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Sialic acid content and surface hydrophobicity of group B streptococci

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SUMMARY

The sialic acid content and the cell-surface hydrophobicity index of 40 group B streptococci (GBS) strains were assessed. GBS isolated from invasive infections (virulent strains) presented an increased level of sialic acid content (1·4%) when compared with GBS isolated from asymptomatic patients (0·53%). Treatment of GBS strain 85634 with neuraminidase resulted in a decrease (about 25%) in the net negative surface charge as assessed by cell electrophoresis. This finding suggests that sialic acid residues are important anionogenic groups exposed on GBS cell surface. *N*-acetylneuraminic acid was the only sialic acid derivative characterized in the strain 85634 as evaluated by gas-liquid chromatography. GBS from different serotypes presented a hydrophobic index mean value of 0·9. Even though the sialic acid contributed effectively to the negative charge on GBS cell surface, no difference was observed in the hydrophobic index when virulent and avirulent strains were compared.

INTRODUCTION

Group B streptococci (GBS) are major agents of sepsis and meningitis in neonates and young infants. Usually the invasive infections are caused by strains of serotype III, although any serotype can produce these infections [1].

Some virulence factors have been characterized and seem to be involved in the pathogenesis of GBS diseases. Studies in humans and animals have shown that type-capsular polysaccharides play important roles in invasive infections [1]. The type III polysaccharide is a polymer containing glucose, galactose, *N*-acetyl-glucosamine and *N*-acetylneuraminic acid (sialic acid) moieties. The important role of the type III capsular antigen and of its terminal sialic acid moiety in GBS virulence was verified by Rubens and co-workers [2]. They demonstrated that the insertion of Tn*916* into the chromosome of a type III GBS clinical strain produced a isogenic mutant, COH 31-15, that lacked capsular polysaccharide and was avirulent when compared with the wild-type strain in a neonatal rat sepsis model.

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Another major virulence factor, lipoteichoic acid, seems to be involved in the adherence of different GBS serotypes to vaginal, neonatal and epithelial cells [3]. Group B streptococci bind to fetal and embryonic cells in a two step fashion, with hydrophobic interactions mediating the initial contact followed by ionic or hydrophilic forces due to the glycerophosphate backbone [4]. Although these and other GBS products have been implicated in the neonatal infections [5–7], the factors involved in the prevalence of type III diseases have not been completely elucidated. We therefore carried out a study to determine whether virulent type III strains isolated from disseminated infections in babies produce higher amounts of sialic acid residues when compared with GBS isolated from health carriers. An additional purpose of this study was to determine the hydrophobic index among different serotypes of GBS strains to verify the possibility of a correlation between adherence to hydrocarbon and GBS type III virulence.

MATERIALS AND METHODS

Strains

GBS strains were obtained from the Centro de Referência para Estreptococos, Universidade Federal do Rio de Janeiro, Brazil. The strains were identified as group B and serotyped as described previously [8]. GBS were stored after lyophilization and recovered in Brain Heart Infusion broth (BHI; Difco). Aliquots of 0·5 ml of these cultures were inoculated into 100 ml of the same medium and incubated at 37 °C for 18 h. A volume of 5 ml was used for determination of surface hydrophobicity and the 95 ml remaining were used for sialic acid extraction.

Determination of sialic acid

GBS isolates were grown in 100 ml of Brain Heart Infusion for 18 h at 37 °C. Cells were recovered by centrifugation, washed once in 0·01 M of PBS and lyophilized. After determinations of the weight of lyophilized cells, the extractions were carried out as described by Kamerling and colleagues [9] but with the following modifications. GBS cells were resuspended in 1 ml of water pH 2·0 (acidified with formic acid). The suspension was warmed for 1 h at 70 °C. After centrifugation the extract was storaged at 4 °C and the cells were again extracted with 1 ml of 0·1 M HCl for 1 h at 80 °C. Both extracts were mixed and used for sialic acid dosage as described [10]. For the strain designed as 85634, the polysaccharide was extracted from a 4 l culture after dialysis for 24 h against 100 ml of distilled water, with two changes. Sialic acids in combined dialysates were purified by ion-exchange chromatography as described previously [11]. Gas-liquid chromatography of the tri-methylsilyl derivates of sialic acid methyl esters, using 3% OV-17 in stationary phase, was also carried out as described elsewhere [12].

Surface hydrophobicity

The hydrophobicity index was determined in quadruplicate by a modification of a method described previously [13]. The cells were harvested, washed with 50 mM sodium phosphate buffer, pH 7·4 and suspended in the same buffer at a turbidity of 0·4 ($OD_{660\,nm}$). Aliquots of 2·5 ml were mixed with 1 ml of xylene and

after vortexing for 2 min, the tube was left for 20 min at room temperature to allow separation of the two phases. The aqueous phase was collected and the turbidity was read at 660 nm. The hydrophobicity index (HI) was calculated using the following equation:

$$HI = (A_{660} \text{ control} - A_{660} \text{ test})/A_{660} \text{ control},$$

where A_{660} control = optical density of the strains before xylene treatment (0·4) and A_{660} test = optical density of the strains after xylene treatment [13].

Microelectrophoresis

Cells were fixed for 1 h at room temperature using 2·5% glutaraldehyde in 0·1 M phosphate buffer, pH 7·2. The electrophoretic mobility (EPM) of GBS was determined in a Zeiss Cytopherometer, by timing the passage of cells through a calibrated graticule when a current of 6 mA and a gradient of 5·5 V cm^{-1} was applied to the electrophoresis chamber. Cell mobility was timed in alternative directions to minimize electrode polarization. Instrument calibration was controlled by measuring the electrophoretic mobility of glutaraldehyde-fixed normal human erythrocytes. The measurements, in alternative directions, were made on 60 individual cells, which were suspended in NaCl solution (ionic strength 0·145 mol dm^{-3}, pH 7·2 at 23·6 °C). Electrophoretic mobility was determined with the aid of the following equation:

$$EPM = (d/t) \times (D/V),$$

where d = distance (in μm) covered by cells during measurements (usually 16 μm); t = time in sec (required by cells to cover the distance d); D = the distance between the two electrodes (18 cm); V = the potential applied to the electrodes [14].

Enzymatic treatment

Cells were washed once in PBS (0·01 M phosphate buffer and 0·15 M NaCl, pH 6·0), and incubated for 30 min at 37 °C in the presence of 0·2 U/ml neuraminidase from *Clostridium perfringens* (Sigma, type X) at pH 6·0. After incubation, the cells were washed once in PBS, fixed in glutaraldehyde and used for determination of the cellular electrophoretic mobility.

RESULTS

A total of 40 GBS was studied. These strains belonged to serotypes Ia, Ia/c, Ib/Ibc, II, III, IV and V. Between four and six strains from each type were used. Five out of 40 strains belonged to serotype III, and were isolated from blood, liquor or placenta of patients with invasive infections (virulent GBS). The other strains were isolated from the vaginal tract of pregnant or non-pregnant women (GBS strains from carriers).

The sialic acid of a type III GBS designated as 85634 was extracted, purified and the content determined. The sialic acid was a *N*-acetylneuraminic acid as verified by gas-liquid chromatography (Fig. 1). No significant difference was observed when the contents before and after sialic acid purification were compared (0·48 and 0·5%, respectively). Based on these results, the determination of sialic acid for all the strains was done immediately after extraction. Among the GBS

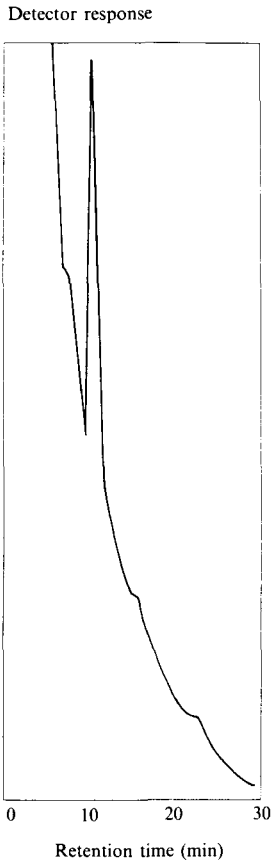

Fig. 1. Gas-liquid chromatography on 3% OV-17 of trimethylsilyl derivatives of sialic acid from the group B streptococci, showing the NeuNAc peak which has the same retention time as the NeuNAc standard.

isolates from carriers, bacteria belonging to serotypes IV, III and Ib/Ibc showed the lowest level of sialic acid content (0·46, 0·53 and 0·62%), followed by type Ia, II, Ia/c and V that presented respectively 0·72, 0·75 and 0·98%. A marked difference was verified when we compared the sialic acid content observed in GBS obtained from carriers with the values found in the virulent type III strains (1·4%; Fig. 2).

The hydrophobic index was also studied for all the GBS above. The strains were extremely homogeneous with respect to their surface hydrophobicity. No significant differences were observed between GBS isolated from healthy carriers and virulent strains except for Ib/Ibc, which presented a mean index of 0·8. For all other serotypes the mean values were higher than 0·9 (Fig. 3). The studies of cell electrophoresis carried out with the strain 85634 confirmed that the GBS have a net negative surface. The EPM was calculated as $-0{\cdot}872 + 0{\cdot}102$ μm s^{-1} V^{-1} cm, when the strains were suspended in a saline solution with an ionic strength of 0·145 mol dm^{-3} at 23·6 °C, pH 7·2. The treatment of the GBS with neuraminidase reduced (by about 25%) the mean EPM of the cells (data not shown). The population of the strain 85634 was heterogeneous in terms of surface charge with

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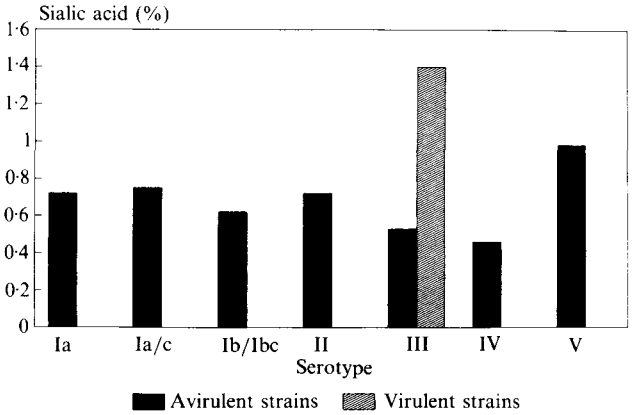

Fig. 2. Increased level of sialic acid contents in the capsular polysaccharide of five group B virulent strains. The sialic acid was measured using a colorimetric assay as described in Materials and Methods. Each value is the mean of four determinations. The results were expressed in percentage of sialic acid, and represent the amount of polysaccharide per 100 mg mass of cells.

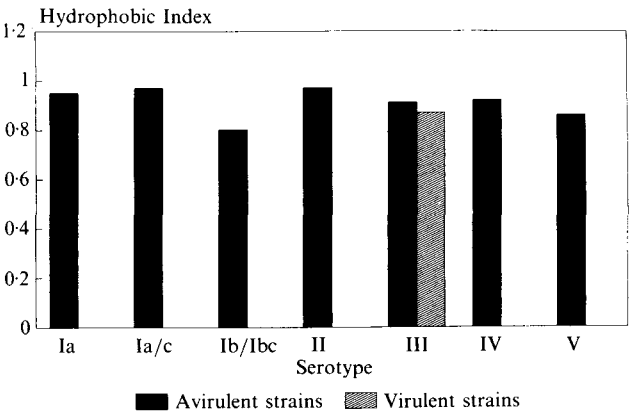

Fig. 3. Surface hydrophobicity of 40 group B streptococci determined after cell adhesion to hydrocarbon as described in Materials and Methods. Each value is the mean of four determinations.

EPM values varying from $-0{\cdot}6$ to $-1{\cdot}0\ \mu m\ s^{-1}\ V^{-1}$ cm. After neuraminidase treatment the EPM of the cells varied from $-0{\cdot}4$ to $-1{\cdot}0\ \mu m\ s^{-1}\ V^{-1}$ cm (Fig. 4).

DISCUSSION

Our results demonstrate that the amount of sialic acid in GBS strains isolated from patients with invasive infection was significantly higher (1·4%) than that observed (0·53%) for strains isolated from carriers. This result is supported by earlier observations [15], although, in the present paper, a larger number of strains, different methods for sialic acid extraction and content determinations were used. In that previous study, it was observed that serotype III strains resistant to opsonization by antibody-containing sera had a higher percentage of sialic acid (1·02%) compared with 0·59% for the type III sensitive strains. These data strongly suggest that the ability of type III virulent strains to produce

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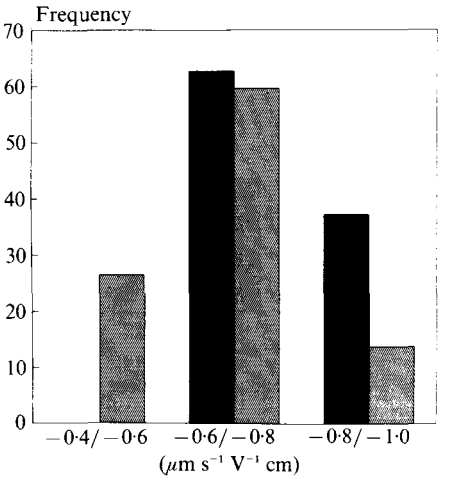

Fig. 4. Distribution of the electrophoretic mobilities (EPM) of a populations untreated [■] and neuraminidase treated [▧] group B streptococci (85634).

increased levels of this polysaccharide may be an important factor involved with the predominance of type III in severe GBS infection. It was clearly demonstrated before that the capsular sialic acid is a critical virulence determinant for type III GBS strains and that the surface sialylation aids the bacteria in evading the host defence cells [5]. Also, a reduction of 70% in the sialic acid content of the bacterial surface was enough to cause a marked reduction of the virulence [15]. Comparatively, other important virulence factors, the cellular lipoteichoic acids, seem to be present in higher amounts among clinical isolates from infants with early- or late-onset disease than asymptomatic isolates from healthy infants [4]. Therefore, it is possible that the 5 virulent strains studied in the present paper, with the highest content of sialic acid among all the 35 avirulent GBS tested belong to a unique clone, adding to these data the fact that 4 of 5 strains were isolated from the same hospital in a period of approximately 4 months. Recently it was demonstrated that the potential for virulence in most bacterial species is non-randomly distributed among phylogenetic lines [16]. Other impressive results were obtained by Musser and co-workers [17], who demonstrated that in the United States a single clone of unusually high virulence is responsible in major part for the high morbidity and mortality caused by GBS type III organisms. A similar phenomenon was described recently by McGuinness and colleagues [18]. Working with another bacterium, *Neisseria meningitidis*, they observed that a point mutation in the *por A* gene was associated with the widespread distribution of infections due to B:15:P1.7,16 meningococci. The *por A* gene is responsible for the expression of class 1 protein in meningococci.

Although surface hydrophobicity has been frequently associated with bacterial adherence and phagocytosis [19, 20], it was homogeneously distributed among the GBS strains and no significant differences were observed between GBS isolates from carriers and virulent strains.

The fact that treatment with neuraminidase reduced the GBS negative charge by only 25%, seems to indicate that, although the carboxyl groups of sialic acid

residues contribute effectively to the negative surface charge, other surface anionic groups are also involved. Previous studies have shown that usually carboxyl, sulphate and phosphate residues exposed on the cell membrane are responsible for the negative cell surface charge [21, 22].

In conclusion, the content of sialic acid seems to be an important factor for GBS type III virulence, since all pathogenic GBS tested presented a higher level of this product, when compared with strains obtained from healthy carriers. Though the sialic acid residues are exposed on the cell surface of GBS and contribute effectively for the negative surface charge of these bacteria most of the GBS studied presented the same ability to adhere on hydrocarbon. The high content of sialic acid presents on type III virulent strains does not significantly contribute for variations on hydrophobic index of these strains.

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