The phenotypic relationship of *Neisseria polysaccharea* to commensal and pathogenic *Neisseria* spp.

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Summary. Eight of 22 non-capsulate strains of *Neisseria meningitidis* previously isolated from primary school children were re-identified as *N. polysaccharea* by aminopeptidase reactions and polysaccharide production. *N. polysaccharea* was not identified amongst 91 non-capsulate strains of *N. meningitidis* isolated from adults attending the Genito-urinary Medicine clinic, Westminster Hospital, London. The biochemical reactions of *N. polysaccharea* strains were similar to those of *N. lactamica* and *N. gonorrhoeae*, but *N. polysaccharea* could be distinguished from these organisms by examination of β -galactosidase activity, carbohydrate reactions and polysaccharide production. Sodium dodecyl sulphate-polyacrylamide gel electrophoresis revealed closer similarity of *N. polysaccharea* to *N. lactamica* than to the pathogenic *Neisseria* spp. An additional finding was variation in the position of one of the major proteins of *N. lactamica* in the 34–39-Kda region.

Introduction

Riou *et al.* (1983) first proposed the new species *Neisseria polysacchareae*, later renamed *N. polysaccharea* (Guibourdenche *et al.*, 1986), after investigating approximately 3500 strains of referred *Neisseria* and *Branhamella* spp. Thirteen isolates, from the throats of healthy children, could not be speciated and were subsequently designated *N. polysaccharea*, a species which could be differentiated from *N. meningitidis* by the absence of glutamyl aminopeptidase activity, polysaccharide production and cysteine requirement for growth on Catlin medium. Bouquete *et al.* (1986) subsequently identified 50 *N. polysaccharea* strains from 216 non-capsulate strains of *N. meningitidis* received in a meningococcus reference laboratory (23%), they also investigated 102 non-capsulate meningococcal isolates from a survey of children aged 5–9 years (Saez-Nieto *et al.*, 1985) of which 38 (37%) were identified as *N. polysaccharea*. In this study we examined strains of *N. meningitidis* obtained from a previous survey of primary school children, aged 4–11 years (Cann *et al.*, 1987), as well as isolates obtained from adults attending the Genito-urinary Medicine clinic, Westminster Hospital, in order to assess the prevalence of *N. polysaccharea* strains in adults and children. We also examined the relation-

ship between *N. polysaccharea* and *N. lactamica* which is a common commensal of the nasopharynx in young children.

Materials and methods

Isolates

A total of 251 strains was examined: 22 non-capsulate isolates of *N. meningitidis* were obtained from a previous survey of nasopharyngeal carriage in primary school children undertaken because of a cluster of cases of invasive meningococcal disease caused by a serogroup B, 15P1.16 strain (Cann *et al.*, 1987); 91 meningococcal strains were collected during 1 year from adult patients attending the Genito-urinary Medicine clinic; 138 strains of *N. lactamica* were also collected from the study of primary school children. All strains were isolated from the posterior nasopharynx and stored in 10% glycerol peptone at -70°C . The 113 isolates were identified as *N. meningitidis* by conventional laboratory methods: they were gram-negative, oxidase-positive cocci which were negative for β -galactosidase (ONPG) and produced acid from the oxidation of glucose and maltose but not sucrose or lactose. *N. lactamica* strains were identified by a positive ONPG reaction. Strains were serogrouped by slide agglutination with Wellcome meningococcal agglutinating sera against groups A, B, C, W135, X, Y, Z. Serotyping was performed by co-agglutination with monoclonal antibodies generously supplied by Drs W. Zollinger and J. Poolman. The serotyping antibodies used were P1.2, 3, 9, 15 and 16, 2a, 2b, 2c and 15.

Biochemical tests

γ -L-Glutamyl and L-hydroxyproline aminopeptidase activity was measured by a non-quantitative modification of the method described by Ison *et al.* (1982). A heavy suspension of each organism was made in saline and assayed in duplicate for both enzymes in a flat-bottomed microtitration plate (Dynatech). A strain of *N. meningitidis* B, 15P1.16 and a *N. lactamica* strain were included as controls. Those isolates that gave negative results for γ -L-glutamyl aminopeptidase were subcultured on nutrient agar (Oxoid) containing horse serum 5% and dextrose 5% at 36°C in reduced oxygen tension (i.e., in a candle jar) with *N. meningitidis* B, 15P1.16 and an *N. lactamica* strain as controls. Polysaccharide production was detected by the addition of a drop of Gram's iodine solution (potassium iodide 2%, iodine 1%) to a colony which became black immediately if positive (personal communication, J. Knapp, Center for Disease Control, Atlanta). This test could also be performed on the growth on the sucrose slope from the initial carbohydrate identification tests. Those isolates that gave negative results for γ -L-glutamyl aminopeptidase were subcultured at 22°C and 36°C, on Thayer Martin agar, in reduced oxygen tension, with *N. meningitidis* and *N. lactamica* as controls.

SDS-PAGE

The 22 non-capsulate meningococcal isolates plus a group of B, 15P1.16 isolates from children, 20 meningococcal isolates of different sero-groups and sero-types from adults and 20 *N. lactamica* strains were prepared for sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). Each isolate was inoculated into Tryptone Soya Broth (Oxoid) and incubated overnight, at 36°C on a gyratory shaker at 150 rpm. The growth was centrifuged to obtain a cell pellet which was washed in saline, re-centrifuged and resuspended in saline. The protein concentration of the suspension was estimated by

the method of Lowry *et al.* (1951) and diluted with saline to a concentration of 2 μ g/ μ l. The standardised cell suspensions were then diluted 1 in 2 with sample buffer (SDS 4%, mercaptoethanol 10%, glycerol 20% in 0.125 M Tris/HCl). Electrophoresis of these samples was performed with 12.5% polyacrylamide gels in a discontinuous buffer system (Laemmli, 1970) for 1 h. The following mol.-wt standards (Kda) were applied: bovine serum albumin 66; ovalbumin 45; pepsin 34.7; trypsinogen 24; β -lactoglobulin 18.4; and lysozyme 14.3. Gels were stained with Brilliant Blue (Sigma Chemical Company, P.O. Box 14508, St Louis, MO 63178, USA) and dried with a slab gel drier (LKB, 232 Addington Road, Selmsdon, S. Croydon CR2 8YD). Two gonococcal lysates, Kellogg strain F62 and strain G7 (Johnston *et al.*, 1976) were provided by Dr C. Ison as controls.

Results

Table I shows the reactions distinguishing *N. meningitidis*, *N. polysaccharea*, *N. lactamica* and *N. gonorrhoeae*. Eight isolates from children but none of the adult strains showed the characteristics of *N. polysaccharea*. Our previous study had been performed as three cross sectional surveys within a 1-year period. Seven of these eight isolates were from different children with ages ranging from 4 to 11 years (one isolate was carried by the same child in two surveys). Seven were from the school which had suffered cases of meningococcal disease and one was from the smaller control school. This difference in the number of *N. polysaccharea* isolated from each school was not statistically significant. The eight *N. polysaccharea* strains and all 138 *N. lactamica* strains were ungroupable and non-typable. The eight *N. polysaccharea* strains also failed to grow at 22°C, on Thayer Martin agar, in reduced oxygen tension.

Table I. Characteristics discriminating *N. meningitidis*, *N. polysaccharea*, *N. lactamica* and *N. gonorrhoeae*

Test	Results obtained with			
	<i>N. meningitidis</i>	<i>N. polysaccharea</i>	<i>N. lactamica</i>	<i>N. gonorrhoeae</i>
Acid from				
glucose	+	+	+	+
maltose	+	+	+	—
lactose	—	—	+	—
sucrose	—	—	—	—
β -Galactosidase				
activity (ONPG)	—	—	+	—
γ -L-Glutamyl aminopeptidase				
activity	+	—	—	—
L-Hydroxyproline aminopeptidase				
activity	—	+	+	+
Polysaccharide production	—	+	—	—

Figure. SDS-PAGE of *Neisseria* spp. Lanes 1 and 2, *N. meningitidis* B, 15 P1. 16 and ungroupable, non-typable, respectively; lanes 3 and 4, *N. lactamica*; lane 5, *N. polysaccharea*; lanes 6 and 7, *N. gonorrhoeae*.

The figure shows the SDS-PAGE protein profiles of *N. meningitidis* B, 15P1.16 and an ungroupable non-typable meningococcus, *N. polysaccharea*, *N. lactamica*, and *N. gonorrhoeae*. The selection of *N. meningitidis* strains examined showed a variety of SDS-PAGE types as described by Mocca and Frasch (1982). The pattern of bands appears to be very similar in all four species in the high mol. wt region, 60–82 Kda. The presence of a major protein band in the 48–50-Kda region was seen in *N. lactamica* and *N. polysaccharea*, similar to *N. meningitidis*. In *N. gonorrhoeae* a major band was seen in a slightly lower position—44 Kda. Both *N. lactamica* and *N. polysaccharea* produce simpler protein patterns with a second major band seen at 34–36 Kda. In *N. lactamica* strains we observed variation in position of this protein band. Twenty *N. lactamica* strains were examined, of which 11 had a 34–36-Kda band but the other nine had a major band at 37–39-Kda. By this technique, *N. polysaccharea* appears to be more closely related to *N. lactamica* than to either *N. meningitidis* or *N. gonorrhoeae*.

Discussion

We chose to examine meningococcal strains for L-hydroxyproline aminopeptidase as well as γ -L-

glutamyl aminopeptidase and β -galactosidase as a result of the work of D'Amato *et al.* (1978), which showed that profiles based on these three enzymes were the most useful in distinguishing *Neisseria* spp. *N. polysaccharea*, like *N. lactamica* and other commensal *Neisseria* spp., is positive for L-hydroxyproline aminopeptidase. *N. gonorrhoeae* is also L-hydroxyproline aminopeptidase-positive; however, on SDS-PAGE *N. polysaccharea* appears to be more similar to *N. lactamica*.

DNA relatedness studies have shown that *N. gonorrhoeae*, *N. meningitidis*, *N. lactamica* and *N. polysaccharea* form a single genospecies within which the four sub-species can be delineated (Guibourdenche *et al.*, 1986). Clinically, however, it is more useful to regard them as separate species with the meningococcus and gonococcus frequently acting as pathogens whereas *N. polysaccharea* and *N. lactamica* appear to be non-pathogenic and to be found more frequently in the nasopharynx of children than adults.

The similarities observed on SDS-PAGE between *N. polysaccharea* and *N. lactamica*, and between these two species and *N. meningitidis*, give some support to the theory that carriage of commensal *Neisseria* spp. may contribute to the acquisition of immunity to *N. meningitidis*. *N. polysaccharea* and *N. lactamica* have been shown to

cross-react with anti-meningococcal sera, especially of group B (Gold *et al.*, 1978; Saez-Nieto *et al.*, 1985; Boquete *et al.*, 1986) but the precise nature of the role of these two organisms in the acquisition of immunity to meningococcal disease requires further study.

Confusion of *N. polysaccharea* strains with *N. meningitidis* will lead to falsely high estimates of meningococcal carriage and acquisition in a population. In our study of primary school children, the true meningococcal carriage and acquisition rates were, in fact, lower, although this does not affect the conclusions drawn. Our finding of approxi-

mately one third (8 out of 22) of non-capsulate strains of "meningococci" from young children displaying the characteristics of *N. polysaccharea* is similar that of Boquete *et al.* (1986) who studied a similar age group. The ease with which *N. polysaccharea* can be distinguished from *N. meningitidis* by testing for polysaccharide production should prevent future confusion and overestimates of meningococcal carriage rates.

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