SHORT ARTICLE

Immuno-electronmicroscopic demonstration of capsules on group-B streptococci of new serotypes and type candidates

M. RÝC, JARMILA JELÍNKOVÁ,* JITKA MOTLOVÁ* and M. WAGNER†

*Department of Electron Microscopy, Institute of Hygiene and Epidemiology, and *Department of Medical Microbiology, Postgraduate Medical Institute, 100 42 Prague 10, Czechoslovakia, and †Central Institute of Microbiology and Experimental Therapy, 6900 Jena, German Democratic Republic*

Summary. The location of type polysaccharides on the cells of reference strains of group-B streptococci of serotypes IV and V and new type candidates NT6 and 7271 was investigated by electronmicroscopy of the bacteria after incubation with homologous type-specific antiserum. A distinct capsular layer was found on the surface of the cells of all these strains. Sialic acid, an integral part of all the conventional type polysaccharides of group-B streptococci, was also detected in all the strains examined.

Introduction

The classification scheme of group-B streptococci (*Streptococcus agalactiae*) was recently extended by serotypes IV and V (Perch *et al.*, 1979; Jelínková, 1982; Jelínková and Motlová, 1985). Type-specific polysaccharides of the conventional group-B streptococcal serotypes (Ia, Ib, II and III) were found in the capsule as demonstrated both by unlabelled (Mackie *et al.*, 1979) and by ferritin-labelled (Wagner *et al.*, 1980) type-specific antibodies. In all the type polysaccharides of the conventional serotypes, but not in the group polysaccharide, N-acetylneuraminic acid (sialic acid) was detected (Baker and Kasper, 1976; Swenson *et al.*, 1982). Strains of the new group-B streptococcal serotypes IV and V and type candidates were also found to contain sialic acid (Motlová *et al.*, 1986). However, neither the location of the type-polysaccharide antigen nor the presence of capsule in the serotypes IV and V and the type candidates was established. To answer these questions, we studied reference strains of these organisms by immuno-electronmicroscopy after treatment with type-specific antiserum and compared the results with the quantitative determination of sialic acid in the type polysaccharide of the corresponding strains.

Materials and methods

Group-B streptococcus strains

Reference strains of serotypes IV (no. 3139) and V (no. 1169) and the new candidate types NT6 (Wilkinson, 1977) and 7271 (Perch *et al.*, 1979) were used.

Antisera

Group-B streptococcal type antisera were raised in rabbits by the standard immunisation technique (Jelínková, 1977) and absorbed to contain monospecific type-antibodies only, as checked by double diffusion in gel.

Immuno-electronmicroscopy

Streptococcal culture grown for 5 h in Todd-Hewitt broth was washed three times in phosphate-buffered saline (PBS), pH 7.2. To the sediment, 0.25 ml of undiluted homologous type-specific antiserum was added and the samples were incubated for 1 h at 37°C with agitation. After two washes in PBS the cells were fixed with glutaraldehyde 2.5% v/v in 0.1M sodium cacodylate buffer, pH 6.8, at 4°C overnight. The samples were then washed in the same buffer and post-fixed with OsO₄ 1% w/v in R-K buffer (Ryter and Kellenberger, 1958) for 1 h at 22°C. After washing, the bacteria were further fixed with OsO₄ 1% w/v in R-K buffer containing 0.1% of Tryptone (Difco Bacto) at 22°C overnight. The bacteria were then washed, embedded in agar, contrasted with uranyl acetate 0.5% w/v for 90 min at 22°C and embedded

Figs 1-8. Electronmicrographs of ultrathin sections of group-B streptococci, and of these streptococci after incubation with homologous type-specific antiserum. (Bar markers represent 0.2 μm .) 1: serotype-IV strain; 2: serotype-IV strain plus serum; 3: serotype-V strain; 4: serotype-V strain plus serum; 5: type-candidate NT6 strain; 6: type-candidate NT6 strain plus serum; 7: type-candidate 7271 strain; 8: type-candidate 7271 strain plus serum.

in Epon (Serva Feinbiochemica). Ultrathin sections were contrasted with uranyl acetate-lead citrate and evaluated in a JEOL 100 CX-II electronmicroscope at 80 kV. Control cells were incubated with PBS instead of the antiserum.

Quantitative determination of sialic acid

The amount of sialic acid in washed lyophilised bacteria was determined by the thiobarbituric acid method (Aminoff, 1961) as described by Motlová *et al.* (1986). N-acetylneuraminic acid (Sigma) was used as a standard. The mean values were calculated from four determinations.

Results and discussion

The cells of reference strains of the serotypes IV and V not incubated with antisera possessed nearly smooth surfaces with very short filamentous protrusions (figs. 1 and 3), which are believed to be the remnants of condensed capsular material. Incubation of the cells with homologous type-specific antisera led to visualisation of a thick electron-dense layer outside the cell wall (figs. 2 and 4). This layer was found consistently on almost all the cells.

Similarly the type-candidate strains NT6 and 7271 not incubated with antisera had an almost smooth surface (figs. 5 and 7). Incubation of these strains with homologous type antisera led to visualisation of a large capsular layer on the cells (figs. 6 and 8).

Thus the presence of a capsule containing the type polysaccharide was demonstrated on each of the representatives of group-B streptococcal serotypes IV and V and the new type candidates. This finding correlated well with our earlier observation (Motlová *et al.*, 1986) that sialic acid was present in each of these strains, as indicated by agglutination with sialic-acid specific lectin (Wagner, 1982). In the present study, sialic acid was found in the following amounts per 10 mg of bacterial dry weight: serotype IV, 22 µg; serotype V, 45 µg; NT6, 49 µg; 7271, 71 µg. Comparison of sialic acid content with thickness of capsules on ultrathin sections (figs. 2, 4, 6 and 8) did not reveal any quantitative correlation.

A more detailed immuno-electronmicroscopic study of the location of new type-polysaccharide and protein antigens is in progress.

REFERENCES

- Aminoff D 1961 Methods for the quantitative estimation of N-acetylneuraminic acid and their application to hydrolysates of sialomucoids. *Biochemical Journal* **81**: 384–392.
- Baker C J, Kasper D L 1976 Identification of sialic acid in polysaccharide antigens of group B *Streptococcus*. *Infection and Immunity* **13**: 284–288.
- Jelínková J 1977 Group B streptococci in the human population. *Current Topics in Microbiology and Immunology* **76**: 127–165.
- Jelínková J 1982 Frequency of serotypes in group B *Streptococcus* isolates: new type candidates. In: Holm S E, Christensen P (eds) Basic concepts of streptococci and streptococcal diseases. Reedbooks Ltd, Chertsey, pp. 50–51.
- Jelínková J, Motlová J 1985 Worldwide distribution of two new serotypes of group B streptococci: type IV and provisional type V. *Journal of Clinical Microbiology* **21**: 361–362.
- Mackie E B, Brown K N, Lam J, Costerton J W 1979 Morphological stabilization of capsules of group B streptococci, types Ia, Ib, II, and III, with specific antibody. *Journal of Bacteriology* **138**: 609–617.
- Motlová J, Wagner M, Jelínková J 1986 A search for new group-B streptococcal serotypes. *Journal of Medical Microbiology* **22**: 101–105.
- Perch B, Kjems E, Henrichsen J 1979 New serotypes of group B streptococci isolated from human sources. *Journal of Clinical Microbiology* **10**: 109–110.
- Ryter A, Kellenberger E, Birch-Andersen A, Maaløe O 1958 Étude au microscope électronique de plasmas contenant de l'acide désoxyribonucléique. I. Les nucléoides des bactéries en croissance active. *Zeitschrift für Naturforschung* **13b**: 597–605.
- Swenson R M, DeCueninck B, Eisenstein T K, Greber T, Reasavy D, Shockman G D 1982 The soluble antigens of type III, II and Ia group-B streptococci. In: Holm S E, Christensen P (eds) Basic concepts of streptococci and streptococcal diseases. Reedbooks Ltd, Chertsey, pp. 94–95.
- Wagner M 1982 Agglutination of bacteria by a sialic acid-specific lectin of the snail *Cepaea hortensis*. *Acta Histochemica* **71**: 35–39.
- Wagner M, Wagner B, Kubin V 1980 Immunoelectron microscopic study of the location of group-specific and type-specific polysaccharide antigens on isolated walls of group B streptococci. *Journal of General Microbiology* **120**: 369–376.
- Wilkinson H W 1977 Nontypable group B streptococci isolated from human sources. *Journal of Clinical Microbiology* **6**: 183–184.