Immunolabelling of SR protein and serotype polysaccharide in *Streptococcus mutans*

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Immunoelectron microscopy was used to determine the accessibility of gold-labelled monoclonal antibodies, specific for the SR protein and serotype polysaccharide, to the surface of viable cells of *Streptococcus mutans*. The results indicate that both antigens are simultaneously accessible to their respective antibodies.

Introduction

The cell-surface components of *Streptococcus mutans* appear to play an important role both in saliva-mediated bacterial adherence to the tooth surface and in bacterial agglutination (Loesche, 1986). Therefore several efforts to prevent adherence of *S. mutans* have focused on the production of an effective vaccine comprising isolated purified streptococcal surface components (Russell & Mestecky, 1986). Current evidence suggests that serotype polysaccharide (Gibbons & Qureshi, 1979) and antigen I/II, a 140 kDa cell-surface glycoprotein (Russell & Lehner, 1978), also named B (Russell, 1979), P1 (Forester *et al.*, 1983), IF (Hughes *et al.*, 1980) and Pac (Okahashi *et al.*, 1989) are: (i) implicated in the initial interactions of *S. mutans* and salivary glycoproteins (Russell, 1979), (ii) surface exposed and (iii) accessible to antibodies (Douglas & Russell, 1984; Schöller *et al.*, 1988). We have shown that when SR protein (a cell-surface component related immunologically to antigen I/II) is covalently linked to purified serotype *f* polysaccharide (Wachsmann *et al.*, 1985) and orally administered in liposomes, a local anti-protein and anti-polysaccharide immune response is produced (Schöller *et al.*, 1988). The wide distribution of SR proteins in mutans streptococci (Bruyere *et al.*, 1988) and partial cross-reactivity between serotype *f* polysaccharide and other serotype polysaccharides (Schöller *et al.*, 1988) makes these antigens of potential use as a vaccine. However, there was no information available as to the accessibility to antibodies of these antigens when they are in their native form on viable streptococci.

To investigate whether native SR protein and serotype polysaccharide are accessible to antibodies, we used monoclonal antibodies (mAbs) directed to each component, linked to gold particles. The simultaneous binding of these mAbs to their respective epitopes was visualized by immunoelectron microscopy with each mAb labelled with gold particles of a different size.

Methods

Bacterial strain and culture conditions. *S. mutans* OMZ 175 (serotype *f*) was grown overnight in Brain Heart Infusion broth. Bacterial cells were collected by centrifugation, washed three times with phosphate-buffered saline (PBS; 0.15 M-NaCl, 0.01 M-Na₂HPO₄/NaH₂PO₄, pH 7.2) and finally resuspended in PBS.

Monoclonal antibodies. The mAbs used in this study have been previously described and characterized (Schöller *et al.*, 1988; Bruyere *et al.*, 1988). LOSM 11 was obtained after immunization of rats with purified 74 kDa SR protein and fusion of spleen cells with line IR 983 F (Bruyere *et al.*, 1988). K28 (anti-serotype-*f*-polysaccharide) was produced in rats immunized with formalin-killed *S. mutans* OMZ 175 cells according to the standard procedure (Schöller *et al.*, 1988). Both antibodies were of subclass IgG 2a with κ light chains. These antibodies bind to their respective purified antigen, to intact *S. mutans* OMZ 175 cells and interfere with the binding of salivary glycoproteins to *S. mutans* (Schöller *et al.*, 1988; Bruyere *et al.*, 1988).

Immunoelectron microscopy. Protein G (Sigma) was conjugated to either 15 nm or 5 nm colloidal gold particles (Janssen) as described by Bendayan (1987); affinity purified mAbs-protein G-gold complexes were prepared according to the method described by Robinson *et al.*, (1984) for mAbs-protein A-gold complexes. Negative controls were provided by non-relevant rat IgG mAbs.

The coated sides of Formvar-covered carbon-stabilized nickel grids were floated for 1 min at 25 °C on the streptococcal suspension. The grids were then transferred to drops of reagents in the following sequence: (i) 0.5% gelatin in PBS (15 min, 25 °C); (ii) gold-protein G-mAbs complexes (30 min, 37 °C); (iii) five successive drops of PBS; and (iv) 1% methylamine tungstate. For double-labelling experiments,

Abbreviation: mAb, monoclonal antibody

Fig. 1. Binding of mAbs LOSM 11 and K28 to *S. mutans* OMZ 175. A, B, electron micrographs of whole cells of *S. mutans* incubated with LOSM 11 (A) and K28 (B). Note the presence of gold particles complexed to LOSM 11 (anti-protein SR) attached to extruded material in (A). C, D, thin sections of cells incubated with LOSM 11 (C) and K28 (D). E, whole cells of *S. mutans* exposed simultaneously to both mAbs. LOSM 11 was complexed to large (15 nm) gold particles and K28 to smaller (5 nm) particles. F, control incubation of cells with a non-relevant rat mAb. Bars, 0.1 µm.

the grids were incubated after step (ii) for a second 30 min period with complexes containing smaller gold particles. The grids were air-dried and examined in a transmission electron microscope (Jeol 100b) at 80 kV.

To prepare thin sections, bacterial suspensions were centrifuged and the pellet was fixed in 2% (v/v) glutaraldehyde and 2% (v/v) paraformaldehyde in 0.1 M-sodium cacodylate buffer (pH 7.2), post-fixed with 1% osmium tetroxide in the same buffer and embedded in Epon 812. Sections were floated on grids, incubated with 10% (v/v) aqueous sodium metaperiodate (Bendayan, 1984), rinsed in three changes of distilled water and incubated with the reagents described above. The sections were stained with uranyl acetate and lead citrate, and examined in a Jeol 100b transmission electron microscope as above.

Results and Discussion

mAb LOSM 11 bound readily to cells of *S. mutans* OMZ 175. Gold-labelled antibody was localized at the outer surface of intact cells and also bound to extruded cell-wall material, probably representing excreted SR protein (Fig. 1A). These results confirmed previous observation concerning the loose association of antigen-I/II-related proteins with the *S. mutans* cell wall (Russell *et al.*, 1980). Incubation of viable streptococci with the anti-polysaccharide-*f* mAb K28 resulted in a specific cell-wall labelling pattern, and a moderate amount of labelling of the extruded material (Fig. 1B). This localization of the SR and polysaccharide antigens was also confirmed on thin-sections of *S. mutans* cells incubated with either LOSM 11 (Fig. 1C) or with K28 (Fig. 1D). The absence from cross-sections of intracellular labelling of the polysaccharide antigen suggests that the polysaccharide is synthesized at the cell surface, rather than being synthesized in the cytoplasm and then exported into the cell wall. When cells were incubated with non-immune IgG no antibody binding was seen on either cell membrane or extruded cell-wall material (Fig. 1F). To determine the accessibility of both SR protein and serotype polysaccharide, viable *S. mutans* cells were exposed in sequence to antibody LOSM 11 complexed with large gold particles and to antibody K28 complexed with small gold particles (Fig. 1E). Each antibody exhibited binding to its corresponding epitope on the cell surface and the double labelling was not affected by the order of the incubation sequence. This showed that the antibodies do not interfere with each other in binding to viable streptococci. The simultaneous binding of both kinds of antibody to bacterial cells implies direct availability of the corresponding epitopes at the cell surface. Furthermore, the double accessibility of the antigens suggests the absence of steric hindrance for the fixation of the antibodies and the unmasking of the epitope, probably due to their different localization within the cell-wall region.

In conclusion, our results demonstrate the direct accessibility of both antigens on the *S. mutans* cell-surface to antibodies elicited during immunization experiments with a protein-polysaccharide conjugate, and thus confirm the potential role of such a conjugate as an alternative vaccine against dental caries.

References

- BENDAYAN, M. (1984). Protein A-gold electron microscopic immunocytochemistry: methods, applications, and limitations. *Journal of Electron Microscopy Technique* **1**, 243-270.
- BENDAYAN, M. (1987). Introduction of the protein G-gold complex for high-resolution immunocytochemistry. *Journal of Electron Microscopy Technique* **6**, 7-13.
- BRUYERE, T., ACKERMANS, F., KLEIN, J. P., PILLAT, M. & FRANK, R. M. (1988). Monoclonal antibodies against *Streptococcus mutans* 74 kDa cell wall saliva receptor (74 kDa SR): correlation between antigenic structure and saliva binding sites. *FEMS Microbiology Letters* **50**, 211-215.
- DOUGLAS, C. W. I. & RUSSELL, R. R. B. (1984). Effect of specific antisera upon *Streptococcus mutans* adherence to saliva-coated hydroxylapatite. *FEMS Microbiology Letters* **25**, 211-214.
- FORESTER, H., HUNTER, N. & KNOX, K. W. (1983). Characteristics of a high molecular weight extracellular protein of *Streptococcus mutans*. *Journal of General Microbiology* **129**, 2779-2788.
- GIBBONS, R. J. & QURESHI, J. V. (1979). Inhibition of adsorption of *Streptococcus mutans* strains to saliva-treated hydroxyapatite by galactose and certain amines. *Infection and Immunity* **26**, 1214-1217.
- HUGHES, M., MACHARDY, S. M., SHEPPARD, A. J. & WOODS, N. C. (1980). Evidence for an immunological relationship between *Streptococcus mutans* and human cardiac tissue. *Infection and Immunity* **27**, 576-588.
- LOESHE, W. J. (1986). Role of *Streptococcus mutans* in human dental decay. *Microbiological Reviews* **50**, 353-380.
- OKAHASHI, N., SASAKAWA, C., YOSHIKAWA, M., HAMADA, S. & KOGA, T. (1989). Cloning of a surface protein gene from serotype *c* *Streptococcus mutans*. *Molecular Biology* **3**, 221-228.
- ROBINSON, E. N., MCGEE, Z. A., KAPLAN, J., HAMMOND, M. E., LARSON, J. K., BUCHANAN, T. M. & SCHOOLNIK, G. K. (1984). Ultrastructural localization of specific gonococcal macromolecules with antibody-gold sphere immunological probes. *Infection and Immunity* **46**, 361-366.
- RUSSELL, R. R. B. (1979). Wall-associated protein antigens of *Streptococcus mutans*. *Journal of General Microbiology* **114**, 109-115.
- RUSSELL, M. W. & LEHNER, T. (1978). Characterisation of antigens extracted from cells and culture fluids of *Streptococcus mutans* serotype *c*. *Archives of Oral Biology* **23**, 7-15.
- RUSSELL, M. W. & MESTECKY, J. (1986). Potential for immunological intervention against dental caries. *Journal de Biologie Buccale* **14**, 159-175.
- RUSSELL, M. W., BERGMEIER, L. A., ZANDERS, E. D. & LEHNER, T. (1980). Protein antigens of *Streptococcus mutans*: purification and properties of a double antigen and its protease-resistant component. *Infection and Immunity* **28**, 486-493.
- SCHÖLLER, M., FARGEAS, C., PINI, A. & KLEIN, J. P. (1988). Monoclonal antibodies to *Streptococcus mutans* serotype *f* polysaccharide cross-react with the "mutans group" of oral streptococci. *FEMS Microbiology Letters* **51**, 125-128.
- WACHSMANN, D., KLEIN, J. P., SCHÖLLER, M. & FRANK, R. M. (1985). Local and systemic immune response to orally administered liposome associated soluble *Streptococcus mutans* cell wall antigens. *Immunology* **54**, 189-194.