Immunoelectrophoresis for Detection of Polysaccharides in Immune Complexes

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A procedure for detecting pneumococcal capsular polysaccharides in immune complexes is described. Separation of antigen from immune complexes is achieved by electrophoresis at 56°C.

Pneumococcal polysaccharide is present in the circulatory system of some patients with pneumococcal infection (1). By use of radioimmunoassay, G. Schiffman, J. E. Summerville, R. Castagna, R. Douglas, M. J. Bonner, and R. Austrian (Fed. Proc. 33:758, 1974) showed that the polysaccharide in some sera is bound to circulating antibody (6) and is detectable only after acid-pepsin digestion of the sera. We have been interested in devising a simple method which could detect both free and complexed polysaccharide in clinical samples from patients with pneumococcal infection.

Milgrom et al. (3, 4) used immunoelectrophoresis to detect protein and nucleic acid antigens in complexes in serum and tissues of animals with immune complex nephritis. We applied their method to the problem of detecting polysaccharides in immune complexes. The method is based on electrophoresis in a gel medium at 56°C to facilitate dissociation of complexes. Free antigen is then detected by use of either counterimmunoelectrophoresis (CIE) or double immunodiffusion, the former technique providing greater sensitivity.

To evaluate the method, we prepared insoluble immune complexes by precipitating type-specific pneumococcal polysaccharides (obtained from Eli Lilly & Co.) in an excess of pneumococcal antibody at 4°C. We evaluated both type-specific rabbit antibody (Statens Seruminstitut, Copenhagen, Denmark) and human antibody (obtained from a patient who was convalescing from pneumococcal pneumonia). Gel plates were prepared with 1% agarose in barbit-}

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to 45 to 60 min, the antigen migrated an excessive
distance through the gel and was less readily
detected by CIE. With insoluble complexes, an
initial electrophoresis of 30 min at 56°C also
appeared optimal; more prolonged electropho-
resis at 56°C resulted in excessive migration of
the free antigen.

Using the technique worked out with insoluble
complexes, we evaluated clinical samples from
patients with pneumococcal infection. Twenty-
one sera (stored at −20°C) from 14 patients with
bacteremic pneumococcal pneumonia were
available for study. The serotypes of the blood
isolates in these cases were as follows: type 1
(one case), type 3 (three cases), type 4 (four
cases), types 8 and 9 (two cases each), and types
11 and 12 (one case each). Sera were subjected
to electrophoresis at 56°C, along with a control
aliquot at room temperature, and free antigen
was detected with polyclonal pneumococcal anti-
serserum (Omniserum, Statens Seruminstitut). In
addition, all sera were tested by a conventional
method of CIE consisting of a 60-min period of
electrophoresis at room temperature with the
test serum and polyclonal antibody placed in
opposing wells (1). Results are listed in Table 1.
Four sera contained antigen which was detecta-
ble only by use of electrophoresis at 56°C. Fur-
ther tests showed that the serotypes of antigen
in these four sera were type 3 (two sera), type 4
(one serum), and type 11 (one serum). The an-
tigen type corresponded with the serotype of the
pneumococcal isolate from the blood in each
case. The results obtained with electrophoresis
at 56°C suggested that the four sera contained
immune complexes of capsular polysaccharide.

<table>
<thead>
<tr>
<th>Source</th>
<th>Electrophoresis (no. positive/no. tested)</th>
<th>Conventional CIE (no. positive/no. tested)</th>
</tr>
</thead>
<tbody>
<tr>
<td>56°C</td>
<td>Room temp</td>
<td></td>
</tr>
<tr>
<td>Patients</td>
<td>9/14</td>
<td>7/14</td>
</tr>
<tr>
<td>Sera</td>
<td>12/21</td>
<td>8/21</td>
</tr>
</tbody>
</table>

*Electrophoresis was for 30 min at indicated temper-

In support of this possibility, we found that two
of the sera also contained type-specific hemag-
glutinating antibody (titer of 32 in the case of
the type 4 infection and titer of 16 in one of
the cases of type 3 pneumonia). The antibody titer
was less than 4 (i.e., negative) in the remaining
case of type 3 infection, and we could not check
for antibodies in the patient with type 11 infec-
tion because we lacked the necessary purified
capsular polysaccharide of type 11. The clinical as-
cpects of these four cases were not unusual, except
that two of the patients had pleural effusions which
contained capsular polysaccharide and which
could have readily entered the circulation to
form complexes.

Our studies suggest that capsular polysac-
charides can be separated from complexes by
electrophoresis at 56°C and that this approach
can give positive results in some cases where
CIE at room temperature is negative. The num-
ber of additional positive cases which can be
expected by electrophoresis at 56°C is not cer-
tain from the present small study. O'Reilly et
al., using radioimmunoassay, detected complexes of capsular polysaccharide in 17 out of 45 cases of hemophilus infection (5). However, the quantity of complexed antigen in many of their cases was quite low (<0.01 µg/ml), which is below the sensitivity of immunoprecipitin methods, including CIE.

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LITERATURE CITED


