Detection of C-polysaccharide in serum of patients with *Streptococcus pneumoniae* bacteraemia

S H Gillespie, M D Smith, A Dickens, J G Raynes, K P W J McAdam

**Abstract**

**Aim**—To investigate the fate of *Streptococcus pneumoniae* C-polysaccharide antigen in serum in patients with *S. pneumoniae* bacteraemia.

**Method**—In vitro dissociation experiments were performed to demonstrate that C-polysaccharide was masked by ligands in normal and acute phase serum. Serum samples from 22 patients with *S. pneumoniae* bacteraemia were treated to dissociate immune complexes and then tested for C-polysaccharide by enzyme linked immunosorbent assay (ELISA).

**Result**—C-polysaccharide antigen was masked in normal and acute phase serum but could be released by EDTA treatment and detected by ELISA. Antigen was found in six patients ranging in concentration from 2.5 to 200 ng/ml. Patients with detectable antigen were more likely to die than those in whom antigen was not detected.

**Conclusion**—This study demonstrates that C-polysaccharide antigen commonly circulates in patients with *S. pneumoniae* bacteraemia but its presence is masked by ligands present in serum.

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**Keywords:** C-polysaccharide, *Streptococcus pneumoniae*.

*Streptococcus pneumoniae* is the commonest bacterial cause of acute community acquired pneumonia.1 Bacteraemia is an important life-threatening complication of *S. pneumoniae* induced pneumonia which still has approximately 30% mortality despite the availability of antibiotics to which this organism is usually sensitive.2

Although the bacterial capsule is an essential determinant of pathogenicity,3 it is not toxic to the host. Its main activity is to inhibit phagocytosis by preventing contact between the phagocyte surface and complement components deposited on the bacterial cell wall.3 C-polysaccharide is the major cell wall polysaccharide antigen of *S. pneumoniae*. Native C-polysaccharide is a ribitol teichoic acid composed of a repeating oligosaccharide subunit made up of β-D-glucitol p-1-3-α-acetamide-tri-deoxygalactosamine p-1-4-α-D-GalNAc p-1-3-β-D-GalNH2 p-1-1'-ribitol-5-phosphate.4 The immunodominant epitope is made up of the two phosphorylcholine residues attached to the oligosaccharide subunit.4 The *S. pneumoniae* lipoteichoic acid, known as the F antigen, has a similar polysaccharide structure and also contains phosphorylcholine.4

The importance of C-polysaccharide in the pathogenesis of inflammation in *S. pneumoniae* infection is being increasingly recognised. C-polysaccharide and F antigen activate the alternative complement pathway through the phosphorylcholine component. C-polysaccharide is the active cell wall component, as removal of teichoic acid from peptidoglycan diminishes complement activation by cell wall components. The plasma membrane is also a weak activator of the alternative pathway, and this activity resides in the lipoteichoic acid F antigen.6 The components of the complement cascade generated by interaction with C-polysaccharide are thought to be crucial in the generation of an inflammatory reaction in the alveoli7 and the meninges.8 Studies of inflammation in the rabbit model indicate that those fractions of killed *S. pneumoniae* which contain teichoic acid polymers induce inflammation when injected into the cisterna magna.9 Organisms with high spontaneous loss of phosphorylcholine containing components induce a greater inflammatory response in the rabbit meninges.9

The fate of C-polysaccharide in the body is unknown. There is only a single report of serum C-polysaccharide antigen in a splenectomised patient with fulminant bacteraemia.10 There is also a single report of C-polysaccharide antigen being found in urine.11 We postulated that C-polysaccharide antigen was present in serum but masked by ligands such as anti-phosphorylcholine antibody and C-reactive protein (CRP). We have now shown that this is the case experimentally and in a series of patients with *S. pneumoniae* bacteraemia.

**Methods**

**IMMUNOASSAYS**

Microtitration plates (M29A Dynatech, UK) were coated with a 1 in 2000 dilution of a
mouse IgM monoclonal antibody directed against phosphorylcholine (5/88 Universal Biologicals, UK) in 0.06 M bicarbonate buffer (pH 9.6) by overnight incubation at 4°C. After washing with 10 mM Tris containing 0.15 M NaCl and Tween-20 (0.05%) and 1 mM CaCl2 (pH 7.0) (TBSTC) samples were added and incubated for one hour at room temperature. CRP was purified and conjugated with hors eradish peroxidase by the peroxidase method. After further washes, 100 μl CRP conjugate (1.7 μg/ml), diluted in TBSTC, was added and the plates incubated for three hours at room temperature. Then 2,2'-azino-di-3-ethyl-benzthiazoline sulphonate (ABTS) peroxidase substrate (Kirkegaard-Perry, Gaithersburg, Maryland, USA) was added after the plates were washed four times and the optical density read at 405 nm using an automated enzyme linked immunosorbent assay (ELISA) reader (Titertek Multiscan MC, Flow Laboratories, UK). Antigen concentration was quantified by comparison with an antigen standard curve diluted in TBSTC using known quantities of purified C-polysaccharide antigen (Dr J Lui, Rockefeller University).

**Results**

Detection of dilutions of C-polysaccharide antigen in buffer, normal serum, and acute phase plasma are illustrated in fig 1A. It shows that C-polysaccharide antigen cannot be detected in acute phase when it is added to normal serum. The results of assays following immune complex dissociation are illustrated in fig 1B. These data suggest that the standard antigen dilution curve can be reconstituted by treatment of acute phase and normal serum with EDTA.

Serum samples from 22 patients (13 males and nine females; mean age 63 years, median 69 years, range seven months to 92 years) with acute *S pneumoniae* bacteraemia were examined. Pneumonia was the main condition in 17 cases and conformed to a lobar pattern in eight. In four patients bacteraemia was a complication of meningitis and in one bacteraemia was present without evidence of localised infection. A predisposition to infection was found in six patients with chronic obstructive airways disease, in three following acute myocardial infarction and congestive cardiac failure, and in one with immunosuppression due to azathioprin and steroid therapy.

There was a fatal outcome in five patients, directly related to *S pneumoniae* infection in three. A single fatal case was associated with acute pancreatitis and *Pseudomonas sp.* bacteraemia, and in another death occurred suddenly 12 days after acute myocardial infarction.

C-polysaccharide antigen was found in serum from six of the 22 patients with *S pneumoniae* bacteraemia in concentrations ranging from 2.5 to >200 ng/ml. Of these six patients,
Serum C-polysaccharide in S pneumoniae bacteraemia

Table: Outcome of S pneumoniae bacteraemia and antigen in serum

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Antigen positive</th>
<th>Antigen negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Died</td>
<td>3</td>
<td>2*</td>
</tr>
<tr>
<td>Survived</td>
<td>3</td>
<td>14</td>
</tr>
</tbody>
</table>

* One patient died suddenly 12 days after an acute myocardial infarction during acute infection. Another had severe pancreatitis and coincident Pneum septicaemia.

During septicaemia and in the first 48 hours following hospital admission, some patients at higher risk of death from pneumococcal infection which was not influenced by antimicrobial therapy in the first 48 hours following hospital admission. Patients with fulminant disease show profound hypocomplementaemia with consumption of the components of the alternative complement pathway. It has also been shown that in patients with complicated S pneumoniae infection alternative complement pathway factors were significantly depleted when compared with patients with uncomplicated infections.

In view of the importance of teichoic acid components in generating inflammation by activation of the alternative complement pathway the presence of C-polysaccharide antigenemia demonstrated in our bacteraemic patients supports the idea that this antigen has an important role in the pathogenesis of this disease.

Demonstration of circulating C-polysaccharide may also explain the contradictory results of some protection studies using phosphorylcholine binding ligands. Rabbit polyclonal and mouse monoclonal antibodies directed against phosphorylcholine have been shown to confer some degree of protection and this has also been shown for CRP. In one explanation for this protection was thought to be by enhancement of opsonisation and C-polysaccharide is situated deep within the capsule and is not available for opsonisation and therefore opsonisation experiments have indicated that antibodies directed against phosphorylcholine, in contrast to those directed against C-polysaccharide, do not promote phagocytosis for most S pneumoniae serotypes and that the opsonising efficacy of serum can be correlated with levels of anti-capsular but not anti-phosphorylcholine antibody.

It is possible that the mechanism of protection associated with phosphorylcholine binding ligands is due to its ability to bind free C-polysaccharide and prevent overwhelming activation of the complement cascade.

In this study C-polysaccharide was detected in the serum of five of 20 patients with bacteraemic S pneumoniae infection. This is similar to the proportion of patients with S pneumoniae bacteraemia in whom capsular polysaccharide can be detected. C-polysaccharide antigen was found in only six of 22 patients with S pneumoniae bacteraemia although all of the control serum samples were negative for this antigen. As a diag, this test is equivalent to a sensitivity of 27% and a specificity of 100%.

Although this is the first report of the use of C-polysaccharide antigen detection in serum by lysis of immune complexes it is clear that this method is specific but does not provide any further advantages to capsular antigen de-
tection methods already reported. Further studies are underway to determine the importance of C-polysaccharide antigen concentration with respect to outcome.

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