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Cross-Reactive Monoclonal Antibodies for Diagnosis of Pneumococcal Meningitis

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A diagnostic test for the detection of *Streptococcus pneumoniae* meningitis was developed using monoclonal antibodies (MAbs) to phosphocholine (PC) and non-PC determinants of pneumococcal teichoic acids. These MAbs do not recognize other bacteria that commonly cause meningitis. By using a dot blot assay, these MAbs were compared with a polyvalent pneumococcal capsular omniserum and an antiserum made to whole cells for their ability to detect pneumococci in infected spinal fluids. An immunoglobulin M (IgM) anti-PC antibody gave a positive reaction with 16 of 22 (73%) pneumococcal culture-positive spinal fluids. One false-positive result out of 45 pneumococcal culture-negative spinal fluids was also observed. D3114/63, an IgM MAb to non-PC determinants of teichoic acids, detected 15 of 22 of the pneumococcal culture-positive spinal fluids with one false-positive result. IgG2b and IgG3 anti-PC MAbs were less efficient than the IgM anti-PC MAb at detecting pneumococci in spinal fluids. Like the IgM anti-PC MAb, omniserum detected 73% of the culture-positive pneumococcal spinal fluids, with one false-positive result. The use of anti-PC or D3114/63 MAbs instead of a pooled serum such as omniserum has several advantages: (i) use of a single cross-reactive antibody rather than 83 pooled antibodies; (ii) possibility of a higher concentration of reactive antibody, which may increase the sensitivity of the test; (iii) a standardized antibody preparation; (iv) ease of preparation of the antibody; and (v) less expense.

The rapid diagnosis of pneumococcal infections, in particular meningitis, is of critical importance. Since the highest incidence of pneumococcal meningitis occurs in children less than 2 years of age and because of the high case fatality rate of this infection (7, 10), a rapid, reliable diagnostic test would greatly improve the prognosis of patients with this disease. Several assay methods have been studied to determine their effectiveness in diagnosing pneumococcal meningitis, such as counterimmunoelectrophoresis (3, 16, 17), latex agglutination (16, 17), and coagglutination (4, 14, 16, 17). The sensitivities of the methods vary among studies, but in general counterimmunoelectrophoresis is the least sensitive, followed by coagglutination and latex agglutination.

A major difficulty in devising a diagnostic assay for pneumococcal meningitis is the multiplicity of capsular serotypes of *Streptococcus pneumoniae*. Most of the diagnostic assays developed thus far have used a pooled anticapsular serum, commonly omniserum, for the detecting antibody. This type of pooled antiserum suffers from at least two disadvantages. First, because so many different antibodies are included in the pool, the antibody concentration of any one capsular serotype may not be high enough to detect low concentrations of antigen. Second, cross-reactions, in particular with alpha streptococci, have been found to be associated with this antiserum (9). An alternative approach to using a pooled antiserum would be the use of a single antibody which would react with an antigenic determinant common to all pneumococci. Antibodies to the phosphocholine (PC) determinant of the pneumococcal cell wall and to a determinant in the pneumococcal teichoic acid recognized by the monoclonal antibody (MAb) D3114/63 have been shown to react with all pneumococci tested and do not react with the other streptococci tested (13).

The present study was undertaken in part to determine the usefulness of anti-PC and D3114/63 MAbs in the diagnosis of pneumococcal meningitis. The study also allows the comparison of the usefulness of MAbs in different types of assays, as well as comparisons of the relative sensitivities of assays with immunoglobulin M (IgM) and IgG MAbs. The results obtained from comparisons with IgG and IgM anti-PC MAbs are particularly valuable because these particular antibodies are known to have essentially identical idiotypes and binding specificities (1). Thus any differences observed can be assumed to be caused by differences in isotype rather than differences in binding site.

MATERIALS AND METHODS

Spinal fluids. Sixty-one culture-positive pediatric spinal fluids were collected. The culture-positive spinal fluids consisted of the following bacterial species: *S. pneumoniae* (22 fluids), *Haemophilus influenzae* type b (20 fluids), *Neisseria meningitidis* (7 fluids), group B streptococci (7 fluids), *Listeria monocytogenes* (2 fluids), viridans group streptococci (1 fluid), *Staphylococcus aureus* (1 fluid), and *Klebsiella* sp. (1 fluid). The 22 spinal fluids positive for pneumococci contained the following capsular types: type 3 (two isolates), type 6 (six isolates), type 8 (one isolate), type 10 (one isolate), type 12 (one isolate), type 14 (two isolates), type 18 (one isolate), type 19 (three isolates), type 23 (three isolates), type 33 (one isolate), and type 35 (one isolate). Six culture-negative spinal fluids were also obtained and were included as controls. Each spinal fluid was stored at −70°C. Before screening, each spinal fluid was coded and tested in a blind format.

Antibodies. The MAbs and antisera used in this study are listed in Table 1. The production of all of these antibodies has been described in earlier publications (1, 2, 6, 13). Quantitation of the anti-PC antibodies was by Farr assay with ^{14}C-labeled PC chloride. The number of binding sites in

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TABLE 1. Description of the antibodies used to screen spinal fluid samples

Antibody	Antibody type	Isotype	Antibody concn (mg/ml)	Epitope	Reference
140.1C2[a]	MAb	IgG2b(κ)	1.3	PC	2
59.6C5[a]	MAb	IgG3(κ)	0.164	PC	2
HPCM2[b]	MAb	IgM(κ)	2.0	PC	6
D3114/63	MAb	IgM(κ)	? (ascites fluid)	Teichoic acid	13
Anti-R36A	Polyvalent		? (serum)	Whole cell	
Omniserum	Polyvalent pool		? (serum)	Capsule	11, 12

[a] Obtained from Lathum Claflin, University of Michigan, Ann Arbor.
[b] Obtained from Patricia Gearhart, Johns Hopkins University, Baltimore.

each sample was estimated by extrapolation to saturating PC. The amount of each antibody was calculated based on the known molecular weight and number of binding sites of each isotype. The MAbs were produced as ascites fluid in X-linked immunodeficient (*xid*) (CBA/N × BALB/cJ)F_1 mice and diluted 1:40 in phosphate-buffered saline (PBS; pH 7.2). A polyvalent serum was included which was prepared by immunizing rabbits with the heat-killed pneumococcal strain R36A (a rough derivative of the serotype 2 strain D39). Omniserum obtained from Statens Serum Institute (Copenhagen, Denmark), which contains antibodies to all known pneumococcal capsular serotypes, was also included for comparison. A 1:50 dilution in PBS of both the anti-R36A and omniserum was used for testing.

Reactivity of MAbs with pneumococci and other bacteria. *S. pneumoniae* and other gram-positive and gram-negative bacteria were collected and screened for reactivity to anti-PC and D3114/63 MAbs by using the colony blot and lysate blot techniques of McDaniel et al. (13) and Waltman et al. (W. D. Waltman II, L. S. McDaniel, B. Andersson, L. Bland, B. M. Gray, C. S. Eden, and D. E. Briles, Microb. Pathogen., in press). The colony blot procedure is very similar to the dot blot assay, except that blots of bacterial colonies rather than bacterial lysates are tested.

Dot blot assay. Fifteen microliters of the respective spinal fluids or standardizing solutions (i.e., live or heat-killed pneumococci, pneumococcal lysates, or PC-bovine serum albumin [BSA]) were spotted onto sterile nitrocellulose membranes, one membrane for each antibody and a BSA control, allowed to air dry, and blocked for 30 min with a solution of 1% BSA in PBS. The membrane was washed twice in PBS containing 0.05% Tween 20 (PBST) and incubated with the respective antibody at room temperature for 3 h on a rocking platform. After three washes in PBST, a 1:750 dilution (in PBST) of alkaline phosphatase-labeled goat anti-mouse immunoglobulin conjugate (0.2 mg/ml; Southern Biotechnics Associates, Birmingham, Ala.) was incubated for 3 h with the membranes probed with the anti-PC and D3114/63 antibodies. A 1:750 dilution of alkaline phosphatase-labeled goat anti-rabbit immunoglobulin conjugate (0.2 mg/ml; Southern Biotechnics Associates) was incubated for 3 h with the membranes probed with anti-R36A and omniserum antibodies. The membranes were washed three times in PBST, and a solution of 0.5 mg of 5-bromo-4-chloro-3-indolyl phosphate (Sigma Chemical Co., St. Louis, Mo.) per ml in 1 M Tris (pH 8.8) was incubated with the membranes for approximately 60 min at room temperature with gentle rocking. The reaction was stopped by rinsing the membranes with distilled water. Positive reactions were visualized by blue staining areas on the sample spot.

ELISA. An enzyme-linked immunosorbent assay (ELISA) was set up to determine the level of antigen detected by each of the antibodies and to determine the feasibility of making a more rapid diagnostic test. This procedure consisted of absorbing either 100 μl of a solution of 10^8 heat-killed R36A cells per ml (centrifuged in a microdilution plate for 15 min at 1,500 rpm) or 100 μl of 10 μg of PC-BSA per ml (incubated overnight at 4°C) onto a microdilution plate followed by blocking with 1% BSA for 1 h. The subsequent incubations with the antibody and enzyme-labeled conjugate followed standard methods and were identical with those of the dot blot assay, except that incubation was at 37°C. The substrate, a solution of 1 mg of *p*-nitrophenyl phosphate (Sigma) per ml in diethanolamine buffer (pH 9.6) was added after the incubation with the enzyme-labeled second antibody and allowed to react for 30 min at room temperature. The reaction was stopped by adding 50 μl of 3 N NaOH, and the plate was read with a Titertek Multiskan (Flow Laboratories, McLean, Va.) microdilution plate reader at 405 nm. Values greater than twice the background level were considered positive.

To determine the feasibility of making a more rapid diagnostic assay, the ELISA parameters outlined above were modified to determine the optimal conditions necessary to run the assay in the shortest possible time. The titers of antigen, antibody, and conjugate were determined, and the incubation times and temperature were altered.

Determination of assay sensitivity. A serotype 14 strain, BG-215, was grown in 150 ml of Todd-Hewitt broth supplemented with 0.5% yeast extract for 12 h at 37°C. The culture was divided into three parts; 50 ml was used as whole live bacteria, 50 ml was heat killed at 60°C for 30 min, and 50 ml was pelleted and lysed with a lysing buffer (0.1% sodium deoxycholate, 0.01% sodium dodecyl sulfate, 0.15 M sodium citrate). The number of bacteria present was determined by standard plate count on blood agar for the live and heat-killed (before heating) samples, and a protein concentration (Bio-Rad Laboratories, Richmond, Calif.) was measured for the lysate. Tenfold serial dilutions of each preparation were made in PBS, and 15 μl was spotted onto a nitrocellulose membrane and assayed as described above. As a control, PC-BSA was serially diluted in PBS, and 15 μl was added to the membrane and assayed. Samples of 100 μl of the same preparations were tested in an ELISA format, as outlined above, by substituting them for the heat-killed pneumococci as the absorbed antigen.

ELISA inhibition assay. One hundred microliters containing 10^8 heat-killed pneumococcal strain R36A cells was absorbed onto a microdilution plate as described above. Fifty microliters of the individual spinal fluids was serially (twofold) diluted in a microdilution plate, 50 μl of a 1:1,000 dilution of HPCM2, D3114/63, anti-R36A or omniserum was added to each well, and plates were incubated for 3 h at 37°C. Subsequent steps, washing in PBST, incubation with alkaline phosphatase-labeled anti-immunoglobulin, washing in PBST, and incubation with the substrate, followed the

TABLE 2. Accuracy of the dot blot assay for diagnosing pneumococcal meningitis from spinal fluid samples[a]

Antibody	No. with pneumococcal culture		% Sensitivity[b]	% Specificity[c]	% Agreement[d]
	+	−			
HPCM2 +	16	1	72.7	97.8	89.6
−	6	44			
D3114/63 +	15	1	68.2	97.8	88.0
−	−	7	44		
Omniserum +	16	1	72.7	97.8	89.6
−	6	44			
Anti-R36A +	19	26	86.4	42.2	56.7
−	3	19			

[a] MAb HPCM2 was diluted to 50 μg/ml, MAb D3114/63 was diluted 1:40, and the omniserum and anti-R36A were diluted 1:50 for assaying the spinal fluids. + or −, Presence or absence of antigen detected by the antibody, respectively.
[b] The percentage of culture-positive pneumococcal spinal fluids which were found to be positive by the dot blot assay.
[c] The percentage of culture-negative pneumococcal spinal fluids which were found to be negative by the dot blot assay.
[d] The percent agreement between positive and negative culture results and results with the dot blot assay.

ELISA format as described above. The percent inhibition was calculated by dividing the optical density at 405 nm (OD_{405}) value of the test sample by the control (50 μl of PBS instead of spinal fluid) OD_{405} value and multiplying by 100.

RESULTS

The results of the dot blot assay for the detection of pneumococcal antigen in spinal fluids are shown in Table 2. Anti-PC antibody HPCM2 detected 72.7% of the culture-positive pneumococcal spinal fluids. HPCM2 only detected one false-positive result out of 45 culture-negative pneumococcal spinal fluids. This single false-positive spinal fluid was from a case of meningitis in which *N. meningitidis* was isolated.

The D3114/63 antibody detected 68.2% of the pneumococcal culture-positive spinal fluids and one false-positive fluid (Table 2). The single false-positive spinal fluid was the same one detected by the HPCM2 MAb.

The omniserum detected 72.7% of the pneumococcal culture-positive spinal fluids and one false-positive fluid (Table 2). The single false-positive spinal fluid was from a case of group B streptococcal meningitis.

Anti-R36A detected 86.4% of the pneumococcal culture-positive spinal fluids but detected 26 false-positive fluids (57.8%) (Table 2). False-positive spinal fluids detected by this antibody included 11 of 20 *H. influenzae* type b, 5 of 7 *N. meningitidis*, 4 of 7 group B streptococci, 5 of 5 other bacteria, and 1 of 6 control spinal fluids.

Two of the 22 culture-positive pneumococcal spinal fluids were not detected as positive by any of the six antibodies, and two others were only detected by the anti-R36A sera.

To compare the effectiveness of IgG versus IgM anti-PC MAbs, each anti-PC MAb was diluted to 10 μg/ml and used to test six random pneumococcus-positive and four random pneumococcus-negative spinal fluids as well as the level of detecting PC-BSA and live and heat-killed pneumococci (Table 3). The MAb 59.6C5 was slightly better than 140.1C2, but HPCM2 was more sensitive than either of the other two. Based on these results, the difference in the ability to detect positive pneumococcal spinal fluids between the anti-PC antibodies appeared to be due to isotype such that IgM > IgG3 > IgG2b.

The level of pneumococcal antigen detected by each of the antibodies was determined in both the dot blot assay and the ELISA (Table 4). HPCM2 detected as few as 10^7 live or heat-killed pneumococci per ml and detected <20 μg of PC-BSA per ml and 2 μg of PC-BSA per ml by the dot blot assay and ELISA, respectively. D3114/63 detected 10^6 live and 10^8 heat-killed pneumococci per ml by the ELISA. The omniserum detected as few as 10^7 live or heat-killed pneumococci per ml by the dot blot or the ELISA. Anti-R36A

TABLE 3. Determination of isotype importance in detecting pneumococcal meningitis by anti-PC MAbs with the dot blot assay[a]

MAb isotype	No. with pneumococcal culture		% Sensitivity	% Specificity	% Agreement	Level of detection[b]		
						PC-BSA (μg/ml)	Pneumococci (CFU/ml)	
	+	−					Live	Heat killed
IgG2b +	4	1	67	75	70	87	6×10^7	6×10^7
−	2	3						
IgG3 +	4	2	80	60	70	353	6×10^7	$>10^8$
−	2	2						
IgM +	5	0	83	100	90	87	6×10^7	6×10^6
−	1	4						

[a] The IgG2b, IgG3, and IgM anti-PC MAbs used were 140.1C2, 59.1C5, and HPCM2, respectively. All antibodies were assayed at 10 μg/ml. + or −, Presence or absence of antigen detected by the antibody, respectively. See footnotes *b*, *c*, and *d* of Table 2.
[b] Minimum amount of protein or number of live or heat-killed pneumococci detected by the respective anti-PC MAbs. Fifteen microliters of each solution was assayed.

TABLE 4. Level of pneumococcal antigen detected by four antibodies with the dot blot assay and the ELISA[a]

Assay	Antibody	Pneumococcal antigen (CFU/ml)			PC-BSA (μg/ml)
		Live	Heat-killed	Lysate	
Dot blot	HPCM2	2×10^7	1×10^7	4.0	<20
	D3114/63	$>10^8$	7×10^7	>373	>853
	Omniserum	2×10^7	1×10^7	4.0	>853
	Anti-R36A	2×10^6	7×10^5	0.4	126
ELISA	HPCM2	1×10^7	1×10^7	0.9	2
	D3114/63	1×10^6	1×10^8	250	>853
	Omniserum	2×10^7	2×10^7	4.7	>853
	Anti-R36A	7×10^5	7×10^5	0.2	7

[a] Values represent the minimum number of pneumococci or amount of protein detected by the respective assay. The dot blot assay used 15 μl of solution, and the ELISA used 100 μl of solution.

antiserum detected 10^6 live and heat-killed pneumococci per ml by the dot blot assay. The D3114/63 and omniserum antibodies did not react with PC-BSA, as would be expected.

Studies to determine the feasibility of shortening the assay time were conducted by using an ELISA format with heat-killed pneumococci as the antigen. The concentrations of antibody and conjugate were varied with the length and temperature of incubation to determine the conditions necessary to run the assay in the shortest possible time without compromising sensitivity (Table 5). By diluting a positive ELISA reaction and comparing visual assessment with spectrophotometric values at 405 nm, we found that an OD_{405} of 0.3 or greater was sufficient to visually distinguish a positive reaction from a negative reaction. The data indicate that using an OD_{405} of 0.3 as an endpoint, the test could be run in less than 60 min if 8 μg of the HPCM2 antibody per ml and 2 μg of the enzyme-labeled conjugate per ml were used and if the incubation times were 15 min for both the antibody and conjugate and 10 min for the substrate; each incubation was at 37°C.

The possibility that an inhibition assay would provide a more rapid test system was evaluated by using heat-killed pneumococci as the solid-phase antigen. Randomly selected positive and negative serially diluted spinal fluids were mixed with the screening antibody in the microtiter wells coated with heat-killed pneumococci. The percent inhibition necessary to separate positive and negative samples was selected to be ≥20% for all the antibodies except HPCM2, where ≥40% inhibition was chosen (Table 6). The reason for selecting an inhibition of ≥40% for HPCM2 is evident from Fig. 1, which shows that the percent inhibition of HPCM2 binding by all of the spinal fluids was higher than the percent inhibition of binding with the other antibodies. The inhibition assay with HPCM2 successfully predicted five culture-negative and five culture-positive pneumococcal spinal fluids; one of the positive fluids was one not detected by the dot blot assay. D3114/63 detected four of five positive fluids (the one missed was also not detected by the dot blot assay) but gave three false-positive reactions. Omniserum detected four of five positive fluids but gave two false-positive reactions. Anti-R36A antiserum detected only two of five positive spinal fluids and one false-positive reaction.

A survey of pneumococci and other gram-positive and gram-negative bacteria showed that IgM anti-PC MAbs react with the lysates and/or colony blots of all of 300 pneumococcal isolates tested. The MAb D3114/63 reacted with all of 79 pneumococcal isolates by the colony blot assay and with 93% of pneumococci by lysate blotting. Both anti-PC and D3114/63 antibodies were highly specific. With the exception of two weak reactions for the anti-PC MAbs with *N. meningitidis*, the anti-PC and D3114/63 MAbs did not react with any of 62 isolates of other bacteria including group A, B, C, D, and G and viridans group streptococci, *L. monocytogenes*, *H. influenzae*, *N. meningitidis*, and *Escherichia coli*.

DISCUSSION

Of the antibodies tested, the IgM anti-PC MAb and the omniserum gave comparable results. They both detected 73% of the pneumococcal culture-positive spinal fluids and detected only 2% of the spinal fluids that were either normal or were culture positive for an organism other than the pneumococcus. Omniserum detected one pneumococcus-positive spinal fluid that HPCM2 missed, but HPCM2 detected one fluid that omniserum missed. Thus, although the anti-PC MAb did not perform better than the omniserum, it should be much easier to produce in large quantities for use in standardized assays.

Anti-PC antibodies of three different isotypes, IgM, IgG2b, and IgG3, were tested. Comparisons of these antibodies demonstrated greater sensitivity of IgM than IgG antibodies. This difference is undoubtedly the result of the isotype, because these MAbs are all of the T15 idiotype and have been shown to have essentially identical binding sites (1). The greater efficiency of the IgM over the IgG antibodies is probably the result of the higher valence of IgM as opposed to IgG antibodies.

The D3114/63 was as specific as the IgM anti-PC antibody and detected only one less pneumococcal culture-positive spinal fluid. D3114/63 reacts with a different determinant on the pneumococcal C carbohydrate than does the anti-PC antibody (13). Although D3114/63 is not superior to the anti-PC antibodies for the detection of pneumococcus-infected spinal fluids, it may be useful along with anti-PC antibodies for the identification of pneumococci isolated from patients. Together the two MAbs detected 82% of the culture-positive spinal fluids.

TABLE 5. Antibody and conjugate titration to determine optimal concentration and incubation[a]

Concn (μg/ml)		Incubation time (min) for conjugate	OD_{405} for antibody incubation time (min):		
Conjugate[b]	Antibody[c]		15	30	45
2	8	15	0.443	0.488	0.519
		30	0.830	0.834	0.854
		45	0.878	0.989	1.136
2	4	15	0.395	0.452	0.566
		30	0.648	0.718	0.952
		45	0.918	1.089	1.314
1	8	15	0.245	0	0.284
		30	0.469		0.462
		45	0.630		0.654
1	4	15	0.247		0.286
		30	0.386		0.443
		45	0.664	0.	0.740

[a] Solid-phase antigen was 10^7 heat-killed R36A per well. Incubation was at 37°C. The substrate was incubated for 10 min and the reaction was read as OD_{405}.

[b] Alkaline phosphatase-labeled goat anti-mouse immunoglobulin.

[c] Anti-PC MAb HPCM2.

TABLE 6. Detection of pneumococcal antigens in spinal fluids of patients with meningitis by four antibodies based on an inhibition ELISA[a]

Antibody	% Inhibition	No. with pneumococcal culture		% Sensitivity	% Specificity	% Agreement
		+	−			
HPCM2 +	≥40	5	0	100	100	100
−		0	5			
D3114/63 +	≥20	4	3	80	40	60
−		1	2			
Omniserum +	≥20	4	2	80	40	70
−		1	3			
Anti-R36A +	≥20	2	1	40	80	60
−		3	4			

[a] See footnotes of Table 2.

Four of the pneumococcal culture-positive spinal fluids were not detected by the anti-PC, D3114/63 antibodies, or omniserum. Presumably the antigen concentration of these spinal fluids was lower than the level of detection. Studies quantitating the level of pneumococci in spinal fluid have demonstrated a wide range of antigen levels. Feldman (5) found in five infants with pneumococcal meningitis a range of 450 to 2.6×10^7 pneumococci per ml of spinal fluid (mean, 3.87×10^5 pneumococci per ml). Olcen (14) found concentrations of <10 to 3×10^7 pneumococci per ml of spinal fluid (mean, 8×10^5 pneumococci per ml). Greenwood et al. (8) studied the levels of pneumococci in 28 adults with meningitis and found a range of 2×10^6 to 1×10^9 pneumococci per ml.

The false-negative spinal fluids in our study may have been taken early in the infection before sufficient antigen concentrations had developed for detection. Our assays to determine the level of antigen detected by the antibodies under the defined conditions of this study indicate that 10^7 live or heat-killed pneumococci per ml, 4 μg of lysate protein per ml, or 2 μg of PC-BSA per ml is necessary for positive tests with the different assay procedures using the anti-PC

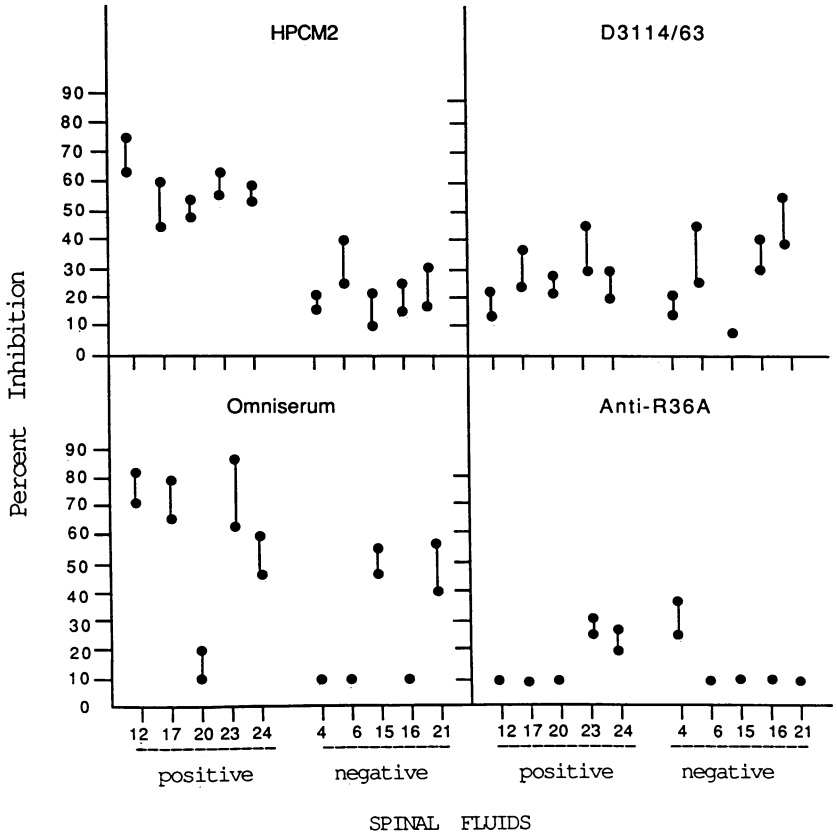

FIG. 1. Percent inhibition by five pneumococcal culture-positive and five culture-negative spinal fluids tested by an ELISA with four antibodies. Duplicate results of each test are shown.

MAb, HPCM2. These antigen concentrations are within the range normally found in pneumococcal meningitis as described above. Various pneumococcal antigen preparations were tested, since the form of the pneumococcal antigen (whether particulate or soluble) in spinal fluid is unknown.

Although anti-R36A antibody was the most sensitive, it is not specific enough to be useful as a screening reagent. However, with suitable absorption such an antiserum might react more specifically with pneumococcal antigens.

A direct ELISA was used to provide a quantitative assay to determine the conditions that would yield a useful result in the shortest possible time. We found that by adjusting the concentrations of the antibody and conjugate the assay could be completed with 15-min incubations for both antibody and conjugate followed by a 10-min incubation with the substrate. The assay time could probably be shortened further by directly conjugating the anti-PC antibody to alkaline phosphatase, thereby eliminating one of the incubation steps. If a similar protocol could be developed for the dot blot assay, this type of modification would be important for the development of an assay that would be useful in a clinical setting.

The inhibition ELISA was examined as an alternative diagnostic assay, since an inhibition format would circumvent the initial absorption step of binding the spinal fluid to the plate necessary in a conventional direct ELISA. The results indicated the feasibility of this method with the HPCM2 anti-PC antibody.

Sorensen (15) found that anti-PC MAbs would bind to beta-lipoprotein in serum and spinal fluids and concluded that these antibodies could not be used for the detection of PC-containing antigens in these fluids because of nonspecific reactions. Our results do not show any problems with nonspecific reactions among the anti-PC MAbs tested. Possibly the difference in the assay technique employed may explain the nonspecific reactions.

Overall, we feel that our results are quite significant because they indicate that, at least in this assay system, readily available IgM anti-PC MAbs are as useful as a mixture of polyclonal antisera for the detection of pneumococcal antigens in biologic samples. IgM antibodies with essentially identical specificity to the IgM anti-PC antibody that we have used have been frequently produced in several laboratories (1, 6). With proper care of the cell lines it should be much easier to provide these MAbs than the mixture of immune sera currently in use in most pneumococcal diagnostic assays.

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