

Serotypes of group B meningococci

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SYNOPSIS Strains of group B meningococci isolated from cases of sporadic infection, epidemic infection, and from patients unassociated with clinical meningococcal infection have been examined by a serological typing technique. The majority of group B meningococci from clinical infections in the UK have serotype 2 antigen. Such strains were relatively uncommon among carriers who had no association with meningococcal disease.

Meningococci of group B and group C have been subdivided by serological typing schemes (Frasch and Chapman, 1972a and b; Gold and Wyle, 1970). This subdivision is based on the presence of protein antigens (Frasch and Gotschlich, 1974) rather than the group-specific polysaccharides, and it appears that these antigens are not confined exclusively to particular serogroups. The meningococci isolated from clinical infections in the UK are predominantly group B, and it seemed important to ascertain which serotypes are most prevalent. We have followed the typing system of Frascch and Chapman but, instead of their precipitin/bactericidal techniques, we have used bacterial agglutination. While this is not as refined as the original method, it is perhaps easier to perform on a number of strains and provides the essential epidemiological information.

Methods

SERA

Strains of 11 serotypes (types 1, 2, 4, 5, 6, 7, 8, 9, 10, 11, 12) were kindly supplied by Dr Frascch and these strains were used to prepare antigens for the production of antisera in rabbits. Initially the criterion of adequate potency was the production of clear precipitin lines with antigenic extracts of the homologous strains (Frasch and Chapman, 1972b). Latterly bacterial agglutination titres of 1/1280 or greater were regarded as adequate.

BACTERIAL SUSPENSIONS

Meningococci were grown overnight in a candle jar on heavily seeded Mueller Hinton agar. The growth was harvested as a thick suspension in phosphate buffered saline pH 6.8 and then heated in a boiling

water bath for 30 minutes. This concentrated suspension was stored at 4°C and was used in this form for absorption of sera. Suspensions for agglutination were prepared by diluting the concentrated suspension to Brown's tube No. 2 opacity.

ABSORBED SERA

The antiserum prepared from each type was absorbed with a poly-suspension containing the heterologous types. Type 2 serum was not absorbed with types 7 and 10. After absorption the titre of the serum was checked to detect undue loss of potency and for reactions with heterologous types. Sera were regarded as satisfactory if the homologous titre was 1/160 or greater and cross-reactions were minor or absent. Under these conditions a serum was used at a dilution of 1/40 for the test.

TYPING

A boiled diluted suspension of the test strain was added to dilutions of each antiserum and incubated overnight at 50°C. A positive typing reaction was recorded when there was complete (4+) or near complete (3+) agglutination in one tube but not in others (except where antigenic relationships were expected). Strains reacting with either a number or none of the sera were classed as untypable.

GROUP B STRAINS

Meningococci are regularly submitted to the Manchester Public Health Laboratory for grouping and sulphonamide sensitivity tests from many parts of England. A number of strains isolated from sporadic clinical cases and received during the winter of 1974/75 have been included in this study. In addition, group B meningococci isolated from clinical infections in Scotland were kindly made available by Dr R. J. Fallon. In recent years cir-

cumscribed outbreaks of infection due to group B meningococci have occurred in Monmouth, Bolton (Farries *et al*, 1975), and Devon (Easton *et al*, 1974), and representative strains from these outbreaks were provided by Dr Patricia Bradstreet.

Meningococcal strains unassociated with any clinical infections were obtained by culturing routine sputum specimens. These were plated on selective blood agar and incubated in a candle jar.

Sensitivity to sulphadiazine was estimated by methods described previously (Abbott and Graves, 1972).

Results

GROUP B STRAINS FROM SPORADIC CASES

The results of typing group B meningococci isolated from sporadic cases of infection are shown in table I. About a quarter of these strains are from Scotland, but the actual distribution of serotypes in these strains was no different from that in the rest. We have classed all the type 2-associated strains together (2; 2, 7; 2, 7, 10; and 2, 10) although the majority of these reacted predominantly with the type 2 antiserum.

GROUP B STRAINS FROM OUTBREAKS

The outbreak of 82 meningococcal infections in the Bolton area during 1971-74 was associated with sulphonamide-sensitive group B meningococci. A sample of 20 strains from clinical infections during this period were all found to be type 2.

Ten group B strains isolated during the outbreak of infection in Monmouth were available for examination; nine of these were type 2 and one belonged to type 1. Twelve strains from the Devon outbreak were group B, eight being type 2 and four failing to type.

MENINGOCOCCI FROM SPUTUM

Eighty strains were isolated from sputum samples and the results of grouping these strains are shown in table II. Group B was the commonest serogroup (42), but when these strains were typed, only five were type 2 (table III). In general, these strains were more difficult to type than those from clinical cases, more often being rough or autoagglutinable, and 21 were untypable.

SULPHONAMIDE SENSITIVITY

Sensitivity tests were performed on many of the strains from clinical cases, and the distribution of the sensitivities of the type 2 strains compared to non-type 2 strains shows no significant difference (table IV).

	Serotype									
	1	2-ass	4	5	6	8	9	11	12	UT
No. of strains	10	150	7	5	1	4	4	0	1	69

Table I Serotypes of 251 group B meningococci isolated from sporadic cases of meningitis and septicaemia

UT = untypable Types 2, 7, and 10 included as type 2-associated

	Group								
	A	B	C	X	Y	Z	29E	W135	NG
No. of strains	3	42	6	0	4	0	9	3	13

Table II Groups of 80 meningococci isolated from sputum

NG = not groupable

	Serotype									
	1	2-ass	4	5	6	8	9	11	12	UT
No. of strains	2	5	3	0	3	3	2	1	2	21

Table III Serotypes of 42 group B meningococci from sputum

UT = untypable Types 2, 7, and 10 included as type 2-associated

Serotype	No. of strains inhibited by sodium sulphadiazine (µg/ml)							Total
	Sensitive				Moderately resistant	Resistant		
	0.4	0.8	1.6	3.2	6.4-10	50	>100	
Type 2-ass	1	9	57	44	2	3	1	117
Other types	5	17	28	21	0	0	2	74

Table IV Sulphadiazine sensitivity, serotype 2 compared with other serotypes

Discussion

Bacterial agglutination with absorbed sera was found to be a satisfactory method of serotyping group B meningococci, although initially comparison of our results with those obtained by Dr Frasch was helpful in the interpretation. Wild strains were found to show some variation in their agglutinability compared with the type strains provided by Dr Frasch. It is possible that with more refined methods the number of untypable strains in this series might have been reduced.

In this study we have examined the serotypes of group B meningococci from various sources in the UK. We found that 60% of the strains from sporadic

clinical infections carried the type 2 antigen. A similar excess of type 2-associated strains in a collection of group B meningococci has been reported from the USA (Frasch and Chapman, 1973). In addition, we have examined meningococci isolated from routine samples of sputum submitted to our laboratory, using these as a source of meningococci not associated with either sporadic or epidemic meningococcal infection. It was of interest that half these meningococci were group B, and type 2-associated meningococci formed only a small proportion (12%) of these strains. The result emphasizes the importance of the presence of type 2 antigen in strains that cause group B meningococcal disease. Examination of representative strains from outbreaks of meningococcal infection that have occurred in recent years showed that the serotype 2 was also associated with epidemic meningococcal disease in the UK. These findings, together with those published from the USA, confirm that most group B meningococcal infection is type 2-associated. The only other major element in the strains examined was the group that was untypable by the present technique.

When we examined sulphonamide sensitivity of the different serotypes we could detect no significant difference between the sensitivity of type 2 strains and the other types. This contrasts with the type 2 strains from the USA which are reported to have a slightly increased resistance to sulphonamide (Frasch and Chapman, 1973). This difference presumably represents a geographical difference in the prevalence of sulphonamide sensitivity, and resistance is not, therefore, necessarily associated with the type 2 antigen.

In the development of vaccines against group B meningococci, antigens other than the group polysaccharide are clearly important. The serotype

antigens appear to be highly immunogenic and stimulate the production of bactericidal and, presumably, also protective antibodies. Knowledge of the distribution of serotypes associated with disease will be advantageous when making the choice of antigens to be included in a vaccine.

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