Cross-Reactions of Lipopolysaccharides of \textit{Pseudomonas aeruginosa} in Antipneumococcal and Other Antisera

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Lipopolysaccharides of the seven Fisher immunotypes of \textit{Pseudomonas aeruginosa} gave cross-precipitation in many antipneumococcal sera. The reaction of \textit{Pseudomonas} type IV in type 25 antipneumococcal serum was immediate and heavy: 93\,\mu\text{g} of antibody nitrogen per ml. Correlations are described, mainly between the structures of the O-chains of the immunotypes and their specificities as shown by the cross-reactions.

Development and use of vaccines containing immunogenic polysaccharides of multiple serological types of microorganisms are increasing. Cross-reactions of these polysaccharides are of interest, since it is possible that such reactions might induce a measure of protection against pathogens not included in a given vaccine (24). The study of cross-reactions has also brought to light many correlations between chemical structure and immunological specificity (see, for example, references 7–10 and 12) and has been a potent tool in the assessment of evolutionary relationships.

We now summarize cross-precipitations of the lipopolysaccharides (lpI through lpVII) (15; D. Horton, J. R. Lubbers, D. Riley, G. Rodemeyer, and R. Saeki, Am. Chem. Soc. C. S. J. Chem. Congress, abstr. no. 49, 1979) of seven antigenic types (5, 6) of \textit{Pseudomonas aeruginosa} in type-specific antipneumococcal sera (anti-Pn), in an anti-\textit{Klebsiella} serum, and in a serum raised against \textit{Salmonella paratyphi} A. The preparation, properties, and criteria of the lipopolysaccharides are reported in reference 15.

Immunochromal methods used have been described previously (8, 12). The data given in Table 1 are mainly qualitative, since there was only one rapid, heavy reaction in the entire series. All tests were begun with the tubes immersed in ice water, usually with 1 ml of antiserum (equine unless raised in a mule [M] [1] or a rabbit [R]) and 40\,\mu\text{g} of lipopolysaccharide. The contents of the tubes were mixed, and the racks were allowed to stand in a cold room at 3 to 5°C for 1 week, after which the tubes were read on a scale of 0 to +++. (For rough equivalents in antibody N

<table>
<thead>
<tr>
<th>Anti-Pn no. (homologous N°)</th>
<th>Precipitation reading for lipopolysaccharide immune type:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I</td>
</tr>
<tr>
<td>3. 792C (600)</td>
<td>+</td>
</tr>
<tr>
<td>4. 609C (2,390)</td>
<td>−</td>
</tr>
<tr>
<td>6. 681C (724)</td>
<td>+</td>
</tr>
<tr>
<td>7. 937C (887)</td>
<td>+ + ±</td>
</tr>
<tr>
<td>9. 623C (1,655)</td>
<td>+ +</td>
</tr>
<tr>
<td>10. 627C (864)</td>
<td>−</td>
</tr>
<tr>
<td>14. 635C (1,010)</td>
<td>+ +</td>
</tr>
<tr>
<td>16. 594C (872)</td>
<td>+</td>
</tr>
<tr>
<td>20. 616 (355)</td>
<td>+ +</td>
</tr>
<tr>
<td>22. 566 (878)</td>
<td>+ ±</td>
</tr>
<tr>
<td>23. 912C (418)</td>
<td>+ + +</td>
</tr>
<tr>
<td>\textit{M}25SC (620)</td>
<td>+ + ±</td>
</tr>
<tr>
<td>\textit{M}25SC (480)</td>
<td>+ + ±</td>
</tr>
</tbody>
</table>

* First number (e.g., 3.) is designation for Pn type; second number (e.g., 792) is equine serum identification number; C indicates absorption with Pn group-specific C-polysaccharide; subscript numerals indicate bleed number. M, Anti-Pn serum raised in a mule.

† Homologous N, Precipitable type-specific antibody nitrogen (\mu\text{g/ml}).

§ Scale is − through +++. See the text for further explanation.

† Anti-Pn 6, pool 2, was used for lpIV through lpVII.

' Quantitatively, 93\,\mu\text{g} of nitrogen per ml. This cross-reaction is reciprocal. A human anti-lpIV, diluted with saline, gave a reading of + + ± with Pn S25.
precipitated per milliliter, see reference 10, p. 68.) Additional amounts of 40 to 80 μg of lipopolysaccharide were added, and the process was repeated.

The repeating units of the cell wall O-chains of the seven Fisher immunotypes of P. aeruginosa are shown below (all sugars are in the pyranose form):

I →2)-L-Rha-α-(1→3)-D-QuinFoS-α-(1→4)-D-Glc-α-(1→4)-D-ManImA-(1→6)
\[ \text{OAc} \]

II →2)-D-Glc-β-(1→3)-L-FucNAc-α-(1→3)-D-FucNAc-β-(1→6)
\[ \text{OAc} \]

III →4)-ManImA-β-(1→4)-Gul-1,2-(NAc)\text{A-α-(1→3)}-FucNAc-QuinFoS-α-(1→4)-L-Rha-α-(1→4)-L-GalNAcA-α-(1→3)-D-QuinFoS-β-(1→6)
\[ \text{OAc} \]

IV →5)-L-Rha-α-(1→4)-L-GalNAcA-α-(1→3)-D-QuinFoS-β-(1→6)
\[ \text{OAc} \]

VI →6)-D-Glc-β-(1→2)-L-Rha-β-(1→3)-L-Rha-β-(1→6)
\[ \text{OAc} \]

VII →4)-D-ManImA-β-(1→4)-D-ManNAcA-β-(1→3)-D-FucNAc-β-(1→6)
\[ \text{OAc} \]

The abbreviations used are as follows: Gal, galactose; Fuc, fucose; Glc, glucose; Man, mannose; Rha, rhamnose; GalA, galacturonic acid; Gua, guluronic acid, etc.; GalNAc, N-acetylgalactosamine, etc.; QuinFoS, N-formylquinovosamine; and ManImA, 1-acetyl-2-methyl-(β-D-mannopyranosyl)uronic acid)-3,2-dilimidazoline (D. Horton and M. G. Schweitzer, Am. Chem. Soc. Meet., abstr. MBTD 138, 1985; for types III and IV, D. Horton, personal communication).

Because all tests in anti-Pn 1, 5, 8, and 11; R13, 15, R17, 18, and 27; and anti-Klebsiella 1 were + and tests were − to + in anti-Pn 2, 12, and 19 and anti-Salmonella paratyphi A, these antisera are omitted from Table 1.

**P. aeruginosa type I.** Cross-precipitation of the lipopolysaccharide in anti-Pn 6 is due either to its 1,3-linked d-QuinFoS, which is chemically and hence serologically related to 1,3-bound d-Glc in pneumococcal (Pn) S6 (22), or to a loose fit of the ManA derivative into antibody sites designed for the ribitol phosphate of Pn S6, or to both. The reading of +++ in anti-Pn 7 is explained by the occurrence of 1,2-linked l-rhamnose (l-Rha) and 1,4-bound d-Glc in Pn S7 (3), both of which are also present in Pn S22 and account for the reaction in anti-Pn 22 (C. C. Barbadori and M. B. Perry, Can. J. Chem., in press). Because of the d-Glc, d-QuinFoS, and d-ManNAcA in Pn S9 (13, 25), a stronger reaction in anti-Pn 9 might have been expected. As has been noted earlier (e.g., references 7 and 22) 1,2-bound l-Rha and other sugars act like somewhat hindered nonreducing lateral end-groups. Pn S19 also has 1,2-linked l-Rha (20), but lPf gives no definite precipitation with the one available anti-Pn 19, equine serum 931. In Pn S23 (8, 18) there are lateral end-groups of l-Rha. In anti-Pn 14 the 1,4-linked d-Glc (2, 19) in lPf and Pn S14 (19) and an analog (11) in lPf would seem responsible; the structure of Pn S25 is not known.

**Type II substance.** Precipitation in anti-Pn 3, 6, 9, and 23 seems due to some cause other than similarly linked sugars in the repeating units of the O-chain or of the Pn polysaccharides. It is possibly due to the Glc or GlcNAc of lipid A or the core. Alternatively, the horses which produced the antisera might have suffered a previous inapparent infection with a cross-reacting *Pseudomonas* strain. With this reservation, +++ in anti-Pn 4 is predictable from the →3)-l-FucNAc(1→3)-d-GalNAc of Pn S4 (14, 17).

Pn S22, with its lateral nonreducing end-group of d-Glc and 1,3-linked d-galactofuranose (M. B. Perry, personal communication) would very likely stimulate the formation of antibodies reactive with the 1,2-linked d-Glc and 1,3-linked d-FucNAc of lPf. The structure of Pn S25 is not known, but it contains d-GalA and when hydrolyzed and chromatographed gives other spots with the mobilities of Gal, GalN, and GlcN (4). If its GlcN is 1,2-linked or the Gal or GalN is

**P. aeruginosa type III.** Except for (+ +) in anti-Pn 6, possibly for the same reason as with lPf, lPfII showed only − to (+) in the other antisera.

**P. aeruginosa type IV.** The high concentrations of aminosugars in this lipopolysaccharide (see immunotypes above; D. Horton, personal communication) made it seem likely that the only immediate, heavy cross-reaction in this entire series (in anti-Pn 25) is due to these sugars and Gal, especially because Gal, GalN, and GlcN are indicated as constituents of Pn S25 (4). One or more of the aminosugars may also be responsible for the other cross-reactions of type IV.

**P. aeruginosa type V.** It is reasonable to ascribe the weak cross-reaction in anti-Pn 9 to the chemical relationship of d-QuinFoS to d-Glc. These sugars are 1,3-linked in lPf and Pn S9, respectively (21, 25). The l-L-GalNAcA of lPf might fit loosely into anti-Pn 10 sites intended for ribitol phosphate (23). There is no evident cause for the reactions in anti-Pn 14 and 23. Precipitation in anti-Pn 25 may be for one of the reasons given for lPfV. **P. aeruginosa type VI.** There is no clear basis for the few weak cross-reactions shown in Table 1. As in other instances, infection of the horses with cross-reacting microorganisms might be responsible. This applies also to *P. aeruginosa* type VII.

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**LITERATURE CITED**