Glycobiology vol. 5 no.1 pp. 97–104, 1995

A 23 kDa membrane glycoprotein bearing NeuNAcα2-3Galβ1-3GalNAc O-linked carbohydrate chains acts as a receptor for *Streptococcus sanguis* OMZ 9 on human buccal epithelial cells

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Streptococcus sanguis **colonizes several human oral surfaces, including both hard and soft tissues. Large salivary mucin-like glycoproteins bearing sialic acid residues are known to bind various *S.sanguis* strains. However, the molecular basis for the adhesion of *S.sanguis* to human buccal epithelial cells (HBEC) has not been established. The present study shows that *S.sanguis* OMZ 9 binds to exfoliated HBEC in a sialic acid-sensitive manner. The desialylation of such cells invariably abolishes adhesion of *S.sanguis* OMZ 9 to the cell surface. A soluble glycopeptide bearing short sialylated O-linked carbohydrate chains behaves as a potent inhibitor of the attachment of *S.sanguis* OMZ 9 to exfoliated HBEC. The resialylation of desialylated HBEC with CMP-sialic acid and Galβ1,3GalNAc α2,3-sialyltransferase specific for *O*-glycans restores the receptor function for *S.sanguis* OMZ 9, whereas a similar cell resialylation with the Galβ1,4GlcNAc α2,6-sialyltransferase specific for *N*-glycans is without effect. Finally, the same resialylation reaction carried out with CMP-9-fluoresceinyl-sialic acid as a substrate yields exfoliated HBEC bearing fluorescence on a single 23 kDa protein, when using the α2,3-sialyltransferase as the catalyst. The latter finding demonstrates that this 23 kDa cell surface glycoprotein bears NeuNAcα2-3Galβ1-3GalNAc O-linked sugar chains, a carbohydrate sequence which is recognized by *S.sanguis* OMZ 9 on exfoliated HBEC. In similar experiments carried out with a buccal carcinoma cell line termed SqCC/Y1, *S.sanguis* OMZ 9 did not attach in great numbers to such cultured cells, and these cells were shown to not express membrane glycoprotein bearing α2,3-sialylated O-linked carbohydrate chains.**

Key words: bacterial adhesion/buccal mucosa/receptor/sialyltransferase/*Streptococcus sanguis*

Introduction

Essential prerequisites for bacteria to become permanent members of the oral microbial ecology are sorption and adherence to oral surfaces (Christensen *et al.*, 1985). Oral bacteria exhibit specific tropisms towards the various types of biological surfaces within the mouth (enamel, epithelium, bacteria themselves) (Gibbons, 1989). In this regard, it has recently become apparent that among the specific molecular recognition mechanisms involved, many are based on lectin–carbohydrate interactions (Mergenhagen *et al.*, 1987). Compared with the multitude of specificities already described for microbial lectins (Ofek and Sharon, 1990), carbohydrate-binding proteins of oral bacteria found so far express specificity for a limited number of complex oligosaccharides. Thus, most oral *Actinomyces* can bind lactose and β-galactosides (Mergenhagen *et al.*, 1987), whereas various strains of *Streptococcus sanguis* specifically bind sialic acid-containing structures (Murray *et al.*, 1982; Demuth *et al.*, 1990). *Streptococcus sanguis* colonizes several human oral surfaces, including both hard and soft tissues (Gibbons, 1989). The success of tooth colonization by *S.sanguis* is supposed to depend upon an equilibrium between adherence of the microorganism to the saliva-coated tooth surface and saliva-dependent bacterial aggregation, the latter process being understood as a host defence mechanism for preventing mouth colonization. Terminal sialic acid residues on salivary glycoproteins are known to play a major role in modulating saliva-induced aggregations of *S.sanguis* (McBride and Gisslow, 1977; Levine *et al.*, 1978). Moreover, a lectin on *S.sanguis* G9B cell surface was seen to specifically interact with sialylated sugar chains of human salivary glycoproteins (Murray *et al.*, 1982; Bergey *et al.*, 1986). A large acidic mucin-like salivary glycoprotein has also been shown to interact through a calcium-dependent binding with a specific bacterial receptor on *S.sanguis* M5, using sialic acid as the primary ligand (Demuth *et al.*, 1990). Besides this clear involvement of sialylated glycoproteins in such saliva-induced bacterial aggregations, the role of sialylated glycoproteins as receptors for *S.sanguis* adhesion to saliva-coated hydroxyapatite has not been clearly established (Liljemark *et al.*, 1989). Morris and McBride (1984) have proposed to distinguish between a pH-sensitive and a sialidase-sensitive binding site. On the other hand, Cowan *et al.* (1987a) have allotted only a modest role to sialic acids in the initial sorbtion of *S.sanguis* 10556 to saliva-coated hydroxyapatite, these residues appearing responsible for the transition to high-affinity binding sites however.

By contrast with the numerous studies devoted to sialylated carbohydrates as ligands mediating saliva-dependent *S.sanguis* aggregations, or addressing questions regarding their precise role in *S.sanguis* adhesion to hard surfaces, studies focused on sialylated cell surface glycoconjugates as possible receptors for the adhesion of *S.sanguis* to human oral epithelium have not been reported. *Streptococcus sanguis* OMZ 9 has been seen to bind to many types of surfaces, including polystyrene, saliva-coated hydroxyapatite, guinea pig erythrocytes and human buccal epithelial cells (HBEC) (Neeser *et al.*, 1988, 1994). The aim of the present work was to determine the structure of the receptor for *S.sanguis* OMZ 9 on HBEC. For this purpose, measurement of bacterial adhesion to normal exfoliated HBEC

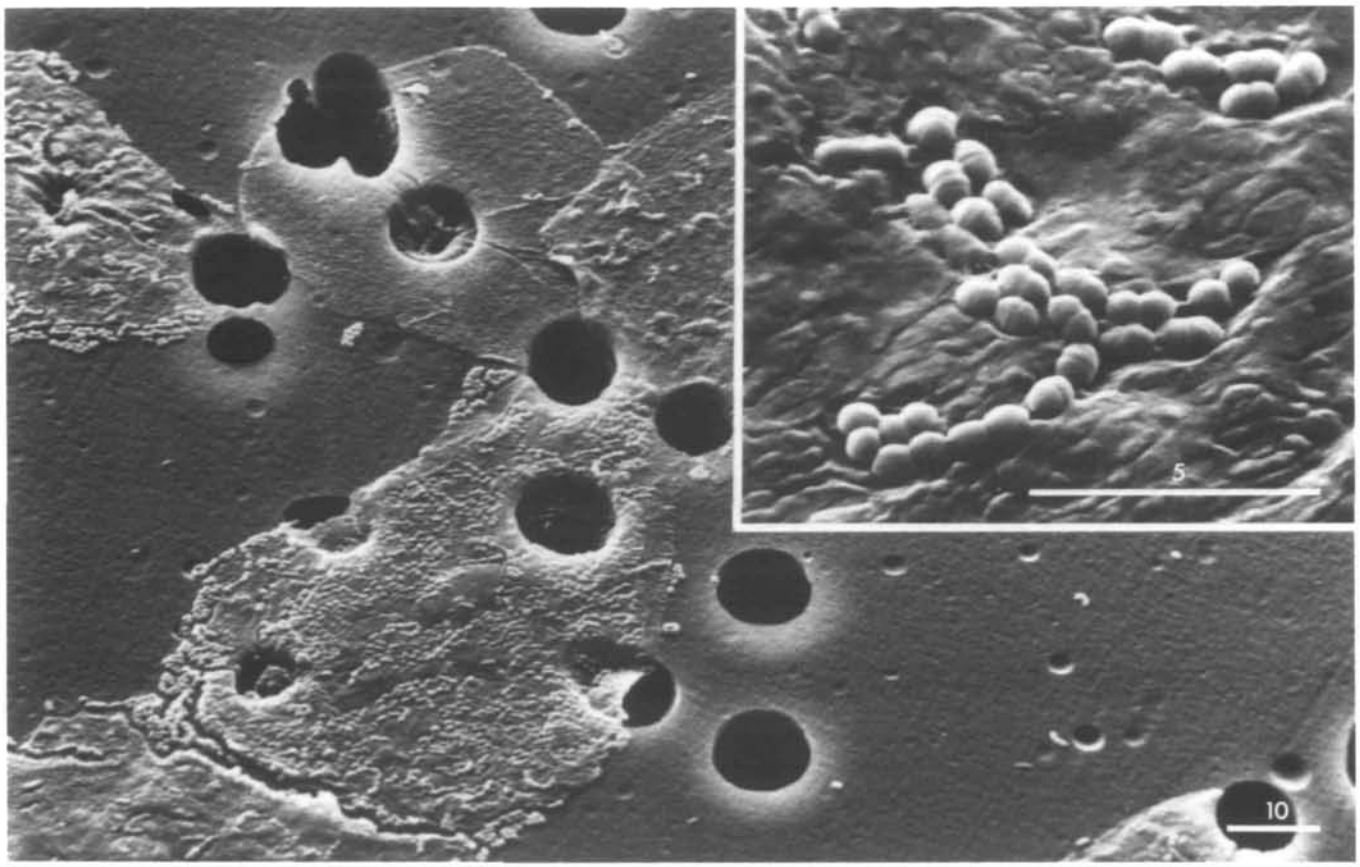

Fig. 1. Scanning electron micrographs of *S.sanguis* OMZ 9 binding to HBEC held by polycarbonate filters. Bars in μm. Magnifications are 1200× (main panel) and 7600× (inset panel).

was used as the main experimental assay. The effect of sialidase treatment of the cells on *S.sanguis* OMZ 9 adhesion was evaluated, as well as the effect of a competition with a soluble glycopeptide bearing sialylated sugar chains of known structures. Purified sialyltransferases were used to produce cell surface sialyloligosaccharides of defined sequences (Rogers and Paulson, 1983), and the same enzymes were also used in conjunction with CMP-9-fluoresceinyl sialic acid to bring fluorescence on the molecule(s) acting as receptor(s). For comparative purposes, similar experiments were performed with cultured HBEC obtained from a squamous cell carcinoma termed SqCC/Y1 (Reiss *et al.*, 1985). The successful restoration of a receptor function for *S.sanguis* OMZ 9 on desialylated exfoliated HBEC by using a pure sialyltransferase correlated with the fluorescent labelling of a single cell surface glycoprotein. As a result, the precise carbohydrate sequence and the cell surface glycoprotein bearing such sugar chains were jointly identified, leading to the complete characterization of the receptor for *S.sanguis* OMZ 9 on normal exfoliated HBEC.

Results

Adhesion of S.sanguis OMZ 9 to exfoliated HBEC

An assay previously established for measuring the adhesion of pathogenic yeast to HBEC (Brassart *et al.*, 1991) was found to be useful for the present purpose. Negligible background values were obtained in the determination of microbial adhesion, since the presence of bovine serum albumin (BSA) in the incubation buffer totally prevented bacterial adhesion to the polycarbonate filters. Preliminary experiments indicated that incubation of 50×10^3 HBEC with increasing concentrations of a ^{14}C-labelled bacterial suspension led to an increase in the ratio of bacteria attaching to the cells, until saturation was reached (final bacterial OD > 1). Scanning electron micrographs showed bacterial binding to HBEC held by the filter (Figure 1). This result illustrates the usefulness of the assay conditions at bacterial OD = 0.5 for determining specific bacterial binding to exfoliated cells, whereas adhesion to the filter was virtually absent (Figure 1). Based on the specific radioactivity associated with the bacterial populations incubated in this assay, an estimate of the extent of *S.sanguis* OMZ 9 attachment to HBEC can be calculated to a mean value of 300 bacteria/buccal cell.

To control both the degree of sialylation of exfoliated HBEC and the effect of the sialidase treatment used for preparing the desialylated cells, the amount of *N*-acetylneuraminic acid (NeuNAc) residues hydrolysed by this enzyme was quantitated. Exfoliated HBEC released ~40 ng NeuNAc/10^4 cells, a value which is in good agreement with previously reported determinations (Davis and Gibbons, 1990). The effect of HBEC desialylation on the extent of bacterial adhesion was then investigated. The adhesion of *S.sanguis* OMZ 9 to HBEC was seen to be strictly dependent on sialic acid-bearing receptors, since desialylation of HBEC prior to the adhesion experiment invariably led to the total prevention of bacterial attachment (As-Cells; Figure 2). Experiments were also carried out to examine whether sialic acid or sialylated soluble complex

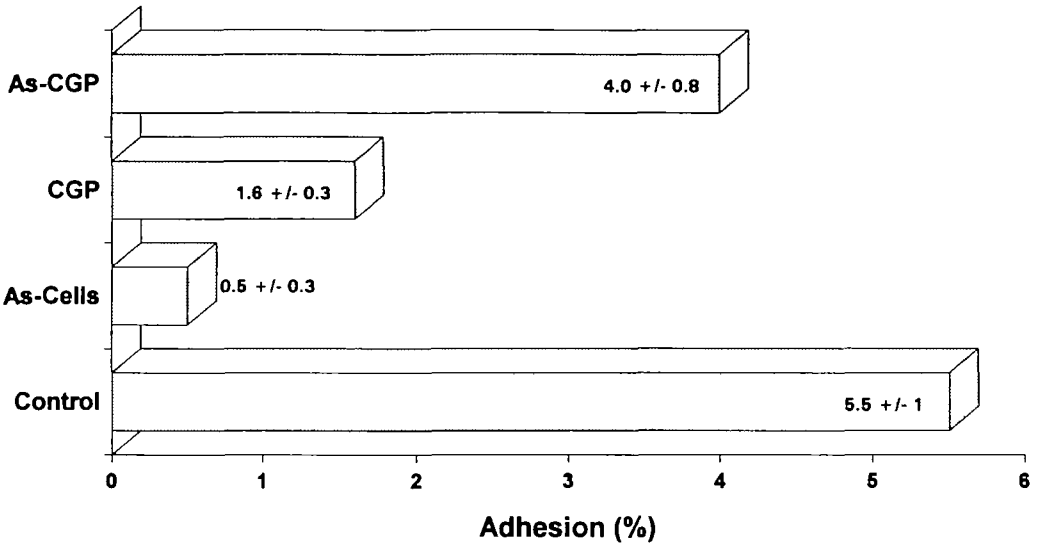

Fig. 2. Adhesion of ^{14}C-labelled *S.sanguis* OMZ 9 to HBEC. The effects of either cell desialylation (As-Cells) or 10 mg/ml of CGP and As-CGP are shown. They are expressed as the proportion of the total radioactivity in suspension remaining with the adhering microorganisms attached to HBEC after washing. The data represent mean values of three experiments with their SD.

a) NeuNAcα(2→3)Galβ(1→3)GalNAc

b) Galβ(1→3)GalNAc
6
NeuNAcα2

c) NeuNAcα(2→3)Galβ(1→3)GalNAc
6
NeuNAcα2

Fig. 3. Structures of the three main carbohydrate chains of bovine CGP. (NeuNAc = *N*-acetylneuraminic acid; Gal = galactose; GalNAc = *N*-acetylgalactosamine). From Van Halbeek *et al.* (1980).

carbohydrates inhibited the binding of *S.sanguis* OMZ 9 to HBEC. At a concentration of 10 mg/ml, the caseinoglycomacropeptide (CGP)—a glycopeptide from bovine whey which bears the short sialylated O-linked carbohydrate chains shown in Figure 3—behaved as an excellent competitive adhesion inhibitor. By contrast, its desialylated analogue (As-CGP) was much less effective (Figure 2).

For comparative purposes, adhesion of *S.sanguis* OMZ 9 to cultured monolayers of HBEC obtained from a squamous carcinoma was also assayed. It was found that *S.sanguis* OMZ 9 failed to attach in great numbers to these SqCC/Y1 cell layers. Indeed, the average score of microbial attachment to such cultured cells ranged around 40 bacteria/buccal cell (results not shown).

Host cell receptor specificity for S.sanguis OMZ 9

For defining the terminal oligosaccharide structure recognized by the streptococcal adhesion factor on HBEC, commercially available purified mammalian sialyltransferases (ST) were used. For this purpose, aliquots of asialo-HBEC were reacted with CMP-NeuNAc and one of the two following sialyltransferases: the Galβ1,3GalNAc α2,3-ST specific for sugar chains O-linked to glycoproteins or located on glycolipids, and the Galβ1,4GlcNAc α2,6-ST specific for the sequences found on oligosaccharides N-linked to glycoproteins. Preliminary assays indicated that exfoliated HBEC were very resistant to structural breakdown, even after prolonged incubations in diluted solutions containing Triton X-100 and glycerol. Consequently, these components were not removed from the commercial enzyme solutions. The results of the resialylation experiments are shown in Figure 4. Clearly the reaction with the α2,3-ST yielded HBEC with a restored receptor function for *S.sanguis* OMZ 9, whereas the resialylation of asialo-cells with the α2,6-ST specific for *N*-glycans had no effect (Figure 4).

By using a fluorescent neuraminic acid derivative for the resialylation reactions, we also investigated the feasibility of visualizing such resialylated buccal cells bearing different oligosaccharide sequences. With the commercial CMP-9-fluoresceinyl-sialic acid donor, we found that asialo-, exfoliated HBEC were readily resialylated with both the α2,3-ST (Figure 5a) and the α2,6-ST (Figure 5b). This result indicates that such desialylated cells expressed different sugar chains at their surface, acting as acceptors either for one or for the other ST. By contrast, the same experiment performed with cultured tumorous buccal cells (SqCC/Y1) yielded fluorescent resialylated cells only after reaction with the α2,6-ST specific for *N*-glycans (Figure 5f), but not after reaction with the α2,3-ST enzyme (Figure 5e). Finally, fluorescent cell samples freshly resialylated with the different sialyltransferases and stemming from both exfoliated and cultured cells were dissolved in SDS and subjected to SDS–PAGE analysis. Interestingly, from exfoliated HBEC, resialylated glycoproteins of low mol. wt (20–30 kDa) were identified as bearing most of the fluorescence attached to the cell surface after reaction with either one or the other ST (Figure 6, lanes 2 and 3). In addition, exfoliated HBEC sequentially desialylated and resialylated with the α2,3-ST yielded a single 23 kDa fluorescent

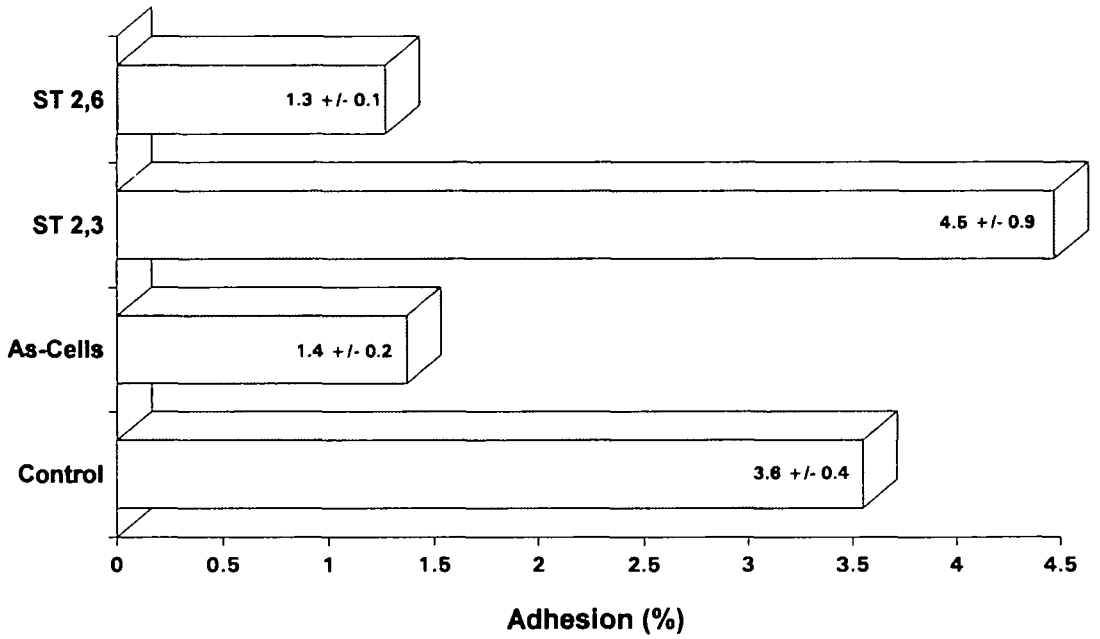

Fig. 4. Adhesion of ^{14}C-labelled *S.sanguis* OMZ 9 to native (Control), asialo (As-Cells) and resialylated (ST 2,3 and ST 2,6) HBEC. The data are expressed as in Figure 2.

glycoprotein (Figure 6, lane 2). By contrast, SDS–PAGE analysis of fluorescence-bearing resialylated SqCC/Y1 cultured cells showed only fluorescent glycoproteins of higher mol. wt (50–100 kDa), and only after reaction with the α2,6-ST (Figure 6, lanes 5 and 6).

Discussion

The ability of several *S.sanguis* strains to specifically bind to sialylated carbohydrate chains of salivary glycoproteins has already been demonstrated and discussed (Mc Bride and Gisslow, 1977; Bergey *et al.*, 1986; Demuth *et al.*, 1990). However, it was never clearly established that the activity of such a streptococcal lectin could also mediate bacterial adhesion to HBEC. *Streptococcus sanguis* OMZ 9 has been shown to bind to saliva-coated hydroxyapatite beads by a mechanism sensitive to acidic polypeptides, including bovine milk casein, CGP, its desialylated analogue (As-CGP) and caseinophosphopeptides (Neeser *et al.*, 1994). It was therefore apparent that the adhesion of *S.sanguis* OMZ 9 to hard surfaces involved mainly ionic interactions, as already observed for other *S.sanguis* strains and other oral streptococci (Doyle *et al.*, 1982; Cowan *et al.*, 1987b; Satou *et al.*, 1988; Koga *et al.*, 1990). By contrast, we show here that the adhesion of *S.sanguis* OMZ 9 to HBEC is strictly dependent on the presence of sialic acid residues on the cell surface, and that such an adhesion is potently inhibited by CGP but not by As-CGP. Thus, sialic acids seem to be the key configuration for the specific attachment of *S.sanguis* OMZ 9 to the buccal mucosal epithelium.

For analysing the sialyloligosaccharide receptor specificity involved in the binding of *S.sanguis* OMZ 9 to HBEC, we used the methodology previously developed by James Paulson and co-workers in their studies of cell surface receptors for animal viruses (Paulson, 1985). We took advantage of the commercial availability of two purified sialyltransferases: the Galβ1, 3GalNAc α2,3-ST and the Galβ1,4GlcNAc α2,6-ST. It is clearly established that both these enzymes exhibit a strict specificity for their respective terminal oligosaccharide structures recognized as acceptors. The majority of the studies involving such enzymatic restorations of cell surface sialyloligosaccharides with defined sequences have been performed from desialylated red blood cells (Paulson, 1985). However, similar treatments of tissue culture cells for investigating receptor determinants in viral infections have also been reported (Markwell and Paulson, 1980; Fried *et al.*, 1981). In the present study, the α2,3-ST clearly restored the receptor function for *S.sanguis* OMZ 9, previously destroyed by the sialidase treatment of HBEC. The use of a fluorescent neuraminic acid derivative for performing similar resialylation reactions from desialylated HBEC finally led to the identification of the single 23 kDa fluorescent glycoprotein acting as an acceptor for the α2,3-ST. Thus, this HBEC membrane glycoprotein bears NeuNAcα2-3Galβ1-3GalNAcα O-linked sugar chains, the carbohydrate sequence specifically restored by the α2,3-ST, and specifically recognized by *S.sanguis* OMZ 9 on HBEC.

It should be noted that SqCC/Y1 tumorous buccal epithelial cells showed minimal microbial attachment, as compared to the exfoliated cells from normal mucosa (HBEC). Tumorigenesis is generally coupled to multiple genetic changes, resulting in altered phenotypic properties of tumorous cells as compared to normal cells. Moreover, aberrations in the cell surface carbohydrate structures have now been established as a universal characteristic of malignant transformation of cells, and cancer has been referred to as a molecular disease of the cell membrane glycoconjugates (Hakomori, 1989; Bhavanandan, 1991). In this regard, glycosylation of annexins I and II by SqCC/Y1 cells has already been examined (Goulet *et al.*, 1992). Also, the SqCC/ Y1 cell line has been seen to exhibit significant changes in glycolipid composition, when differentiated in the absence of serum (Tatsumura *et al.*, 1988). Finally, the ability of these cells to synthesize glycosaminoglycans differed markedly from

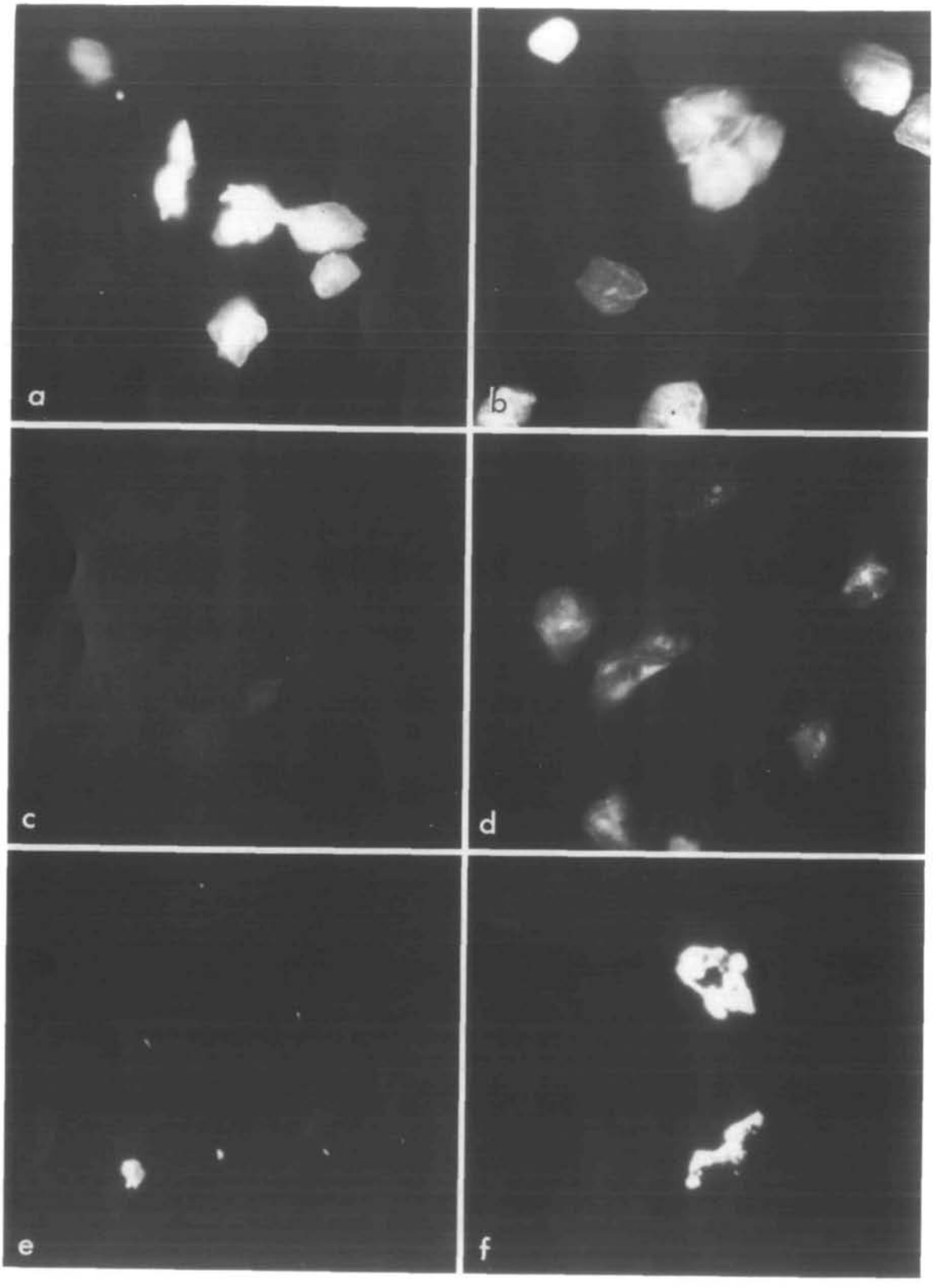

Fig. 5. Fluorescence micrographs of exfoliated HBEC (**a–d**) and cultured SqCC/Y1 cells (**e, f**). The desialylated cells were treated with the CMP-9-fluoresceinyl-sialic acid donor, in conjunction with either α2,3-ST(a, e) or α2,6-ST (b, f). Controls were obtained by incubating exfoliated HBEC without the fluorescent reagent (c) or without sialyltransferase (d).

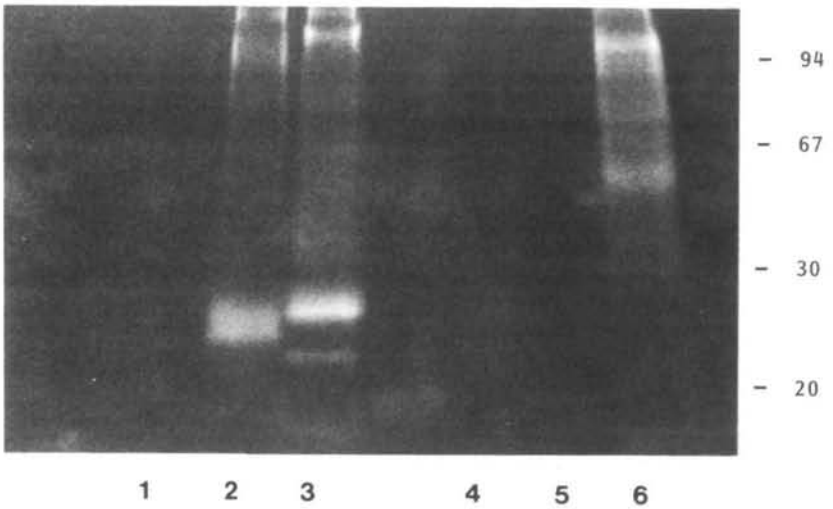

Fig. 6. SDS–PAGE analysis of total cell extracts from exfoliated HBEC (lanes 1–3) and cultured SqCC/Y1 cells (lanes 4–6). The desialylated cells were treated with the CMP-9-fluoresceinyl-sialic acid donor, in conjunction with either α2,3-ST (lanes 2 and 5) or α2,6-ST (lanes 3 and 6). Controls were obtained by incubating the fluorescent reagent without sialyltransferase (lanes 1 and 4).

that of normal keratinocytes (Reiss *et al.*, 1986). Thus, changes in cell surface glycosylation may affect the cell-to-cell interactions that participate in regulating growth and differentiation of the epithelium, also the ability of such pheno plastic and neoplastic cells to be identified *in vivo* by immunologically based recognitions, and finally the expression of particular determinants which can act as specific receptors for bacteria.

Another explanation may be envisaged, to understand the difference observed between exfoliated HBEC and cultured tumorous SqCC/Y1 cells, regarding the expression of receptors for *S.sanguis* OMZ 9. The labellings yielded by the treatment of both these cell types with the specific sialyltransferases and a fluorescent neuraminic acid derivative revealed very different membrane glycoprotein patterns. It has been reported that in the oral cavity, certain salivary components may interact with the epithelial cells to be finally cross-linked to the cell cytoskeleton by epithelial transglutaminases (Bradway *et al.*, 1989). In such a case, the surface of HBEC obtained by exfoliation would be very different from that of (normal and tumorous) cultured cells. Interestingly, the determination of type I transglutaminase in differentiating normal and neoplastic (SqCC/Y1) human keratinocytes has recently retained attention (Moore *et al.*, 1993). Thus, further studies are required, involving the growth of various normal and tumorous cell types from the oral mucosa, cultured in the presence and in the absence of saliva, to understand the origin of the 23 kDa cell surface receptor for *S.sanguis* OMZ 9.

Materials and methods

All reagents were of the highest quality available from Sigma Chemical Co. (St Louis, MO) or Fluka (Buchs, Switzerland), unless otherwise stated.

Caseinoglycopeptide derivatives

Bovine CGP was isolated from a whey protein concentrate (Danmark protein, Videback, Denmark) by protein precipitation with a trichloroacetic acid solution (12% final, w/v). CGP desialylation was performed by mild acid hydrolysis (H_2SO_4, 25 mM, 2 h at 80°C). Both these CGP derivatives were purified on a G-50 Sephadex column (Pharmacia, Uppsala, Sweden), using a 0.1 M acetic acid buffer.

Bacterial growth conditions and radiolabelling

Streptococcus sanguis OMZ 9 was cultured in fluid universal medium (FUM), a previously defined medium (Gmür and Guggenheim, 1983). The bacteria were pre-cultured for 7 h at 37°C and then grown for 14 h before harvesting. The bacteria were metabolically labelled by the addition of [^{14}C]acetic acid (94 mCi/mmol; 100 μCi/10 ml tube; CEA, Gif-sur-Yvette, France). Specific radioactivity (c.p.m./10^8 cells) was between 300 000 and 350 000.

Bacterial adhesion to HBEC and inhibition studies

HBEC were freshly collected by gently rubbing the buccal mucosa of several donors in the authors' laboratory. The cells were pooled, washed as previously described (Brassart *et al.*, 1991) and finally suspended in a Hank's balanced salt solution containing 0.2% BSA, 0.02% each Ca^{2+}, Mg^{2+} (Hank's/BSA), in a concentration of 10^5 cells/ml. After harvesting, ^{14}C-labelled bacteria were washed three times and suspended (OD = 1.0, λ = 650 nm) in Hank's/BSA solution, with or without an inhibitor to be tested (10 mg/ml). Then, 0.5 ml aliquots of the HBEC suspensions were mixed with 0.5 ml of the bacterial suspensions in plastic tubes which were rotated at 80 r.p.m. for 1 h at 25°C. These mixtures were finally filtered as previously described (Brassart *et al.*, 1991). Bacterial adhesion was determined by radiometric counting. Desialylated HBEC were obtained by incubating 1.5×10^5 washed cells (1 h at 37°C) in 0.2 ml of 150 mM NaCl containing 10 mM $CaCl_2$ and 0.2 U of sialidase from *Vibrio cholerae* (Behring, Marburg, FRG). Then the cells were collected, washed three times with Hank's/BSA and used in adhesion studies as described above. The sialic acid residues released from HBEC membranes by this enzymatic hydrolysis were quantitatively determined by colorimetry (Jourdian *et al.*, 1971).

Scanning electron microscopy

For ultrastructural studies, bacteria attached to HBEC were fixed on the filter for 30 min at room temperature with 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4). After washing with this buffer, cells were dehydrated in a graded series (50–100%) of ethanol. Wet cells were dried after substitution with liquid CO_2 in a critical-point dryer (Polaron Equipment Ltd, Watford, UK) and coated with gold (SEM coating unit E5100; Polaron). Samples were viewed using a Philips 505 SEM microscope.

Culture conditions for SqCC/Y1 cells and measurement of bacterial adhesion

SqCC/Y1 cells were kindly provided by Prof. A.C.Sartorelli, New Haven, CT. The cells were routinely grown in a serum-containing medium termed SqCC/Y1 medium, as previously described (Sundqvist *et al.*, 1991). Prior to an assay for bacterial adhesion, the cells were cultured in 6-well plates in SqCC/Y1 medium until the cells covered the surface area. To avoid serum-dependent interactions in the assay, the cells were then incubated for 48 h in a serum-free medium termed EMA, developed for both normal and tumorous

buccal epithelial cell cultures (Sundqvist *et al.*, 1991). Bacterial adhesion was measured by using metabolically ^{14}C-labelled bacteria, as previously described (Neeser *et al.*, 1989). The cell layers were then washed with Hank's/BSA before they were exposed to the bacterial suspensions (OD = 0.5 in 1 ml of Hank's/BSA) for 1 h at 37°C. Subsequently, unbound bacteria were removed by multiple washing, and the buccal cells were dissolved with the adhered organisms in a NAOH solution (0.2 N). Bacterial adhesion was quantitated using liquid scintillation counting, as previously described (Neeser *et al.*, 1989).

Resialylation of desialylated HBEC for adhesion studies

Freshly collected HBEC were pooled and pre-treated as described above. Exfoliated cells (1.2×10^6) were subjected to desialylation by incubation (1 h at 37°C) in 2 ml of 150 mM NaCl containing 10 mM $CaCl_2$ and 2 U of sialidase from *V.cholerae*. These cells were then washed twice in a resialylation buffer [25 mM MOPS (pH 6.25) containing 75 mM NaCl, 100 mM glucose, 10 mg/ml BSA, and supplemented with 115 U/ml penicillin, 115 U/ml streptomycin, 1.3 μg/ml amphotericin B and 150 μg/ml gentamicin]. Such desialylated HBEC were divided into three samples. Resialylation reactions were performed by using either Galβ1,3GalNAc α2,3-ST from porcine liver or Galβ1,4GlcNAc α2,6-ST from rat liver, with CMP-NeuNAc in both cases; all these reagents being from Boehringer Mannheim, FRG. The procedure was adapted from that described by Rogers and Paulson (1983), except that the enzyme solutions were used as received from the supplier, without removing Triton X-100 and glycerol. Typically, 4×10^5 desialylated HBEC were suspended in the resialylation buffer described above (40 μl) and mixed with CMP-NeuNAc (500 μg in 10 μl of resialylation buffer). Then, either 5 mU of the α2,3-ST solution or 25 mU of the α2,6-ST solution were added to the cell suspensions, which were incubated for 24 h at 37°C. A sample of 4×10^5 native and intact exfoliated HBEC was incubated in 75 μl of resialylation buffer supplemented with Triton X-100 (0.22%) and glycerol (17%), to be used as a control. After completion of the enzymatic reactions, the cells were collected, washed three times with Hank's/ BSA and used in adhesion studies as above.

Resialylation of desialylated HBEC and SqCC/Y1 cells with fluoresceinyl-NeuNAc residues

Experiments were performed either with freshly collected exfoliated HBEC (1.6×10^6 cells), or with SqCC/Y1 cells (4×10^6) obtained from cell layers cultured as described above, and further treated with a dispase II solution (Boehringer Mannheim, FRG) to gently detach the cells from the plastic. All cells were washed, desialylated, and again washed prior to resialylation, as already described. Samples of each cell type (exfoliated HBEC and SqCC/Y1) were subjected to the different resialylation reactions studied here (with the α2,3-ST and the α2,6-ST, respectively), but in two steps: a first incubation period (4 h at 37°C) was carried out by using CMP-9-fluoresceinyl-NeuNAc (Boehringer Mannheim, FRG, 5 μg in 10 μl of resialylation buffer) as a glycosyl-donor nucleotide; after that time, a solution of CMP-NeuNAc (625 μg in 15 μl of resialylation buffer) was added to these samples, which were further incubated for 20 h at 37°C. Samples of desialylated cells were used as controls after an incubation period (4 h at 37°C) with the fluorescent probe, but in the absence of any sialyltransferase. All cell samples were washed three times with a phosphate-buffered saline solution (pH 7.2) before being examined in a fluorescence microscope equipped with an epi-illuminator.

Gel electrophoresis

Fluorescent cell samples obtained as above were also suspended in 50 μl portions of a solution of Triton X-100 (1%) and thoroughly shaken, prior to being mixed and heated with the incubation buffer for gel electrophoresis. SDS–PAGE (gradient from 10 to 20% acrylamide) was performed by the method of Laemmli (1970). The gel was finally examined under a UV lamp. Molecular weight markers were from Pharmacia (Uppsala, Sweden).

Acknowledgements

We thank K.Sundqvist and A.Pfeifer for help and advice with culturing the buccal epithelial cells, and E.Kolodziejczyk for expert assistance in fluorescence microscopy.

Abbreviations

As-CGP, asialo-caseinoglycomacropeptide; BSA, bovine serum albumin; CGP, caseinoglycomacropeptide; FUM, fluid universal medium; HBEC, human buccal epithelial cell; MOPS, morpholinopropanesulphonic acid; NeuNAc, *N*-acetylneuraminic acid; OD, optical density; ST, sialyltransferase.

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Received on June 29, 1994; accepted on September 29, 1994