Radioimmunoassay of capsular polysaccharide antigens of groups A and C meningococci and *Haemophilus influenzae* type b in cerebrospinal fluid

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SUMMARY Sensitive radioimmunoassays capable of measuring 0.5 ng/ml of the *Haemophilus influenzae* type b polysaccharide and 2 ng/ml of the groups A and C meningococcal polysaccharides were developed and used to detect these substances in cerebrospinal fluid (CSF). Polysaccharide of the causative agent was detected in the CSF of 14 out of 15 patients with *Haemophilus influenzae* type b meningitis, in 18 out of 23 patients with group A, and in two out of four patients with group C meningococcal meningitis. In some cases the antigen could be detected even after three days of antibacterial treatment. No false positive reactions were seen. The assay procedure could be shortened to approximately three hours. These assays could be useful in routine diagnostic work and epidemiological investigations.

Soluble bacterial antigens have been shown to be present in the cerebrospinal fluid (CSF) of patients with meningitis (Rake, 1933), and their demonstration by countercurrent immunoelectrophoresis (Greenwood *et al.*, 1971; Sillanpää *et al.*, 1975) or latex agglutination (Whittle *et al.*, 1974) has been used as a diagnostic test. These methods do not always give positive results even in cases verified by bacterial cultures. Assuming that tests with increased sensitivity would give further positive results, we decided to study the use of radioimmunoassays for the detection of these polysaccharides. We present here results obtained with a highly sensitive and rapid radioimmunoassay for meningococcal and *Haemophilus influenzae* type b polysaccharides in CSF.

Material and methods

CEREBROSPINAL FLUID SAMPLES

CSF was received from definite or suspected cases

of bacterial meningitis from several hospitals in different parts of Finland. There was a preponderance of cases caused by group A meningococci because of an epidemic in 1973-75 (Mäkelä *et al.*, 1975; Peltola *et al.*, 1976).

RADIOIMMUNOASSAYS

The tyramine derivatives of the capsular polysaccharides of group A and group C meningococci (MenA, MenC) (Axén *et al.*, 1967; Gotschlich *et al.*, 1972) were received from Dr E. Gotschlich (the Rockefeller University, New York, NY 10021) and that of type b *Haemophilus influenzae* (Hi b) (Robbins *et al.*, 1973) from Dr J. Robbins (Bureau of Biologics, Federal Drug Administration, Bethesda, Maryland 20014). These were iodinated with Na¹²⁵I (Radiochemical Centre, Amersham) as described by Greenwood *et al.* (1963). As standard antigens we used the tyramine derivative of group C meningococcal polysaccharide without iodination, the lyophilised group A polysaccharide vaccine (lot 572 Merck Sharp and Dohme) (Peltola *et al.*, 1976), and the lyophilised *Haemophilus influenzae* type b vaccine prepared by Dr Porter Anderson (Peltola *et al.*, 1976; Anderson *et al.*, 1972). The standard antigens were solubilised in phosphate buffered saline containing 0.02% azide and 1% fetal bovine serum

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free of immunoglobulins (Microbiological Associates).

The antisera were diagnostic sera for routine serotyping (Difco). They were diluted in fetal bovine serum to give 30 to 60% binding of the antigen.

In the assays, 50 or 100 μ l of sample or standard antigen, 100 μ l of antiserum dilution, and 50 μ l of labelled antigen (0.01 μ g/ml) were incubated at +4°C for 20 or 2 hours. The immunoglobulins with bound antigen were precipitated by adding an equal volume of saturated ammonium sulphate followed after 30 to 60 minutes by 50% saturated ammonium sulphate to give a final volume of 2 ml. The precipitate was collected by centrifugation and counted for radioactivity. Concentration of CSF was performed by immersing a sack of dialysis tubing containing the sample in Sephadex G-10 powder.

Results and discussion

Comparison of standard inhibition curves obtained using incubation of the assay tubes for 20 or 2 hours (Figure) showed that sufficient binding occurred after two hours to allow detection and quantitation of the polysaccharides. The detection limit was about 0.5 ng of the *Haemophilus influenzae* type b polysaccharide and about 2 ng of meningococcus group A or C polysaccharides. The shorter incubation caused about a two-fold decrease in the sensitivity of the assay. This makes radioimmunoassay 10 to 50 times more sensitive than earlier methods such as countercurrent immunoelectrophoresis (Coonrod and Rytel, 1972) or latex agglutination (Whittle *et al.*, 1974; Leinonen and Herva, 1977), which detect 20-50 ng/ml of the bacterial polysaccharides.

A total of 64 CSF samples were assayed for all three polysaccharides (Table). Polysaccharides discordant with the type of bacteria identified by culture were not found. Nineteen samples from various groups when tested using two-hour incubation in the assay gave results concordant with those obtained with the longer incubation.

In most cases of untreated meningitis the antigen content in CSF seems to be high enough to be detectable by the earlier methods; we found over 20 ng/ml of antigen in 90% of untreated cases. However, in six samples, four of which were taken one to three days after the beginning of antibacterial therapy, the amount of *H. influenzae* type b antigen was below 20 ng, with as little as 0.5 ng/ml found in three samples. Diagnosis by antigen determination is especially valuable in this situation, where antibacterial therapy has rendered the cultures negative. Our finding of the presence of *H. influenzae* type b antigen in CSF a few days after the beginning of therapy is in accordance with earlier results (O'Reilly

Figure Inhibition by unlabelled antigen of binding of ^{125} I labelled capsular polysaccharides of *Haemophilus influenzae* type b (H i b) and groups A and C meningococci (MenA, MenC) to specific antiserum. Solid line = 20-hour incubation; broken line = 2-hour incubation. Percent antigen bound = $\frac{A}{T} \times 100 - \frac{B}{T} \times 100$, where T = total activity, A = activity of the precipitate, and B = background activity as determined from tubes prepared without antiserum. This was 5% for the H i b polysaccharide and 5 and 15% for the MenA and MenC polysaccharides, respectively.

et al., 1975) where this polysaccharide was found to persist even longer in spite of appropriate treatment. Residual antigen was found also in treated cases of meningococcal meningitis, but less often than was the case with the H i b polysaccharide (3 out of 9 and 4 out of 5 cases, respectively). This may be due to faster elimination of the meningococcal polysaccharides.

As a further control of specificity we tested CSF samples from four patients with group B meningococcal meningitis and 18 control samples. These were negative for the three antigens tested. It would be important to develop a test to detect group B meningococcal disease, but the small molecular size and poor immunogenicity of the group B polysaccharide have so far hampered such attempts.

Table Detection by radioimmunoassay of capsular polysaccharide antigens of *Haemophilus influenzae* type b (H i b) or meningococci of groups A (MenA) or C (MenC) in cerebrospinal fluid of patients with meningitis

Number of patients	Causative agent*	Amount of antigen detected in CSF†, (ng/ml)			Number of patients with antibacterial therapy before sampling
		H i b	MenA	MenC	
8	H i b	50 to > 100	—	—	0
3	"	3.5 to 18	—	—	1
3	"	~ 0.5‡	—	—	3
1	"	—	—	—	1
15	MenA	—	100 to > 1000	—	1
3	"	—	26 to 60	—	1
5	"	—	—	—	4
2	MenC	—	—	150, 160	1
2	"	—	—	—	2
4	MenB	—	—	—	—
18	No specific pathogen	—	—	—	—

*Based on culture of CSF or blood or, in three cases, on specific antibody rise.

†These were not always the same samples which had given the positive culture result.

‡Polysaccharide detectable only after concentration of the samples.

The severity of bacterial meningitis and the epidemiological importance of meningococcal disease warrant a maximum effort at their rapid and accurate diagnosis. The radioimmunoassays we describe may be a contribution to this end.

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