EPIDEMIOLOGY

Distribution of serovariants of group B streptococci in isolates from England and Norway

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Summary. The distribution of capsular polysaccharide antigen (CHO) types, surface-exposed c proteins α (c^α) and β (c^β) and an R-protein antigen was examined in 334 group B streptococci (GBS) isolates from three groups of patients hospitalised in England and Wales or Norway. The isolates were from 108 carriers, 67 cases of neonatal infection and 154 cases of adult infection. Each group contained all CHO types (Ia, Ib, II, III, IV, V and NT); type III strains predominated except in the adult infected group. Strains within each CHO type could be further subdivided by the protein markers into five subtypes by a combined typing system. The proportion of type Ib and type III strains in the neonatal infection cases and of type Ib strains in the adult infection cases significantly outnumbered isolates of these serotypes among the carrier strains. Twenty-nine different serovariants were identified; 24, 13 and 23 serovariants among the carrier, neonatal infection and adult infection isolates, respectively. Certain CHO antigen-protein associations were identified, notably those between Ia/ c^α , Ib/ $c^{\alpha\beta}$ and III/R. The proportion of invasive isolates that expressed protein was not higher than in the carrier isolates. All CHO-type Ib isolates contained a c protein, but 7% of the Ib isolates did not contain any of these proteins. These findings indicate that this combined typing approach may be useful in examining epidemiological problems associated with GBS.

Introduction

Group B streptococci (GBS) are major causes of neonatal sepsis and meningitis and are also recognised as occasional pathogens in adults.¹ Traditionally, GBS have been classified or typed serologically on the basis of the capsular polysaccharide (CHO) antigens designated Ia, Ib, II and III. Typability and discrimination in the CHO typing system are poor and, therefore, alternative methods of typing GBS isolates have been explored, e.g., bacteriophage typing,² multilocus enzyme electrophoresis³ and restriction endonuclease analysis.^{4,5}

More recently it has been realised that, in addition to the CHO antigens, GBS may produce surface-localised c and R protein antigens.^{6,7} The c protein was called Ibc protein⁸ and comprises two proteins, the α and β antigens.⁹ Both proteins have been characterised extensively.^{10–12} It has been shown that GBS isolates vary in the expression of both α and β protein¹³ and others have shown a similar picture for the strepto-

coccal R protein antigens, of which the R4 protein is particularly prevalent in GBS.¹⁴ To date however, there has been no systematic assessment of the typing potential of a system that combines these different serological approaches.

The present study was designed to explore whether the combined use of CHO and protein antigens in a GBS typing system could increase discrimination and typability. A total of 334 GBS strains collected in hospitals in England and Wales and Norway during 1990–1993 was tested and data on serovariant distribution amongst carriers and cases of infection were assessed.

Materials and methods

Isolates examined

Of a total of 334 GBS examined, 233 isolates came from England and Wales and 101 from Norway. The strains were from infected patients or carriers and were referred from different hospitals throughout the two countries, from 1990 to 1993. All patient isolates were from sporadic cases and duplicate outbreak isolates

were not included. No GBS epidemic was recorded in either country during the period of collection. GBS from carriers ($n = 108$) were skin isolates from neonates (35) or vaginal isolates (73) from adults. Of the 226 isolates from invasive GBS infection, 68 were from neonates, 45 from blood cultures and 23 from cerebrospinal fluid or pus; of the 158 isolates from adults, 101 were from blood cultures and 57 from pleural effusions or pus.

Preservation and culture

The isolates were preserved in Greaves's medium at -80°C . Preparations of GBS for testing were made from bacteria cultured on blood agar at 37°C for 18 h.

Testing of antigenic markers

The bacteria were identified by the Streptex kit (Murex) as recommended by the manufacturer. For isolates from England and Wales, CHO type was determined by immunodiffusion in agar gel with HCl extracts and rabbit antisera.¹⁵ Isolates from Norway were tested for CHO antigen by a direct immunofluorescent assay with fluorescein isothiocyanate-conjugated IgG from rabbit antisera.¹⁶ Surface-exposed protein markers of GBS, the c proteins α (c^{α}) and β (c^{β}), were tested by an indirect immunofluorescent assay with monoclonal antibodies (MAbs);^{17,18} a presumed R protein was examined by a MAb produced recently.¹⁹ Slides with GBS were prepared and the tests were performed and interpreted as described previously.¹⁶

Results

Test performance

Only 25 isolates produced equivocal results when tested for the CHO or protein markers and these were re-examined. Isolates from England and Wales were serotyped by immunodiffusion in agar gel and those from Norway by an immunofluorescent assay; 15

isolates were tested by both methods with similar results. Isolates for CHO and protein markers were subcultured and tested repeatedly for up to 15 years and no reduction in antibody activity has been noted.

Overall serotype and subtype distribution

The CHO type and subtype distribution among GBS from England and Wales and Norway were similar. Of the 334 GBS isolates examined, 303 (91%) belonged to one of the CHO types Ia, Ib, II, III, IV or V (table I). The remaining 31 (9%) isolates were non-typable and have been considered as a separate serotype (type NT). Type III occurred most frequently, followed by type Ib, Ia and type II.

One or two of the protein antigen markers were expressed by 279 (84%) of the isolates—c protein by 171 (51%) and R protein by 108 (32%). Of the c proteins c^{α} alone occurred with the highest frequency, followed by the $c^{\alpha\beta}$ combination and c^{β} alone. No isolate expressed both c and R proteins.

Strains of all seven CHO types expressed protein, but some of the CHO antigens were more likely to be associated with one or two particular proteins than with other proteins. This association is shown in fig. 1. The Ia subtype Ia/ c^{α} (74% of Ia isolates) greatly outnumbered other Ia subtypes ($p < 0.001$) and the Ib subtype Ib/ $c^{\alpha\beta}$ (42%) and the type III subtype III/R (75%) outnumbered other type Ib and type III subtypes ($p < 0.001$). None of the 71 type Ib strains expressed the R antigen and five (7%) of the Ib strains also failed to express c protein. No particular subtype predominated in type II strains. Of the protein antigen markers, c^{α} was most widely distributed among isolates of different CHO types; 29 serovariants were found among the 334 isolates (table I).

Distribution of CHO and protein markers in GBS of different clinical origins

Tables II and III show the distribution of the markers in carrier and invasive isolates and fig. 2 illustrates the predominant subtypes within the most

Table I. Distribution of capsular antigen (CHO) types and protein markers among 334 GBS isolates from England and Wales and Norway

CHO serotype	Protein antigen marker*					Total number
	c^{α}	$c^{\alpha\beta}$	c^{β}	R	None	
Ia	43	8		1	6	58 (17.4)
Ib	15	37	14		5	71 (21.3)
II	11	11	3	7	3	35 (10.5)
III	2	1		85	25	113 (33.8)
IV	9		1		1	11 (3.3)
V	2	1	1	7	7	18 (5.4)
NT	8	4		8	8	28 (8.1)
Total number	90	62	19	108	55	334
(%)	(26.9)	(18.6)	(5.7)	(32.3)	(16.5)	(100)

NT, non-typable.

None of the putative marker combinations c^{α}/R , c^{β}/R , and $c^{\alpha\beta}/R$ was detected.

Fig. 1. Association of protein antigens with CHO antigen type: □, no protein antigen; ■, c^α; ▨, c^β; ▩, c^{αβ}; ▤, R.

Table II. Distribution of GBS capsular antigen types by clinical sources

Source of GBS	Number of isolates	Number (%) of strains of serotype						
		Ia	Ib	II	III	IV	V	NT
Carriers	108	24 (22)	9 (8)	10 (9)	35 (32)	9 (8)	10 (9)	11 (10)
Neonatal infection	67	7 (10)	14 (21)*	5 (7)	35 (52)†	2 (3)	3 (5)	1 (2)
Adult infection	159	27 (17)	49 (30)‡	20 (12)	43 (27)	0	5 (3)	15 (9)

*p < 0.02 when carriers and neonatal infection cases were compared by χ^2 test.

†p < 0.01 when compared to carriers by χ^2 test.

‡p < 0.001 when compared to carriers by χ^2 test.

Table III. Distribution of the c^α, c^β and R protein antigens in GBS by clinical source

Source of GBS	Number of isolates	Number (%) of strains with protein antigen				
		c ^α	c ^β	c ^{αβ}	R	None
Carriers	108	34 (32)	4 (4)	9 (8)	41 (38)	20 (19)
Neonatal infection	67	14 (21)	3 (4)	9 (13)	25 (37)	16 (24)
Adult infection	159	42 (26)	12 (8)	44 (28)*	41 (26)	20 (13)

*p < 0.001 when compared to carriers by χ^2 test.

common CHO antigen types. The carrier isolates from neonates and adults were grouped together, because the serovariant distributions were very similar.

Strains of the major CHO types, Ia, Ib and III, represented 62%, 83% and 74% of carrier, neonatal infection and adult infection isolates, respectively. Compared with the carrier isolates, GBS that expressed the CHO antigen Ib or III occurred with greater frequency in cases of neonatal infection and strains that expressed the Ib antigen in adult cases (table II). Strains of serotypes II, IV, V and NT, with

or without the expression of protein markers, were isolated from carriers and cases. Of the protein markers, the c^{αβ} combination occurred more frequently in the adult infection isolates than in the carrier isolates (table III). This was clearly related to the high frequency of type Ib isolates in adults with infections and the fact that many (52%) of Ib isolates possessed the c^{αβ} combination. None of the other CHO antigen-protein associations occurred with higher frequency in the invasive isolates than in the carrier isolates. Subtype Ia/c^α occurred in 79% of the type Ia carrier

Fig. 2. Distribution of subtype antigens in the most common CHO antigen types: □, Ia/c^α; ▨, Ib/c^{αβ}; ■, III/R; ■, other (○) CHO types; □, other subtypes with CHO antigen types Ia, Ib and III.

strains and 67% of case isolates, subtype Ib/c^{αβ} in 56% and 51% and subtype III/R in 83% and 69%, respectively.

Of the 29 different serovariants detected in the whole strain collection, 24 variants were detected among the carrier strains, 13 among the neonatal infection isolates, and 23 among the isolates from adults with GBS infections.

Discussion

The c proteins α and β have been well documented 10–12 as well as the characters of the MABs to these proteins used in this study.^{17,18} The identity of the protein targeted by the third MAB is less certain, but some observations are consistent with this being an R protein.¹⁹ Also, work in progress has shown that this MAB cross-reacts with the R4 protein studied by Flores and Ferrieri¹⁴ and also with protein Rib described by Ståhlhammer-Carlemalm *et al.*²⁰ These data suggested that the target for the anti-R protein MAB is the R4 protein.

The distribution of GBS CHO types in our strain collection largely corresponds with that of other countries.^{14,21,22} Type III GBS was the most frequent, as in other GBS strain collections.^{14,21,22} Strains of all seven CHO types included isolates that expressed one or two of the protein markers or neither. Of all the isolates tested, 51% produced c protein (27% α , 6% β and 19% both α and β) and 32% expressed the R protein.

Certain capsular CHO types were associated with particular protein markers: the type Ia antigen with c^α, type Ib with the c^{αβ} combination and type III with the R protein.¹⁴ However, each of the protein markers may also be produced by strains of other CHO types consistent with the supposition that expression of the genes encoding these proteins does not depend on synthesis of the favoured CHO antigen. The c proteins α and β can be expressed by the same GBS isolate, most frequently by type Ib isolates.

Simultaneous expression of R and c protein was not detected, although combined expression of these proteins has been reported as a rare event.¹⁴ Hitherto it has been considered that type Ib GBS always produce c protein but 7% of our Ib isolates did not. It is tempting to speculate as to whether these strains harbour c protein gene(s) that are not expressed.

Whereas the CHO antigen determination enabled classification of the 334 isolates into seven different categories, the combination of this typing method with protein antigen testing enabled identification of 29 different serovars; thus the combined approach may prove useful in epidemiological studies of GBS. It is not known how this approach will compare with DNA typing methods used previously.^{4,5}

The prevalence of certain CHO types among the invasive isolates was higher than among the carrier strains, particularly type III and to a lesser extent Ib strains in infected neonates, and Ib strains in adult infections. Although the individuals from whom the carrier strains were isolated, neonates and adult women, may not match the groups with clinical disease, particularly the adults, who were both women and men, these observations are in accord with the anticipated role of GBS capsular antigens as virulence factors, type Ib and type III antigens being potent virulence factors.^{7,23} An alternative explanation might be that insufficient immunoprotection against type Ib and type III GBS is more common than against GBS of other CHO types. The levels of immunoprotection in the populations studied are not known.

Despite the dominance of type Ib and type III GBS among the invasive isolates, isolates of all the serotypes, whether they produced protein or not, could be invasive. Also, these results show that the proportion of invasive isolates that expressed a single protein or the c^{αβ} combination, corresponded to that of the carrier isolates. On the basis of these findings it would seem that the surface-exposed proteins have little if any importance in the virulence of GBS; this was also noted by Chun *et al.*²¹

Despite this, reported data have indicated an important role for these proteins as targets for protective antibodies.^{24,25} Our findings support the notion that expression of invasiveness by GBS requires the interaction of multiple microbial factors, not only the surface-exposed antigens.

In summary, testing of GBS with antibodies to the surface-exposed c proteins and an R protein greatly enhanced the power of the conventional CHO antigen

typing system to identify unrelated GBS isolates. Furthermore, the findings are consistent with the role of CHO serotype antigens in the expression of invasiveness by GBS, particularly the type Ib and type

III antigens, and the surface-exposed proteins may be less important in this context.

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