SHORT REPORT: LIPOPOLYSACCHARIDE HETEROGENEITY AMONG BURKHOLDERIA PSEUDOMALLEI FROM DIFFERENT GEOGRAPHIC AND CLINICAL ORIGINS

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Abstract. Heterogeneous patterns were obtained for lipopolysaccharide (LPS) from 1,327 Burkholderia pseudomallei isolates by sodium dodecyl sulfate–polyacrylamide gel electrophoresis, silver staining, and immunoblot analysis. Two LPS serotypes (A and B) possessing different ladder profiles and a rough LPS without ladder appearances were identified. All three LPS types were antigenically distinct by immunoblotting. The predominant type A (97%) produced the lowest amount of biofilm. The two less common types (smooth type B and rough type) were found more in clinical than environmental isolates and more in Australian isolates than Thai isolates. These isolates were more often associated with relapse than with primary infection.

Melioidosis is a potentially fatal infectious disease endemic in southeast Asian countries and northern Australia.1,2 It has a broad clinical spectrum, ranging from a seropositive but asymptomatic condition to acute fatal septicemia. The causative agent is Burkholderia pseudomallei, a gram-negative bacillus found in soil and water in areas endemic for infection. Seropositive asymptomatic individuals may harbor the organisms for several years. Inappropriate or inadequate antibiotic treatment is associated with a high rate of relapse and no vaccine is currently available. Diagnosis remains a problem and a definitive diagnosis still relies on the isolation and identification of bacteria from clinical specimens. Attempts to develop reliable immunologic and molecular assays to replace the more time-consuming bacteriologic diagnosis have been made, but these new diagnostic methods remain unvalidated.3–7 We reported a highly reliable latex agglutination test based on the use of monoclonal antibodies specific for a 200-kD exopolysaccharide antigen present only in B. pseudomallei.3,8 It was highly sensitive and specific for the detection of B. pseudomallei antigen in overnight broth hemocultures taken from patients suspected of having septicaemic melioidosis.9

Burkholderia pseudomallei is generally considered to be a rather homogenous species, but colony variation occurs and variation in antibiotic susceptibility profiles and biochemical profiles using a number of commercial bacteriological identification systems have been reported.1,2,9 In addition to these phenotypic variations, we recently reported physicochemical and antigenic heterogeneity of the lipopolysaccharide (LPS) prepared from B. pseudomallei9,10 that could be responsible for false seronegativity in the patients with B. pseudomallei infection. A recently described case of seronegative bacteremic melioidosis caused by a B. pseudomallei strain possessing an ambiguous biochemical profile may have a similar explanation because the investigators based their conclusion on the LPS-based enzyme-linked immunosorbent assay system used for antibody detection.9

In our initial communication, we reported that 96% of the LPS from clinical isolates possessed a sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) silver-staining ladder profile referred to as a typical ladder and approximately 3% exhibited different ladder characteristics referred to as an atypical ladder.10 The remaining 1% had no ladder detectable in the high molecular weight region but possessed a low molecular weight silver-staining band below the 29-kD marker that was designated as a no ladder LPS.10 All three LPS types were antigenically distinct, as evident from immunoblot reactivity against pooled sera from patients from whom the typical or atypical LPSs were isolated.10 To investigate the possible biologic significance of the different LPS phenotypes, we have now expanded our study to include environmental isolates as well as clinical isolates from patients with different clinical manifestations.

The LPSs were extracted from 1,327 isolates (Table 1) by proteinase K digestion and tested with pooled patient sera as previously described.11 In this large series of isolates, the proportion of isolates possessing different LPS patterns was similar to that in our initial report, i.e., 97:2:1 (Figure 1A and Table 1). Overall, 99% of the isolates possessed smooth type LPS exhibiting two different ladder profiles. These two LPS types were serologically distinct (Figure 1), most likely reflecting two different repeating polysaccharide side chains. We refer to the most common serotype (97%) as serotype A and the less abundant one (2%) as serotype B (corresponding to typical and atypical LPS, respectively, in the original report).10 The remaining 1% without a ladder profile and negative seroreactivity (tested against sera from patients from whom isolates with serotype A and type B LPSs were recovered) was considered to be a rough LPS (no ladder in the original report) and most likely represented only a lipid A core oligosaccharide. Our observation therefore differs from a previous report showing that B. pseudomallei isolates exhibited homogeneous SDS-PAGE patterns.12 The different LPS patterns of the isolates do not correlate with ribotypes13 or pulsed-field gel electrophoresis (PFGE) profiles.14 How-
ever, when quantitated for a capacity to produce biofilm,\textsuperscript{15,16} the three LPS types differed from one another (Figure 2). Those with a rough LPS (without ladder appearance) exhibited the highest biofilm-producing capacity and those with a smooth serotype A profile showed the lowest mean biofilm-producing capacity. Whether biofilm production is important in the pathogenesis of melioidosis is still unclear. However, we recently showed that it is most likely unrelated to the virulence of \textit{B. pseudomallei}.\textsuperscript{15}

Results of analyses of LPS data of \textit{B. pseudomallei} isolates from different geographic regions and clinical manifestations are shown in Table 2. It is evident that a much higher proportion of the Australian isolates possessed the two less common LPS types (19.3%) in comparison with the Thai isolates (2.7%) (relative risk [RR] = 7.3, 95% confidence interval [CI] = 3.7–14.1, \(P < 0.0001\), by Fisher’s exact test). The LPSs of clinical isolates were more heterogeneous than the LPSs from environmental isolates. All environmental isolates had the smooth type LPS, and all except one belonged to serotype A. This atypical environmental isolate was obtained from soil on a goat farm where a \textit{B. pseudomallei} outbreak had been reported. It exhibited the same PFGE profile as found in one of the isolates obtained from an infected goat. It is therefore possible that this environmental isolate was passed from an infected animal to the soil.

Since LPS has been implicated as one of the virulence factors for a number of gram-negative bacteria, including \textit{B. pseudomallei},\textsuperscript{17} we investigated possible associations between the LPS types and various clinical parameters of the patients from whom these isolates were obtained (Table 2). There was no association between the LPS type and disease severity (e.g., fatal versus non-fatal, and septicemic versus localized), clinical manifestations (neurologic versus non-neurologic), or underlying risk factors (diabetic versus non-diabetic). The two least abundant LPS patterns were found more in isolates obtained from patients with relapse melioidosis than from those with primary infection (RR = 6.6, 95% CI = 2.2–19.6, \(P = 0.012\), by Fisher’s exact test). This suggests that the bacteria with these less common LPSs survive in the host better than those with the more common LPS type. Since each LPS type is antigenically distinct, antigenic polymorphism among the \textit{B. pseudomallei} LPS may allow the bacteria to evade host immunity.

We identified one \textit{B. pseudomallei} isolate from a primary and relapse infection that had the same PFGE pattern and ribotype but different a LPS type (patient 4; Table 3). However, the role of specific immunity in the control of melioidosis remains uncertain. These uncommon LPS types were not associated with a survival advantage \textit{ex vivo} because bacteria with different LPS phenotypes were equally resistant to killing and equally able to replicate in 30% normal human serum (data not presented).

Variability in LPS has been observed to exhibit different degrees of surface colonization.\textsuperscript{18} The interrelationship between LPS and biofilm synthesis and the possible involve-

\begin{table}[h]
\centering
\caption{Heterogeneity of \textit{B. pseudomallei} lipopolysaccharide (LPS)}
\begin{tabular}{|c|c|c|c|c|}
\hline
Origin & No. of isolates & Type A (%) & Type B (%) & Rough type (%) \\
\hline
Human & & & & \\
Thailand & 1,056 & 1,028 (97) & 22 (2) & 6 (1) \\
Australia & 37 & 28 (76) & 6 (16) & 3 (8) \\
China & 4 & 2 (50) & 1 (25) & 1 (25) \\
Cambodia & 2 & 2 (100) & 0 & 0 \\
Bangladesh & 1 & 1 (100) & 0 & 0 \\
\hline
Animals & & & & \\
Australia & 11 & 11 (100) & 0 & 0 \\
Hong Kong & 19 & 19 (100) & 0 & 0 \\
Malaysia & 11 & 11 (100) & 0 & 0 \\
\hline
Soil & & & & \\
Australia & 3 & 2 (67) & 1 (33) & 0 \\
Thailand & 127 & 127 (100) & 0 & 0 \\
Laos & 47 & 47 (100) & 0 & 0 \\
\hline
Water & & & & \\
Australia & 1 & 1 (100) & 0 & 0 \\
NTCC* & 8 & 8 (100) & 0 & 0 \\
\hline
Total & 1,327 & 1,287 (97) & 30 (2) & 10 (1) \\
\hline
\end{tabular}
\footnotesize{* NTCC strains 1668, 4845, 4846, 6700, 7133, 7383, 8016, and 8707 were used for comparison.}
\end{table}
ment in host adhesion and host persistence have been previously reported in *Pseudomonas aeruginosa* and *P. fluorescens*. We reported herein that the *B. pseudomallei* isolates with a rough LPS appeared to have a higher capacity to produce biofilm (Figure 2). Although it is logical to predict that such a combination may allow the organism to evade host defenses and therefore survive better inside the host, our most recent report that the biofilm by itself is probably unrelated to the virulence of *B. pseudomallei*. The involvement of LPS in the pathogenesis of melioidosis remains inconclusive. Further biochemical characterization and structural elucidation of the different O-polysaccharide side chains and the lipid A of *B. pseudomallei* LPS should provide additional insights into the possible role of LPS in pathogenesis, diagnosis, and vaccine development.

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### Table 2

<table>
<thead>
<tr>
<th>Variable</th>
<th>No.</th>
<th>Smooth type A (%)</th>
<th>Smooth type B (%)</th>
<th>Rough type (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thailand</td>
<td>1,056</td>
<td>1,028 (97.3)</td>
<td>22 (2.1)</td>
<td>6 (0.6)</td>
</tr>
<tr>
<td>Australia</td>
<td>52</td>
<td>42 (80.8)</td>
<td>10 (19.2)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Clinical</td>
<td>1,152</td>
<td>1,110 (96.4)</td>
<td>29 (2.5)</td>
<td>10 (0.9)</td>
</tr>
<tr>
<td>Environmental</td>
<td>178</td>
<td>177 (99.4)</td>
<td>1 (0.6)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Primary†</td>
<td>338</td>
<td>324 (96.0)</td>
<td>11 (3.4)</td>
<td>3 (0.9)</td>
</tr>
<tr>
<td>Relapse†</td>
<td>11</td>
<td>8 (72.7)</td>
<td>2 (18.2)</td>
<td>1 (9.1)</td>
</tr>
<tr>
<td>Fatal</td>
<td>173</td>
<td>161 (93.1)</td>
<td>9 (5.2)</td>
<td>3 (1.7)</td>
</tr>
<tr>
<td>Non-fatal</td>
<td>213</td>
<td>199 (93.4)</td>
<td>10 (4.7)</td>
<td>4 (1.8)</td>
</tr>
<tr>
<td>Septicemic</td>
<td>208</td>
<td>193 (92.8)</td>
<td>11 (5.3)</td>
<td>4 (1.9)</td>
</tr>
<tr>
<td>Non-septicemic</td>
<td>179</td>
<td>167 (93.5)</td>
<td>9 (5.0)</td>
<td>3 (1.7)</td>
</tr>
<tr>
<td>Diabetic</td>
<td>177</td>
<td>166 (93.8)</td>
<td>8 (4.5)</td>
<td>3 (1.7)</td>
</tr>
<tr>
<td>Non-diabetic</td>
<td>203</td>
<td>189 (93.1)</td>
<td>10 (4.9)</td>
<td>4 (2.0)</td>
</tr>
<tr>
<td>Neurologic‡</td>
<td>16</td>
<td>11 (68.8)</td>
<td>3 (18.8)</td>
<td>2 (12.5)</td>
</tr>
<tr>
<td>Non-neurologic‡</td>
<td>21</td>
<td>17 (80.9)</td>
<td>3 (14.3)</td>
<td>1 (4.8)</td>
</tr>
<tr>
<td>IHA§</td>
<td>98</td>
<td>84 (85.7)</td>
<td>7 (7.1)</td>
<td>7 (7.1)</td>
</tr>
<tr>
<td>Negative</td>
<td>47</td>
<td>43 (91.5)</td>
<td>4 (8.5)</td>
<td>0 (0.0)</td>
</tr>
</tbody>
</table>

* Parameters in bold in the variable column are significantly different from one another (P < 0.05).
† Data from Thai patients only.
‡ Data from Australian patients only.
§ Indirect hemagglutination test (IHA) commonly used for antibody detection in many areas endemic for infection. A cut-off dilution for positive sera in the present study is 1:160.

### Table 3

<table>
<thead>
<tr>
<th>Patient</th>
<th>Infection</th>
<th>Type</th>
<th>Date obtained</th>
<th>LPS type</th>
<th>Ribotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Primary</td>
<td>Hemoculture</td>
<td>9/6/89</td>
<td>B</td>
<td>17</td>
</tr>
<tr>
<td>2</td>
<td>Primary</td>
<td>Hemoculture</td>
<td>9/6/89</td>
<td>A</td>
<td>17</td>
</tr>
<tr>
<td>3</td>
<td>Primary</td>
<td>Hemoculture</td>
<td>7/22/96</td>
<td>A</td>
<td>4</td>
</tr>
<tr>
<td>4</td>
<td>Primary</td>
<td>Hemoculture</td>
<td>11/14/96</td>
<td>A</td>
<td>4</td>
</tr>
<tr>
<td>5</td>
<td>Primary</td>
<td>Hemoculture</td>
<td>11/15/98</td>
<td>Rough</td>
<td>26</td>
</tr>
<tr>
<td>6</td>
<td>Primary</td>
<td>Sputum</td>
<td>10/3/98</td>
<td>A</td>
<td>23</td>
</tr>
<tr>
<td>7</td>
<td>Primary</td>
<td>Relapse</td>
<td>2000</td>
<td>B</td>
<td>23</td>
</tr>
</tbody>
</table>

![Biofilm production by *Burkholderia pseudomallei* with different lipopolysaccharide (LPS) profiles. Representative isolates possessing type A, type B and rough type LPSs were cultured in biofilm-inducing medium and biofilm production was determined.](image-url) Relative biofilm formation on the y axis represents the ratio of the optical density value at 630 nm of the test strain divided by that of the reference strain used in all experiments. The horizontal dotted lines indicate medians, and the boxes and error bars represent 25th–75th and 10th–90th percentiles, respectively. Differences between the three LPS groups were statistically significant by the Kruskal-Wallis test (type A versus type B; P = 0.0268; type A versus rough type; P = 0.0001; and type B versus rough type; P = 0.0264).
REFERENCES