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Type-Specific Antigens of Group A *Neisseria meningitidis*: Lipopolysaccharide and Heat-Modifiable Outer Membrane Proteins

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The solid-phase radioimmunoassay inhibition method was used to analyze the noncapsular surface antigens of group A *Neisseria meningitidis* for type specificity. By use of antisera prepared against group A strains, three serologically distinct lipopolysaccharide antigens and five outer membrane protein antigens were identified among group A strains from a variety of geographical origins. Two of the lipopolysaccharide antigens were unique to group A strains while the third was similar to those on strains of other meningococcal serogroups. Fractionation of outer membrane proteins in the presence of 2% sodium deoxycholate followed by quantitative inhibition of the typing reactions with the subfractions revealed that the protein responsible for type specificity was not the principal outer membrane protein, but, most likely, the 31,000-dalton, heat-modifiable outer membrane protein. Thus, although group A strains may share a common principal outer membrane protein, typing is feasible using other surface antigens. In a survey of 82 group A strains, 93% were typable with respect to outer membrane proteins.

Recent studies have indicated that the protein and lipopolysaccharide (LPS) antigens of group A *Neisseria meningitidis* are more homogeneous than those of the other meningococcal serogroups (13) and are not extensively shared with strains of the other serogroups (17). Sippel and Quan (13) demonstrated that group A strains all have the same pattern of outer membrane proteins when analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and all share a common outer membrane protein antigen (presumably the principal outer membrane protein). Differences among group A strains, however, have been demonstrated by bactericidal reactions (7, 9, 12, 13) and by solid-phase radioimmunoassay (SPRIA) inhibition serotyping with antisera raised against group B and group C strains (17). In the latter studies, however, only a relatively small percentage of group A strains were typable with respect to either protein or LPS antigens.

In the present study we have applied the SPRIA inhibition serotyping method with antisera raised to group A strains to the analysis of meningococcal group A outer membrane protein and LPS antigens. The results of this analysis indicate that group A strains may be distinguished (typed) on the basis of either LPS antigens or outer membrane proteins of lower molecular weight than the principal outer membrane protein.

MATERIALS AND METHODS

Bacterial strains and growth media. The group A strains that were used for preparation of typing sera (prototype strains) were all isolates from individuals with disseminated meningococcal disease. Their geographical origin was as follows: 139M, Philippines; 120M, Pakistan; 7880, Pacific Northwest, United States; 7851, Brazil; 7889, Finland; 106, Morocco. Bacterial strains were from the culture collection of the Department of Bacterial Diseases, Walter Reed Army Institute of Research, or were kindly provided by the following: R. H. Weaver, Center for Disease Control, Atlanta, Ga.; A. R. Ronald, University of Manitoba, Manitoba, Canada; P. H. Mäkelä, Helsinki, Finland; and J. McLeod Griffiss, Walter Reed Army Institute of Research, Washington, D.C. Strains were stored in the lyophilized state or frozen in skim milk at −60°C and were with several exceptions tested within six passages of the original isolation.

Unless otherwise indicated, bacteria were routinely grown on BYE agar plates or, for preparation of antigens, a modification of the medium of Watson and Sherp (11).

Preparation of antigens. The outer membrane complex (OMC) was prepared as previously described (17), and LPS was prepared by the phenol-water method of Westphal et al. (14). Whole organisms to be used for determination of serotype by SPRIA inhibition were prepared by suspending cells from an overnight BYE agar plate in 0.15 M NaCl to an optical density of about 5.0 at 650 nm and adding 1/10 volume of 0.1 N HCl. The suspension was allowed to stand at room temperature for 1 h and then centrifuged to pellet the organisms. The cells were washed once in

Dulbecco phosphate-buffered saline (GIBCO Laboratories, Grand Island, N.Y.) and resuspended in the same buffer to the original volume.

Fractionation of the OMC to obtain fraction I and fraction II proteins was performed at room temperature in the presence of sodium deoxycholate (DOC). The OMC at about 5 mg of protein per ml was mixed 2:1 (vol/vol) with a buffer concentrate containing 10% DOC, 0.15 M glycine, and 0.03 M ethylenediaminetetraacetic acid (pH 8.8) and kept at 4°C for 30 min. The mixture was then centrifuged at 80,000 × *g* for 1 h at 25°C, and the supernatant was concentrated by ultrafiltration on a PM-10 membrane (Amicon Corp., Lexington, Mass.) to one-third the original volume. About 3 to 4 ml of this solution was applied to a column (2.5 by 36 cm) of Sephadex G-150 equilibrated with buffer containing 2% DOC, 0.05 M glycine, and 0.005 M ethylenediaminetetraacetic acid, pH 8.8. Protein, as determined by the method of Lowry et al. (10), eluted in two peaks followed by LPS and lipid. The fractions comprising each of the two protein peaks were pooled and concentrated to about 2 to 3 ml by ultrafiltration on PM-10 membranes, and each pool was then rechromatographed on a column (1.6 by 36 cm) of Sephadex G-150 equilibrated as described above. The fractions comprising the main protein peak from each of these column runs were pooled, and the protein was recovered by precipitation with 3 volumes of cold ethanol. The precipitates were recovered by centrifugation, washed twice with absolute ethanol, and suspended in distilled water.

SDS-PAGE (18) and treatment of antigens with subtilisin (17) were performed as previously described.

Preparation of typing sera. Antisera were prepared in rabbits by using viable organisms as previously described (17). Absorption of antisera with bacterial cells was performed as previously described (17), except a 1:1 mixture of fresh organisms and organisms which had been exposed to 0.1 N HCl for 1 h at room temperature was used and the absorption was carried out at 37°C for 1 h followed by overnight incubation at 4°C.

Typing. Typing by SPRIA inhibition was performed as previously described (17) using the OMC at 100 μg of protein per ml or whole organisms at an optical density of 5.0 at 650 nm as test antigen (inhibitor). At this concentration, antigen was present in large excess and normally resulted in maximal inhibition for each antigen. (Further increases in antigen concentration produced no further increase in the percent inhibition.) Whole organisms were taken from overnight plates since it was found that some LPS determinants were not expressed on very young cultures. A strain was considered to have the determinant in question if >50% inhibition of the appropriate reaction was obtained. This endpoint was arbitrarily chosen and probably results in designating as positive some strains with determinants that are closely related but not identical.

Quantitative inhibition. To determine the minimal amount of antigen required to inhibit a given typing reaction by 50% (MIC_{50}), twofold serial dilutions of the antigen were used to inhibit in the standard SPRIA inhibition procedures. The 50% endpoint was obtained by graphical interpolation.

Quantitation of antibody by SPRIA. Antibody to the OMC or LPS was determined by SPRIA as described previously and is based on the correlation of the SPRIA with results of quantitative precipitation tests.

RESULTS

Type specificity of LPS determinants. Rabbit antisera raised against eight group A strains isolated in different parts of the world were tested by SPRIA for antibodies against purified LPS from each of the eight strains to screen for differences in specificity of anti-LPS antibodies. Three distinctly different specificities were apparent (Table 1). Except for the binding of anti-7889 to 7880 LPS, the level of antibody binding to the homologous LPS was about 100-fold higher than the binding to heterologous LPS. The specificities defined by these sera were designated L9, L10, and L11.

The suitability of these three sera as LPS typing sera for group A strains and the relationship of these group A LPS determinants to the eight determinants previously identified on group B and group C strains (17) were investigated by performing a grid inhibition experiment with all 11 antisera (Table 2). Cross-reactions between the group A types (L9–L11) and the group B and C types L1–L8 were confined to L9. Only those sera or antigens of types L1–L8 which exhibited reactions with the group A sera or antigens are given in Table 2. LPS from the prototype strains for L3 and L6 inhibited the L9 antiserum, and LPS from the L9 prototype strain inhibited the L7 antiserum and weakly

TABLE 1. *Specificity of group A LPS typing sera*

Rabbit antiserum	LPS type	Antibody to LPS from indicated strain (μg/ml)[a]		
		120M	7880	7889
Anti-120M	L9	429	5	4
Anti-7880	L10	4	895	11
Anti-7889	L11	3	72	306

[a] As determined by SPRIA.

TABLE 2. *Grid inhibition of group A-associated LPS typing reactions*

LPS typing reaction	Serogroup of prototype strain	% Inhibition by prototype OMC for				
		L3	L6	L9	L10	L11
L4	C			40		
L6	B		97	43		
L7	B			70		
L9	A	85	66	95		
L10	A				98	
L11	A				33	97

inhibited the L4 and L6 antisera. The LPS determinants corresponding to L10 and L11 appeared to be unique to group A strains.

Verification that the antigenic determinants being detected by these LPS typing reactions were actually associated with LPS rather than protein was obtained in two ways. First, treatment of the OMC preparations that were used as inhibitors with subtilisin resulted in a reduction or loss of the capacity to inhibit protein typing reactions, but did not affect or slightly enhanced the capacity to inhibit the LPS typing reactions. Second, when the OMC from the three prototype strains was fractionated into protein fractions I and II and LPS as described above, and the MIC_{50} was determined for the separate fractions, LPS was found, with one exception, to inhibit 100- to 1,000-fold more strongly than the protein fractions. The one exception was protein fraction II, which was found to inhibit 1/10 as well as LPS but was then shown to contain about 10% contaminating LPS as determined by KDO assays. Thus, in all cases inhibition by protein fractions could be attributed to contaminating LPS.

Demonstration of type-specific protein antigens. The same antisera that were tested for type-specific antibodies to LPS were also analyzed for type-specific antibodies to outer membrane proteins.

On the basis of preliminary grid assays, each antiserum was cross-absorbed with a group A strain which had the same LPS type but possibly a different protein type than the strain against which the antiserum had been raised. This was done to remove antibodies to capsular polysaccharide, LPS, and common protein antigens since the solid-phase antigen used for protein typing is the OMC, which contains LPS and some capsular polysaccharide as well as the outer membrane proteins. The level of antibodies to the homologous OMC and purified LPS in five of the sera which appeared to show type specificity (Table 3) was determined by SPRIA before and after absorption. In each case, the absorption reduced the level of antibodies to LPS from about 20% of the total antibody to the OMC to less than 2%. Antibody to the OMC remaining after absorption was 12 to 44% of that originally present.

A grid typing experiment using these five absorbed antisera and the homologous antigens (OMC) was performed to ascertain the degree of specificity they exhibited (Table 4). Although the OMC from four of the five strains inhibited one or more of the typing reactions in addition to the homologous reaction, none of the sera showed the same pattern of inhibition. The five specificities defined by the binding of each of these antisera to the homologous OMC were designated P'19 to P'23. Reactions P'20 and P'21 appeared to be the most closely related, but both were tentatively included in the typing scheme for the purposes of further evaluation. The P'23 reaction was only inhibited by the homologous OMC, although that same OMC inhibited three of the other reactions as well.

Although the inhibition patterns were very reproducible when the same OMC preparations were used, significant variations were occasionally seen when different preparations of OMC from the same strain were tested or when inhibition by whole organisms was compared to that obtained with OMC from the same strain. The three values in Table 4 with a superscript *c* represent the highest values obtained in several experiments. In separate experiments in which different OMC preparations were used, these values were as low as 25 to 30%. The homologous inhibition and the stronger heterologous inhibitions (>75%) were generally not subject to this variation.

To investigate phenotypic stability with re-

TABLE 3. *Effect of absorption on the antibody content and specificity of group A typing sera*

Antiserum	Protein type	Serum antibody to indicated homologous antigen (μg/ml)[a]			
		OMC		LPS	
		Unabsorbed	Absorbed[b]	Unabsorbed	Absorbed
Anti-139M(A)	P'19	710	85	220	1.5
Anti-7880(A)	P'20	1570	580	440	7.6
Anti-7851(A)	P'21	1000	250	210	1.1
Anti-7889(A)	P'22	890	240	190	1.7
Anti-106(A)	P'23	700	310	5.3	0.2

[a] As determined by SPRIA.

[b] Each serum was absorbed with a group A strain which had the same LPS type as the vaccine strain but appeared to have different protein antigens.

TABLE 4. *Grid inhibition of meningococcal group A protein-based typing reactions*

Typing reaction[a]	% Inhibition by OMC from prototype strain for				
	P'19	P'20	P'21	P'22	P'23
P'19	83[b]		45		79
P'20		88	88	58[c]	63
P'21		69[c]	93	70[c]	
P'22				89	60
P'23					87

[a] Binding of appropriate dilution of typing serum to homologous OMC.

[b] Only inhibition values greater than 30% are given. Inhibition of ≥50% is considered positive.

[c] These values were below 50% in some experiments. The values reported are the highest values obtained in three or four experiments.

spect to the expression of these protein determinants, we carried out an experiment to determine the effect of different growth conditions and of repeated passage on the expression of the protein determinants by two strains. Beginning with cells derived from a single colony, the OMC was prepared from liquid cultures of each strain before and after 10 passes on BYE agar. The OMC was also prepared from the strains before passage but after growth on or in three different media and with high and low aeration (Fig. 1). These OMC preparations were tested for the capacity to inhibit the five typing reactions. Inhibitions by the OMC preparations derived from cells grown under different conditions were reasonably consistent, but strain 7889 inhibited P'20 and P'21 much more strongly after passage than before. To determine whether the strain had changed or whether the difference was simply due to variation between two OMC preparations, we also inhibited the five typing reactions with whole organisms before and after the 10 passes. With the whole cells no differences resulting from passage were observed, and both the pre- and postpassage cells of strain 7889 inhibited the P'20 and P'21 reactions about as strongly as the postpassage OMC had. In addition, whole cells of strain 7893 inhibited the P'22 reaction more strongly than any of the 7893 OMC preparations had. In general, the whole cells appeared to express the type-specific protein determinants more strongly than the OMC preparations.

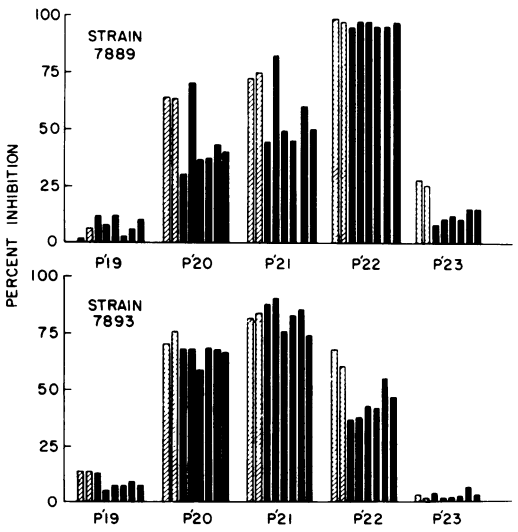

FIG. 1. *Inhibition of group A protein typing reactions by antigens from two group A strains grown under a variety of conditions. From left to right, the bars in each group are whole cells (cross-hatched bars) derived from a single colony before (bar 1) and after (bar 2) 10 passes on BYE agar; OMC (solid bars) from cells grown in modified Watson-Sherp liquid medium before (bar 3) and after (bar 4) 10 passes on BYE agar; OMC from cells grown on BYE agar (bar 5), in Müller-Hinton broth (bar 6), or modified Watson-Sherp medium with low (bar 7) or high (bar 8) aeration.*

The P'19 and P'23 typing reactions were not inhibited to a significant extent by either whole cells or any of the OMC preparations, indicating the absence of these two determinants on strains 7889 and 7893.

Of the 18 protein serotype determinants identified on group B and group C strains (17), only P'6 (previously designated P6) was found on any of the group A strains that were tested (6 of 50). None of the five prototype strains for the group A outer membrane protein types had the P'6 determinant. The group A types P'19 through P'23, therefore, appeared to be distinct from any of the 18 protein serotypes that have been identified on group B and group C strains. Nevertheless, it is likely that some of the group A protein determinants or similar determinants were also found on non-group A strains since the OMC from 7 of 16 type P2 strains of serogroups B and C were found to inhibit the P'22 or P'23 serotype reactions or both at a level of 50% or greater.

Localization of the type-specific protein determinants. Since outer membrane proteins of all group A strains had been shown to have the same SDS-PAGE pattern and share a common major protein antigen, it appeared unlikely that the type-specific protein determinants we had identified on group A strains were associated with the principal outer membrane protein. SDS-PAGE of the OMC from the five prototype strains for the group A protein types confirmed that the principal outer membrane proteins were all of the same molecular weight. The protein nature of the determinants was indicated both from the fact that anti-LPS and anti-capsular polysaccharide antibodies had been removed from the typing sera by absorption (Table 3) and by the observation that the determinants were sensitive to proteolytic enzymes. Treatment of the OMC from the five prototype strains with subtilisin resulted in a 20 to 90% decrease in the inhibitory capacity of the OMC (at 100 μg of protein per ml) in the homologous typing reaction as compared to that for the controls to which no enzyme was added. No decrease in the inhibitory capacity of the LPS component of the OMC toward the homologous LPS typing reaction was observed.

To identify the protein(s) with which the type-specific determinants were associated, we fractionated the OMC from the prototype strains

for P'6, P'22, and P'23 in DOC to remove LPS and to separate the outer membrane into two subfractions (see above). The two fractions, designated I and II, were analyzed along with the OMC by SDS-PAGE (Fig. 2). Fraction I consisted principally of two major proteins with apparent molecular weights of 46,000 and 38,000. The band with a molecular weight of 38,000 was consistently the most dominant in group A OMC preparations and in view of its low solubility in DOC most likely corresponded to the principal outer membrane protein which has generally been associated with serotype specificity in group B and C strains. Fraction II contained principally a protein with a molecular weight of 31,000 which shifted its position on gels to a lower apparent molecular weight if heated in 2% SDS at 40°C for 1 h rather than at 100°C for 10 min (Fig. 2). The heat-modifiable behavior of this protein(s) was reported earlier for a group B strain (16) and has recently been studied in more detail by Frasch and Mocca (4). This protein appears analogous to the protein II* of *Escherichia coli* (5) and *Neisseria gonorrhoeae* (8). The OMC, along with the separated fractions I and II, was then tested to determine the minimum concentration of each that would give 50% inhibition of the appropriate typing reactions (Table 5). For each of the type specificities associated with group A strains (P'22, P'23, and P'6), fraction II inhibited manyfold more strongly than fraction I. In fact, fraction II was severalfold more active than the intact OMC, which indicates that the active component had been enriched in fraction II. In contrast, when the OMC from a group B type P2 strain was fractionated in the same way, the P2 serotype reaction was inhibited more strongly by fraction I, which is consistent with the localization of that determinant on the principal outer membrane protein. The results indicate that the determinants corresponding to P'22, P'23, and P'6 are not located on the principal outer membrane protein, but rather constitute a new class of type-specific proteins which are present in the outer membrane in addition to the principal outer membrane protein. Since fraction II consists principally of the 31,000-molecular-weight, heat-modifiable outer membrane protein(s), the type-specific determinants are most likely associated with this protein(s). We have designated

TABLE 5. *Inhibition of homologous typing reactions by OMC and two protein subfractions*

Typing reaction	OMC from strain	MIC_{50} of indicated antigen (μg/ml)			
		OMC	Fraction I	Fraction II	Fraction I/II
P'23	106(A)	5	40	0.75	67
P'22	7889(A)	1	5	0.12	42
P'6	32I(C)	1.5	100	0.30	330
P2	99M(B)	1.5	7	>100	<0.07

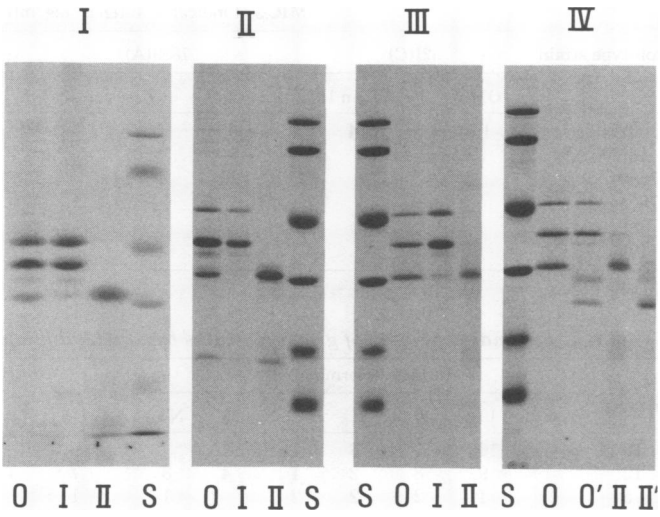

FIG. 2. *SDS-PAGE of OMC and two subfractions obtained by fractionation on Sephadex G-150 in the presence of 2% DOC. (I) Strain 7889 (A:P'22); (II) strain 321(C:P'6); (III) strain 106(A:PP23). Positions: (O) OMC; (I) fraction I protein; (II) fraction II protein; and (S) molecular weight standards. Beginning at the top, the bands are phosphorylase b (94K), albumin (67K), ovalbumin (43K), carbonic anhydrase (30K), trypsin inhibitor (20.1K), and α-lactalbumin (14.4K). For O' and II', the samples were heated at 40°C for 1 h rather than the usual 100°C for 10 min.*

these determinants on the heat-modifiable outer membrane proteins with P' preceding the number to distinguish them from OMC serotype determinants on the major outer membrane protein which are designated with a P.

The type specificity of the fraction II protein(s) was confirmed by determining the MIC_{50} for each of six different typing reactions. These results are compared in Table 6 to those obtained with the homologous OMC. Good agreement between the inhibition patterns obtained with fraction II and the homologous OMC was obtained. The results indicate that all of the protein determinants defined by the typing reactions listed in Table 6 are present on fraction II proteins.

Typing of representative group A strains. A total of 82 group A strains from different parts of the world were typed by using both the LPS and protein typing reactions described above. All but 18 of the strains were isolated from patients with disseminated meningococcal disease; the remainder were isolated from the nasopharynx of asymptomatic carriers. The frequency of occurrence of each of the protein and LPS types is shown in Table 7 according to the geographical origin of the strains. The most commonly occurring protein determinants were P'20 and P'21, which occurred with about equal frequency in all geographical areas. P'19 was detected most frequently among the strains from West Germany and Africa (19 of 22 strains). The P'6 determinant, which frequently occurs together with P2 on group B and group C strains, was only detected on strains isolated in North America. Six of the 10 strains that were positive for P'6 were negative for the other five protein types. P'22 and P'23 were not confined to a particular geographical area, but they did not occur on carrier strains. Only 6 of the 82 strains were nontypable as defined by the OMC inhibiting less than 50% in all six of the typing reactions.

The most common LPS type was L10, which was found in 60 of 82 strains. With one exception, the LPS types L1 through L8 were only found on strains which also had L9. Nearly all strains that had the L9 determinant also had one or more of the determinants L1 to L8, but rarely had L10 or L11. A high percentage of the carrier strains had both the L10 and L11 determinants, whereas case strains from the same area were predominantly L10 only. Only five strains were nontypable with respect to their LPS antigens.

Designation of test strains. The following strains were selected as test strains for the purpose of verifying the specificity of the typing reactions and testing new reagents: 97E(A:P'19,20:L10) for P'19; 7884(A:P'20,23:L10) for P'20 and P'23; 7879(A:P'20,21:L10) for P'21 and L10; 7891(A:P'22:L11) for P'22 and L11; 3843(A:

TABLE 6. *Comparative type specificity of OMC and fraction II protein*

Typing reaction	Prototype strain	MIC_{50} of indicated antigen[a] (μg/ml)					
		32I(C)		7889(A)		106(A)	
		OMC	Fraction II	OMC	Fraction II	OMC	Fraction II
P'6	32I(C)	1.3	0.14				
P'19	139M(A)					0.5	0.9
P'20	7880(A)			20	14	66	14
P'21	7851(A)			1.3	1.1		
P'22	7889(A)		78	0.7	0.06		18
P'23	106(A)					10	5

[a] Only values ≤100 are given.

TABLE 7. *Summary of typing of group A strains by SPRIA inhibition*

Geographical area	No. of strains	Protein determinants[a]							LPS determinants[a]				
		6	19	20	21	22	23	NT[b]	1-8	9	10	11	NT
West Germany	10		10	7	6	1	1				9	1	
Africa	12		9	8	2	1	4	3	7	7	5		
Finland	5		1	2	2	2		1	1			4	1
Brazil	6		1	5	4			1			6		
North America													
Case	30	8	3	22	16	5	10		2	2	25	3	2
Carrier	15	2	1	11	11			1		1	12	10	2
Other	4		2	3	2		1		1	1	3	2	

[a] A single strain was often found to have more than one type-specific protein or LPS determinant or both.
[b] NT, Not typable.

P'19,20,23:L7,9) for L9; and 7881(A:P'6:L10) for P'6.

DISCUSSION

Although strains of group A meningococci appear to be more homogenous with respect to subcapsular antigens than meningococci of other serogroups, it is clear that sufficient differences do exist for them to be subclassified on the basis of LPS antigens, outer membrane protein antigens, or both. To what extent such subclassification or typing can provide useful epidemiological information is not yet clear. LPS typing appears to be the most reproducible and has been found in one instance to distinguish between carrier strains and case strains isolated (1975 to 1977) during an outbreak of group A disease in the U.S. Pacific Northwest. The possible epidemiological significance of this finding is discussed elsewhere (R. E. Mandrell et al., manuscript in preparation). Protein typing of the same strains also revealed some differences between the case strains and carrier strains. Certain protein determinants often found on case strains (P'19, P'22, and P'23) were not found on the carrier strains tested. These findings suggest that protein typing of group A strains may also be able to provide useful epidemiological information. There is no evidence, however, that any of the type-specific determinants that have been identified on group A strains might be a marker for virulence or epidemic potential such as appears to be true for the P2 determinant (1, 6).

The polypeptide responsible for protein type specificity on group A strains is not the principal outer membrane protein which is generally associated with serotype specificity in meningococci. For each type that we have analyzed, the type specificity appeared to be associated with the lower molecular weight, heat-modifiable outer membrane protein, which in our gel system has an apparent molecular weight of 31,000 (after boiling in 2% SDS for 10 min). Differences in serological specificity associated with these proteins are not reflected in molecular-weight changes as seen on SDS-PAGE. In addition, it is common for a single strain to have multiple determinants of this type. This could result from the presence of several different polypeptides of this type, or multiple determinants on the same polypeptide. Indeed, if the sample is prepared for SDS-PAGE by warming to 40°C for 1 h, the 31,000-dalton band of strain 106 appears as two bands at 25,000 and 29,000 daltons (Fig. 2). It may be that the structural differences which result in differences in type specificity in these proteins are relatively minor as compared to the structural differences among principal outer membrane protein serotypes. It is clear that type-specific, heat-modifiable outer membrane protein antigens are also present on group B and C strains since the prototype P'6 strain is a group C strain, and P'6 is often found associated with P2 on group B and C strains. In addition, there is evidence that protein serotype 1 in the system of Frasch (3) and possibly serotypes 9, 10, and 12 (J. T. Poolman, C. T. P. Hopman, and H. C. Zanen, J. Gen. Microbiol., in press) are associated with the heat-modifiable outer membrane protein(s).

We propose that meningococci possess two general categories of type-specific outer membrane proteins. The first is the principal outer membrane protein (molecular weight, 36,000 to 44,000) of which P2 is the prime example (2), and the second is the heat-modifiable major outer membrane protein(s) (molecular weight, about 26,000 to 32,000) which may be present in addition to the serotype protein and is responsible for protein type specificity among group A strains. In instances where multiple protein serotype determinants have been identified on non-group A meningococci, it is likely that only one of these is on the principal outer membrane protein and that the others are on the heat-modifiable outer membrane proteins. It will be important to determine the genetic basis of the antigenic diversity exhibited by the two categories of outer membrane proteins.

The heat-modifiable outer membrane proteins may be important in the host's immune response to the meningococcal infections, since we found that anti-protein bactericidal antibodies present in several human sera after natural infections were directed at these proteins (W. D. Zollinger, H. Hansel, and R. E. Mandrell, manuscript in preparation).

Typing of group A meningococci on the basis of the heat-modifiable outer membrane proteins was in our hands subject to more variability than when the principal outer membrane proteins were involved. The most probable explanation for this variability is the variation in the expression of these protein antigens on the surface of the organisms when grown under different conditions, and/or to greater lability and susceptibility to the action of proteolytic enzymes.

Over 90% of the group A strains tested could be typed on the basis of LPS by using three LPS typing reactions (L9, L10, and L11). L10 and L11 appeared unique to group A strains and partially cross-reactive, while L9 was shared with group B and group C strains and was almost always found in association with one or more of the

types L1 through L8. L9 appeared to be most closely related to L7, although cross-reactivity was also obtained with L4 and L6. The prototype strain for L3 also appeared to have the L9 determinant. These cross-reactions with existing L-types need to be studied further, but L9 was tentatively included in the typing scheme because it was found to be best suited for use in typing group A strains.

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