Immunoelectron Microscopic Study of the Location of Group-specific and Type-specific Polysaccharide Antigens on Isolated Walls of Group B Streptococci

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The ultrastructural locations of the group-specific polysaccharide and the type-specific polysaccharides Ia, Ib, II and III of group B streptococci (Streptococcus agalactiae) were studied on isolated walls by the direct immunoferritin technique. The type polysaccharides were located exclusively on the outer side of the wall on which they formed a distinct capsule. Except for strain 58/59 (type Ia) the thickness of the capsule was characteristic of each strain investigated. In all strains the type-specific ferritin labelling was confined to the outer surface of the capsule. The group-specific polysaccharide could be demonstrated on the inner surface in all strains tested. It could also be demonstrated on the outer surface in strains 59/59 (type Ib) and 8/66 (group B variant) and on most of the walls of strain 58/59 (type Ia). The failure to detect this antigen on the outer side of the walls of strains 60/59 (type II) and 13/63 (type III) and on some walls of strain 58/59 was probably due to the thickness of the type polysaccharide capsule.

INTRODUCTION

Using the immunoferritin technique, we have previously shown that the group-specific polysaccharide of group B streptococci (Streptococcus agalactiae) strains 24/60 (type X) and 25/60 (type R) is located on both the outer and inner surfaces of isolated walls whereas the protein antigens R and X are found only on the outer side (Wagner et al., 1980). Recently, Kasper & Baker (1979) used the same technique to demonstrate the type Ia, II and III polysaccharide antigens of group B streptococci. However, the latter studies were performed only on whole cells, so no conclusion could be drawn as to whether these antigens were also located on the inner side of the wall. In the present paper, we show that in contrast to the group polysaccharide, the type-specific polysaccharides are located only on the outer wall surface.

METHODS

Bacterial strains and growth conditions. The following strains of Streptococcus agalactiae (group B streptococci) from the Collection of Pathogenic Microorganisms (Institute of Hygiene and Epidemiology, Prague) were used: strain 58/59 ('090', orig. R. C. Lancefield, New York), type Ia; strain 59/59 ('H36B', orig. R. C. Lancefield), type Ib; strain 60/59 ('18RS21', orig. R. C. Lancefield), type II; strain 13/63 ('6313', orig. J. Jelinková, Prague), type III; strain 25/60 ('Compton', orig. I. H. Pattison, London), type R; strain 8/66 ('090 variant', orig. R. C. Lancefield), group B reference strain without type antigen.
Fig. 1. Demonstration of type II and III polysaccharides on walls of *S. agalactiae* by ferritin-conjugated homologous type-specific antibody. (a) Strain 60/59 (type II); (b) strain 13/63 (type III). Bar markers represent 0.2 μm.

Bacteria were grown with shaking for 3 to 4 h at 37 °C in Todd–Hewitt broth enriched with Na₂HPO₄ and glucose (Baker & Kasper, 1976).

*Preparation of isolated walls.* Walls were prepared by methods previously described (Wagner et al., 1978).

*Antisera.* Type-specific antisera were prepared in rabbits according to the immunization schedule of Jelinková (1977) using heat-inactivated bacteria of the strains listed above. To remove antibodies directed against the group-specific polysaccharide, the sera were absorbed with cells of strain 8/66 (a group B variant strain without type antigen). The antiserum directed against type Ia was further absorbed with cells of strains 60/59 (type II) and 24/60 (type X). Absorptions were done by mixing 1 vol. cells with 2 vol. serum and incubating for 1 h at room temperature with shaking. The specificity of the sera was checked by the ring precipitation reaction and by counter-immunoelectrophoresis (Pattison et al., 1955; Kubin et al., 1977) with Lancefield extracts.

Group-specific antisera were prepared with strain 8/66 following the same immunization schedule.

*Immunoelectron microscopy.* All labelling experiments were done using the direct immunoferritin tech-
Group B streptococcal antigens

RESULTS AND DISCUSSION

Isolated walls of the strains tested showed the typical trilamellar profile consisting of electron-dense outer layers and an electron-translucent middle layer. The outer wall surface was covered with fuzzy material. The type-specific polysaccharides of group B streptococci were defined as capsular antigens by Lancefield & Freimer (1966). However, as previously reported by Kasper & Baker (1979) and Mackie et al. (1979), these polysaccharides were...
Fig. 4. Demonstration of type Ia polysaccharide on walls of *S. agalactiae* strain 58/59 by ferritin-conjugated homologous antibody. (a) The wall with a large capsule is surrounded by several walls without capsular material that are labelled in a pattern typical of the location of group antigen; (b) the inner side of the capsule-bearing wall is labelled in the same manner as the walls without capsule. Bar markers represent 0.2 μm.

barely visible on sections of whole cells unless they had been labelled with the homologous antibody.

After incubating walls of strains 60/59 (type II) and 13/63 (type III) with ferritin-conjugated antibody directed against the homologous type-specific polysaccharides, heavy ferritin labelling was observed only in a zone around, but apparently not in contact with, the outer surface of the walls (Fig. 1 a, b). Between the labelled capsule and the wall was an
Group B streptococcal antigens

Fig. 5. Demonstration of the group polysaccharide on walls of *S. agalactiae* by ferritin-conjugated group-specific antibody. (a) Strain 58/59 (type Ia); (b) strain 59/59 (type Ib); (c) strain 8/66 (group B variant). Bar markers represent 0.2 μm.

unlabelled area traversed by a few filamentous structures protruding radially from the wall. The thickness of the whole capsule measured 65 to 75 nm in strain 60/59 and 115 to 135 nm in strain 13/63. The unlabelled zones measured 25 to 35 nm and 40 to 60 nm, respectively.

Walls of strain 59/59 (type Ib), after incubation with ferritin-conjugated antibody directed against the type Ib polysaccharide, exhibited only sparse labelling of the outer wall surface. The ferritin was bound to radially arranged thread-like material extruding from the wall, but not forming a continuous covering or capsule (Fig. 2).

In all cases, only the homologous strains were labelled by the conjugates (for example, Fig. 3a, b). This proved their type-specificity and provided evidence that the location of the ferritin particles was not a result of non-specific attachment.

Walls of strain 58/59 (type Ia), after incubation with the ferritin-conjugated antibody directed against the type Ia polysaccharide, showed a different labelling pattern. Some
walls had a very large capsule, up to 300 nm thick. The outer region of the capsule was heavily labelled by ferritin (Fig. 4a, b), whereas the inner part, about 100 to 200 nm thick, remained unlabelled and was traversed by filamentous structures as in strains 60/59 and 13/63. Most of the walls, however, showed little or no capsular material and were labelled on both the outer and inner surfaces. This labelling pattern is typical of the location of group-specific polysaccharides of group B and group C streptococci (Wagner & Wagner, 1975; Wagner et al., 1980). Cross-reactions of the conjugate with walls and whole cells of strains 8/66 (group B variant) and 25/60 (type R) resulting in the same labelling pattern suggested that group-specific antibodies were still accessible to labelling. The presence of
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Fig. 7. Simultaneous demonstration of the type and group polysaccharides on walls of S. agalactiae strain 13/63 (type III) by unlabelled homologous type-specific antiserum and ferritin-conjugated group-specific antibody. Bar marker represents 0.2 \( \mu \)m.

the capsule only on some walls provided evidence that the amount of type antigen may vary from cell to cell within strain 58/59.

In all labelling experiments with type-specific conjugates, the inner surface of the isolated walls was not labelled. The asymmetric location of the type polysaccharides on the wall confirms the capsular nature of these antigens (Lancefield & Freimer, 1966; Kasper & Baker, 1979). A similar asymmetric location is typical of the protein antigens R and X of group B streptococci (Wagner et al., 1980) as well as the proteins T and M of group A streptococci (Wagner et al., 1979; M. Wagner & B. Wagner, unpublished results). Furthermore, the location of both the M proteins and the group B Streptococcus type polysaccharides on long filamentous structures is reminiscent of their similar function as virulence factors (Lancefield, 1962; Lancefield & Freimer, 1966).

As previously demonstrated for strains 24/60 (type X) and 25/60 (type R) (Wagner et al., 1980), the location of the group polysaccharide differs from that of the type antigens. This observation was confirmed by the present studies with ferritin-conjugated group-specific antibody. Walls of strains 59/59 (type Ib) and 8/66 (group B variant) and most of the walls of strain 58/59 (type Ia) exhibited ferritin labelling on both the inner and outer surfaces (Fig. 5a, b, c), whereas labelling of strains 60/59 (type II) and 13/63 (type III) was seen only on the inner wall surfaces (Fig. 6a, b). It can be concluded from the results of the type reactions that the demonstration of the group antigen on the outer wall surface in these strains might be sterically hindered by the densely packed filaments of the type polysaccharide. The same conclusion was drawn from diagnostic immunofluorescent investigations using group- and type-specific antisera (Kubín et al., 1968; Romero & Wilkinson, 1974) and from immunoelectron microscopic studies on a type III strain (Kasper et al., 1978). Labelling experiments on ultrathin sections are in progress to determine whether the group antigen can also be detected on the outer side of the wall in these strains.

For simultaneous demonstration of both the group and type polysaccharide, walls of strain 13/63 (type III) were incubated with unlabelled homologous type-specific antiserum.
and subsequently with the ferritin-conjugated group-specific antibody. The arrangement of the ferritin particles on the inner side of the wall indicated the location of the group antigen (Fig. 7). On the outer wall surface, type polysaccharide capsular material became visible by interaction with the specific antibody. Mackie et al. (1979) explained this visualization of type polysaccharides as a stabilizing effect of the antibody preventing the capsular material from collapsing during the dehydration process. Another possible explanation is that polysaccharides are not stained by conventional methods in electron microscopy, whereas after antibody binding, staining with OsO₄ occurs. The visualized capsule, which was about 85 nm thick, consisted of densely packed fuzzy material and did not show an electron-translucent zone like that seen after ferritin-labelling.

Kasper et al. (1978) concluded from chemical studies that the native polysaccharide antigen of type III possesses both a superficial type-specific determinant and a common group B determinant. However, this assumption was not supported by our findings which provide evidence for a different location of these antigens.

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REFERENCES


