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

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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 CAMPs has been linked to 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 expression in a polynucleotide repeat within lptA can influence gonococcal resistance to CAMPs.

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.

FIG 1 Genetic context of lptA in N. gonorrhoeae FA19. The 3.8-kb region of the FA19 genome shown corresponds to nucleotides 1236150 to 1232381 in N. gonorrhoeae FA1090 (http://www.genome.ou.edu/gono.html and GenBank accession number AE004969.1). serC encodes a putative phosphoserine aminotransferase, NGO1282 encodes a hypothetical gene, lptA encodes a lipid A phosphoethanolamine transferase, and nfnB encodes a putative nitroreductase. The locations of the serC (undefined) and lptA (defined in the legend) promoters are depicted with bent arrows. The lptA transcriptional start point, −10, and −35 promoter elements are shown below the illustration. The approximate sites of annealing for oligonucleotide primers used in RT-PCR experiments (Table 1) are shown with arrows.
The presence of the T-8 tract and PMB MIC of 100 μg/ml onto gonococcal base (GCB) agar plates with or without PMB selection, we identified a colony (strain BB22) that was unable to grow on GCB agar plates containing 10 μg/ml PMB. Therefore, our production of a full-length Lpa enzyme, FA19 total RNA as the template and a radioactively ([γ-32P]ATP) labeled reverse primer (LptA7_R) that anneals 67 bp downstream of the LptA gene was searched against a BLAST database of all the whole-genome shots of these clinical isolates using BLASTN in WUBLAST, in order to identify the genome location of the gene within each of the strains. We used the default blastn parameters and specified hspsepSmax (maximum separation allowed between HSPs [high-scoring segment pair] along subject) to be 100 bp. Sequences of the poly-T tract were then extracted and screened for the presence of a T-8 tract on both the forward and reverse strands of the gene using pattern matching. The results showed that all strains contained a T-8 tract and a full-length LptA sequence with 100% nucleotide identity to FA 1090 (data not presented). Accordingly, we propose that possession of an in-frame LptA gene is a common feature of N. gonorrhoeae isolates. lptA behaves as a PV gene in N. gonorrhoeae, and phase-off variants are hypersusceptible to PMB. The presence of the T-8 tract in the 5′ end of the lptA coding sequence suggested to us that it is a member of the PV gene family possessed by N. gonorrhoeae (10). If so, production of a full-length LptA, PEA decoration of lipid A, and CAMP resistance could differ within a population of gonococci. To test this possibility, we employed a PMB screening procedure since loss of lptA expression renders N. gonorrhoeae hypersusceptible to this model CAMP (3, 5, 7). After replica plating approximately 3,000 colonies of strain FA 1090 (T-8 tract and PMB MIC of 100 μg/ml) onto gonococcal base (GCB) agar plates with or without PMB selection, we identified (approximate frequency of 3.3 × 10−4) a colony (strain BB22) that was unable to grow on GCB agar plates containing 10 μg/ml PMB.

TABLE 1 Oligonucleotide primers used in this study

<table>
<thead>
<tr>
<th>Primer name</th>
<th>Primer sequence</th>
<th>Purpose</th>
</tr>
</thead>
<tbody>
<tr>
<td>LptA6</td>
<td>5’-CGGTTTTTGATTGATCCATGTT-3’</td>
<td>Transcriptional linkage</td>
</tr>
<tr>
<td>LptA7</td>
<td>5’-GGCTTTTCTTCTCGTTTCT-3’</td>
<td>Sequencing of the poly-T tract</td>
</tr>
<tr>
<td>LptA7_R</td>
<td>5’-AAGAATACCAGGAGAAAGG-3’</td>
<td>Primer extension</td>
</tr>
<tr>
<td>LptA8</td>
<td>5’-AGCCTTGCAATGCTACGCG-3’</td>
<td>Transcriptional linkage</td>
</tr>
<tr>
<td>LptA11</td>
<td>5’-CGGCTGCGATTTGCGTTTACG-3’</td>
<td>Primer extension</td>
</tr>
<tr>
<td>LptAstart</td>
<td>5’-TCTAGAAGCAGCTGACTTGTAATG-3’</td>
<td>Sequencing of the poly-T tract</td>
</tr>
<tr>
<td>NGEG_02068_R</td>
<td>5’-CGGCGGAACAGCTTTTTCAATGCT-3’</td>
<td>Sequencing of the poly-T tract</td>
</tr>
<tr>
<td>serC_F</td>
<td>5’-GGACTCCCGACTGATTACG-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 10⁻⁵; 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 proinflammatory 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 isoforms 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 mediated by a PV process.

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

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We declare that we have no conflicts of interest.

**REFERENCES**


