T tract impacts LptA protein length and function. (A) Summary of the lptA PV poly-T tract. The PV poly-T tract comprises nucleotides 172 to 179 of the phase-on lptA open reading frame. Analysis of phase-on and phase-off lptA coding sequences using the ExPASy Translate tool (http://web.expasy.org/translate/) revealed that insertion of a ninth T nucleotide (bolded) in the poly-T tract of strain BB22 results in a frameshift mutation, which would generate a “UAA” stop codon shortly after this PV tract in the lptA mRNA transcript. (B) Impact of the PV poly-T tract on LptA protein length. When in the phase-on state, the lptA poly-T tract (hatched boxes) has 8 nucleotides and lptA encodes a protein 544 amino acids long. Frameshift of the lptA open reading frame (ORF) due to an insertion of a single T nucleotide within this poly-T tract would result in a premature stop codon and subsequent truncation of the LptA nascent polypeptide at just 61 amino acids. Phase-off variants are not predicted to translate the C-terminal sulfatase domain of the LptA protein (13). DUF, domain of unknown function.

ACKNOWLEDGMENTS

We thank Jo-Anne Dillon, Carlos del Rio, and Magnus Unemo for providing N. gonorrhoeae strains and DNA used in whole-genome sequencing (WGS).

This work was supported by NIH grants U19 AI031496 (to P. F. Sparling, University of North Carolina-Chapel Hill), R37 AI021150-29 (W.M.S.), R43 AI09768 (T.D.R.), and R01 AI42053 (A.E.J.) and a VA Merit Award from the Medical Research Service of the Department of Veterans Affairs to W.M.S. W.M.S. was supported by a Senior Research Career Scientist Award from the Medical Research Service of the Department of Veterans Affairs.

We declare that we have no conflicts of interest.

REFERENCES


