Molecules of *Streptococcus gordonii* that bind to *Porphyromonas gingivalis*

Richard J. Lamont,¹ Suchool Gil,¹ Donald R. Demuth,² Daniel Malamud² and Burton Rosan³

Author for correspondence: R. J. Lamont. Tel: +1 206 543 5477. Fax: +1 206 685 3162.
e-mail (Internet) lamon@u.washington.edu

¹ Department of Oral Biology, University of Washington, Seattle WA 98195, USA

^{2,3} Department of Biochemistry² and Department of Microbiology³, University of Pennsylvania, PA 19104, USA

Interbacterial binding is considered an important colonization mechanism for many of the organisms that inhabit dental plaque. *Porphyromonas gingivalis*, a periodontal pathogen, can adhere to species that comprise early plaque, such as *Streptococcus gordonii*. In this study, the molecules of *S. gordonii* G9B that mediate binding to *P. gingivalis* were investigated. Biotinylated surface molecules of *S. gordonii* were extracted and mixed with *P. gingivalis* cells. Interactive streptococcal components were identified by SDS-PAGE of the *P. gingivalis* cells followed by electroblotting, and visualization of the adsorbed streptococcal molecules with streptavidin–alkaline phosphatase. *S. gordonii* molecules of 45 kDa and a doublet of 62/60 kDa were observed to bind to *P. gingivalis*. Polyclonal antibodies raised to the 62/60 kDa proteins inhibited the binding interaction. These antibodies demonstrated an antigenic relationship between the 62/60 kDa molecules and the 45 kDa protein. Both molecules were also antigenically related to, and may be breakdown products of, a larger molecule of 170 kDa which is antigenically related to the P1 antigen of *S. mutans*. Cloning and expression in *Enterococcus faecalis* of the gene for the P1-like molecule from *S. gordonii* M5 resulted in a phenotype that expressed the 62/60 kDa and 45 kDa antigens and was capable of binding to *P. gingivalis*. These results suggest that a P1-like molecule in *S. gordonii* is involved in adherence to *P. gingivalis*. Processing of the P1-like molecule into smaller fragments of 62/60 kDa and 45 kDa may be required for binding activity.

Keywords: *Streptococcus gordonii*, *Porphyromonas gingivalis*, interbacterial binding adhesin, P1

INTRODUCTION

The development of dental plaque involves a succession of microbial species (Kolenbrander & London, 1993; Listgarten *et al.*, 1975; Theilade *et al.*, 1966). Early plaque, consisting mostly of Gram-positive facultative organisms such as streptococci and actinomyces, exists in commensal harmony with the host. Transformation into a potentially pathogenic entity requires the subsequent colonization of mainly Gram-negative anaerobic and facultative species. The early plaque colonizers, predominantly sanguis group streptococci and *Actinomyces viscosus*, can adhere to molecules of the salivary pellicle that coats the enamel surface (Fives-Taylor *et al.*, 1987; Ganeshkumar *et al.*, 1988; Gibbons & Hay, 1988; Lamont *et al.*, 1988b). The colonization of pathogenic organisms, however, is dependent, to some degree, on their ability to adhere to the preformed plaque (Kolenbrander & Andersen, 1986;

Lamont *et al.*, 1992; Schwarz *et al.*, 1987; Slots & Gibbons, 1978). This interbacterial binding phenomenon has been extensively studied and many of the species that comprise mature dental plaque are capable of binding to one another (Kolenbrander & Andersen, 1986; Kolenbrander & London, 1993).

Porphyromonas gingivalis, a Gram-negative anaerobic organism, is considered an important pathogen in many forms of severe adult periodontitis. This species can be recovered in high numbers from diseased periodontal pockets and possesses a number of virulence factors capable of destroying periodontal tissues (Slots & Listgarten, 1988; van Winkelhoff *et al.*, 1988). *P. gingivalis* is a late colonizer of plaque, and can adhere to many of the antecedent commensal plaque organisms, including *Streptococcus gordonii* (Lamont *et al.*, 1992; Stinson *et al.*, 1991). The molecular mechanisms of this binding in-

teraction are only partially understood. The fimbriae of *P. gingivalis* mediate binding to the streptococcus, although additional molecules may also be involved (Lamont *et al.*, 1993). Although a number of *S. gordonii* adhesins that mediate binding to salivary molecules and actinomyces have been described (Fives-Taylor *et al.*, 1987; Kolenbrander & Anderson, 1990; Lamont *et al.*, 1988a), the molecules responsible for binding to *P. gingivalis* remain to be determined. The goal of this investigation was to isolate surface molecules from *S. gordonii* and examine their ability to adhere to *P. gingivalis*.

METHODS

Bacteria and culture conditions. *P. gingivalis* strain 33277 and *S. gordonii* strains G9B and M5 are maintained as frozen stock cultures. *Enterococcus faecalis* strain S161EB-5 was generated by transformation of *E. faecalis* S161 with shuttle vector pAM401 (Wirth *et al.*, 1986) containing a 5.3 kb insert encoding the *S. gordonii* M5 SSP-5 antigen (Demuth *et al.*, 1989). *E. faecalis* strain S161-401 was generated by transformation of S161 with pAM401 that did not contain a streptococcal insert. *P. gingivalis* was cultured in Trypticase soy broth (BBL) supplemented with 1 g yeast extract (Difco) l⁻¹, 5 mg haemin l⁻¹ and 1 mg menadione l⁻¹. Streptococci and enterococci were grown in Trypticase peptone broth (BBL) supplemented with 5 g yeast extract l⁻¹ and 0.5% glucose. Culture was at 37 °C under anaerobic conditions (85% N₂, 10% H₂, 5% CO₂). Numbers of bacteria were determined in a Klett–Summerson photometer. To metabolically label bacteria, 10 µCi (370 kBq) [³H]thymidine (Amersham) ml⁻¹ were added to the media. Resulting specific activities ranged between 6 × 10⁻⁵ and 2 × 10⁻⁴ c.p.m. per cell.

Interbacterial binding assay. Adherence of *P. gingivalis* to streptococci and enterococci was determined by the nitrocellulose blot assay described previously (Lamont & Rosan, 1990). Briefly, cells of one partner of the binding pair were suspended in buffered KCl (5 mM KCl, 2 mM K₂HPO₄, 1 mM CaCl₂, pH 6.0) and 1 × 10⁸ bacteria deposited on nitrocellulose paper in a dot-blot apparatus. The blot was washed three times in KCl containing 0.1% Tween 20 (KCl–Tween). The adsorbed bacteria were incubated for 2 h at room temperature with radiolabelled cells of the other test species suspended in KCl–Tween. After washing to remove unbound organisms, the experimental areas of the nitrocellulose were excised and the amount of interbacterial binding quantified by scintillation spectroscopy.

Antibody inhibition of binding was investigated by incubating (1 h, 37 °C) the radiolabelled bacteria with antibody prior to assay. Bacteria were then collected by centrifugation (10000 g, 10 min), resuspended in KCl–Tween and adherence measured as described above.

Biotinylation of *S. gordonii* surface molecules. A 100 ml overnight culture of G9B was washed in KCl and suspended in 0.1 M NaHCO₃, pH 8.1. *N*-Hydroxysuccinimidobiotin (Sigma) was added to a final concentration of 1 mg ml⁻¹ of cells in suspension. After 3 h at room temperature the cells were recovered by centrifugation (10000 g, 10 min), washed twice and resuspended in KCl.

Preparation of surface molecules. Bacterial surface molecules were prepared from biotinylated and non-biotinylated organisms by the mild sonication procedure developed by Kolenbrander & Andersen (1990). Cells were sonicated in an ice bath

for three 30 s intervals with a Virsonic 475 ultrasonic disrupter emitting 45 W. The cells were allowed to cool for 1 min between ultrasonic treatments. Bacteria were removed by centrifugation (20000 g, 20 min) and the supernatant, containing surface molecules, was filtered (0.22 µm pore size) and stored at –20 °C.

Binding of biotinylated streptococcal molecules to *P. gingivalis*. Biotinylated, extracted G9B surface molecules (100 µg protein) were added to 10¹⁰ *P. gingivalis* cells and incubated for 2 h at room temperature. Controls of *P. gingivalis* cells with non-biotinylated G9B extract were included in all experiments. *P. gingivalis* cells with any attached streptococcal components were washed and recovered by centrifugation at 10000 g for 10 min. After the final wash, the bacteria were resuspended in 50 µl SDS-PAGE sample buffer and the bacterial suspension (10 µl) subjected to SDS-PAGE and blotted onto nitrocellulose paper as described previously (Lamont *et al.*, 1988a, b). After blocking the membrane with PBS containing 0.1% Tween 20 (PBS–Tween), biotinylated streptococcal molecules that bound to the *P. gingivalis* cells were visualized by developing the blot with streptavidin–alkaline phosphatase conjugate (Bio-Rad) and the chromogenic substrates 5-bromo-4-chloro-3-indolyl phosphate and nitroblue tetrazolium (Bio-Rad) according to the manufacturer's instructions.

Antisera and immunoblotting. Production of rabbit polyclonal antiserum to formalinized whole cells of G9B has been described previously (Rosan, 1973). Monospecific polyclonal antibodies to individual streptococcal molecules were prepared by excising the band of interest from Coomassie blue stained 10% (w/v, acrylamide) SDS-PAGE gels of G9B surface extract (50 µg protein). Inoculation of mice and preparation of antisera were performed by the Berkeley Antibody Co., Richmond, CA, USA. Monospecific polyclonal antibodies to the P1 antigen of *S. mutans* were provided by Dr H. F. Jenkinson, University of Otago, New Zealand, and Dr A. S. Bleiweis, University of Florida, FL, USA. Antigen–antibody interactions were visualized by immunoblotting, as described above. Bacterial surface extracts (5 µg protein) were separated by SDS-PAGE and electroblotted onto nitrocellulose paper. The membrane was blocked with PBS–Tween and incubated with antibody diluted in the same buffer for 1 h at room temperature. After washing, antigen–antibody binding was localized with alkaline-phosphatase-conjugated goat anti-mouse, or anti-rabbit, IgG (Bio-Rad) and developed with the chromogenic substrates 5-bromo-4-chloro-3-indolyl phosphate and nitroblue tetrazolium (Bio-Rad) according to the manufacturer's instructions.

Chemical analysis. Protein concentration was estimated by using the Bio-Rad reagent.

RESULTS

Binding of *S. gordonii* surface extract to *P. gingivalis*

Mild sonication removed a variety of components from *S. gordonii* (Fig. 1), many of which reacted with antiserum to whole G9B cells (Fig. 2). To determine which of these molecules bind to *P. gingivalis*, the biotinylated sonic extract was mixed with *P. gingivalis* cells which were then washed to remove unbound streptococcal molecules. Any interactive streptococcal molecules were identified by subjecting the *P. gingivalis* cells and associated streptococcal molecules to SDS-PAGE and electroblotting, followed by detection of the biotinylated streptococcal molecules with streptavidin. Fig. 1 shows that a doublet

Fig. 1. Blot showing binding of *S. gordonii* G9B molecules to *P. gingivalis* 33277. Lane 1, biotinylated G9B surface molecules (2 µg protein); lane 2, 33277 cells with adsorbed G9B molecules; lane 3, control of 33277 cells with non-biotinylated G9B molecules.

Fig. 2. Immunoblot of *S. gordonii* G9B surface extract (5 µg protein) probed with: lane 1, whole G9B antibodies (1:1000); lane 2, 62/60 antibodies (1:1000).

Fig. 3. Immunoblot of *S. gordonii* G9B surface extract (5 µg protein) probed with: lane 1, 62/60 antibodies (1:1000); lane 2, P1 antibodies (1:500).

of 62/60 kDa along with another molecule of 45 kDa bound to the *P. gingivalis* cells.

Antigenic relationship between adhesive streptococcal molecules

To investigate the relationship between the *S. gordonii* molecules that adhered to *P. gingivalis*, the 62/60 kDa doublet was excised from SDS-PAGE gels and injected into mice. The resulting antibodies were examined by immunoblotting with the G9B sonic extract. As shown in Fig. 2, the anti-62/60 antibodies reacted with the homologous antigen in addition to the 45 kDa antigen (which also adhered to *P. gingivalis*) and a molecule of 170 kDa. It would appear, therefore, that the 62/60 and 45 kDa antigens, that bind to *P. gingivalis*, are antigenically related to each other and to a larger molecule of 170 kDa. A possible explanation for this is that the 62/60 and 45 kDa molecules are proteolytic breakdown products of the 170 kDa protein, or that all are components or breakdown products of a larger molecule not seen by SDS-PAGE. When the extraction procedures were repeated in the presence of the proteolytic inhibitors PMSF, TLCK and benzamidine, identical results were obtained. Streptococci, however, possess a wide variety of proteases and complete inhibition of proteolytic activity is not practically attainable. These results, therefore, do not preclude a role for proteolytic enzymes in the generation of the 62/60 and 45 kDa molecules.

The size of the 170 kDa molecule indicated that it may be related to the P1 molecule identified as an adhesin in *S. mutans* and which has homology with the SSP-5 salivary

agglutinin binding molecule found in *S. gordonii* strain M-5. To investigate this possible relationship, antibodies to P1 were reacted with the G9B sonic extract. Fig. 3 shows that P1 antibodies react with the 170 kDa antigen as well as the 62/60 (which did not resolve as separate bands in this experiment) and 45 kDa molecules. The P1 molecule and the 170, 62/60 and 45 kDa molecules are, therefore, antigenically related.

Antibody inhibition of *S. gordonii*-*P. gingivalis* binding

To seek further evidence for the role of the 62/60 kDa and antigenically related molecules in mediating binding of *S. gordonii* to *P. gingivalis*, the ability of the 62/60 antibodies to inhibit this interaction was investigated. Antibodies raised to the 62/60 molecules inhibited binding of *S. gordonii* to *P. gingivalis* by a maximum of 64 % (Fig. 4). Normal mouse serum also showed some inhibition of adherence but this was much less than the inhibition caused by the 62/60 serum (Fig. 4).

Binding of recombinant SSP-5 protein to *P. gingivalis*

The antigenic relationship between the 62/60, 45 and 170 kDa molecules and P1 suggested that the former may represent a homologue of P1 in *S. gordonii* G9B. Therefore, the ability of SSP-5, a P1 homologue in *S. gordonii* M5, to bind to *P. gingivalis* was investigated. *E. faecalis* S161EB-5, a strain that contains the gene for SSP-5 and expresses the molecule on the cell surface, was able to bind to *P. gingivalis*, whereas *E. faecalis* S161-401, which does not

Fig. 4. Inhibition of *S. gordonii* G9B binding to *P. gingivalis* 33277 by 62/60 antibodies (■) or normal mouse serum control (□). Error bars represent standard errors of the means of three experiments.

Fig. 5. Immunoblot of surface extracts from *S. gordonii* M5 (lane 1) and *E. faecalis* S161EB-5 (lane 2) probed with 62/60 antibodies (1:1000).

contain a streptococcal insert, demonstrated little binding activity. [The mean no. ($\times 10^7$) of *P. gingivalis* 33277 bound (input cell no. 8×10^7) was 3.9 ± 0.4 for *S. gordonii* G9B, 2.5 ± 0.3 for *E. faecalis* S161EB-5 and 0.8 ± 0.1 for *E. faecalis* S161-401 (values are means $\pm$ SD, $n = 6$).] Thus, *S. gordonii* P1 related molecules are capable of mediating binding to *P. gingivalis*. An immunoblot of extracted surface molecules from *E. faecalis* S161EB-5 and *S. gordonii* M5 reacted with the 62/60 antibodies revealed that both strains produce the 62/60, 45 and 170 kDa molecules along with a higher molecular mass molecule of around 200 kDa (Fig. 5). This latter molecule is characteristic of the product of the gene for SSP-5 in both M5 and S161EB-5 (Demuth *et al.*, 1988), and may represent non-

proteolytically degraded SSP-5. *E. faecalis* S161-401 surface molecules did not react with the 62/60 antibodies.

DISCUSSION

Adherence of periodontal pathogens such as *P. gingivalis* to antecedent plaque organisms such as *S. gordonii* is considered an important colonization mechanism (Lamont *et al.*, 1992; Slots & Gibbons, 1978). Although fimbriae have been implicated in mediating binding of *P. gingivalis* (Lamont *et al.*, 1993), the streptococcal molecules involved have not been identified. The surfaces of oral streptococci are complex and contain polysaccharides, teichoic acids and a variety of proteins (Lamont *et al.*, 1988a; Rosan, 1973, 1976). Several of these proteins have been identified as adhesins, both for the enamel salivary pellicle and for other bacteria (Fives-Taylor *et al.*, 1987; Ganeshkumar *et al.*, 1988; Kolenbrander & Anderson, 1990; Lamont *et al.*, 1988a). In this study, surface proteins were removed by mild sonication, a process originally developed to release a streptococcal adhesin for *Actinomyces naeslundii* (Kolenbrander & Anderson, 1990). When biotinylated surface proteins of *S. gordonii* G9B were added to *P. gingivalis*, three molecules were observed to bind: a 45 kDa protein and two proteins of 62 and 60 kDa that ran as a doublet on SDS-PAGE gels. The 60 kDa molecule occasionally did not resolve from the 62 kDa molecule. Thus, the 60 kDa protein may be a partial breakdown product that, under certain experimental conditions, is absent due to complete processing. Antibodies raised to the 62/60 proteins also reacted with the 45 kDa molecule. Thus, these molecules are antigenically related and may be breakdown products of a larger molecule of 170 kDa that also reacted with the antiserum. The 170 kDa molecule itself did not appear to bind to *P. gingivalis*. This may indicate that conformational changes, perhaps caused by processing into smaller fragments, are required to induce binding activity in the streptococcal molecules. Thus, the 170 kDa protein *per se* is inactive until the 62/60 and/or 45 kDa fragments are generated. Under our experimental conditions the smaller molecules may have been produced by sonication; however, lower molecular mass fragments of streptococcal P1-like molecules have been found when extraction techniques that do not involve sonication were utilized (Demuth *et al.*, 1988; Jenkinson *et al.*, 1993; Wyatt *et al.*, 1988). A more plausible explanation is that the lower molecular mass molecules were generated by proteolytic activity. Oral streptococci produce a number of proteases that can be released from the cells and may degrade surface proteins (Cowman & Baron, 1991; Floders *et al.*, 1987; Lamont & Rosan, 1989; Rodgers *et al.*, 1990). The importance of proteolytic activity in the co-adherence of *P. gingivalis* and *S. gordonii* was demonstrated by Stinson *et al.* (1992) who found that treating *P. gingivalis* with protease inhibitors reduced binding to streptococci immobilized on CNBr-activated agarose beads. Similarly, Li *et al.* (1991) reported that proteases are involved in the adherence of *P. gingivalis* to *Actinomyces viscosus*, and proteolytic activity is important in the adherence mechanisms of *Trichomonas vaginalis* and *Candida* (Arroyo *et al.*,

1989; Ray & Payne, 1988). Furthermore, the *S. gordonii* G9B adhesin for salivary pellicle is the substrate for an endogenous protease that may serve to induce conformational changes necessary for activity (Lamont *et al.*, 1988b). The relative contributions of *P. gingivalis* and streptococcal proteases to their coadherence will be difficult to determine until the proteases produced by *S. gordonii* are better defined.

Further evidence for the involvement of the 62/60 and antigenically related molecules in adherence was provided by the inhibitory activity of antibodies. Antiserum to the 62/60 antigen inhibited binding of G9B to *P. gingivalis*, in a dose dependent manner, by up to 64%. The failure to produce complete inhibition may indicate that antibodies raised to the adhesin recovered from SDS-PAGE gels do not recognize possible conformational epitopes involved in binding. Alternatively, more than one streptococcal adhesin/adhesin receptor may be involved in adherence to *P. gingivalis*. Previous studies have suggested that a number of molecules may be responsible for *P. gingivalis* binding (Lamont *et al.*, 1993). Binding between *P. gingivalis* and *S. gordonii* may, therefore, involve several interactive components of which the *P. gingivalis* fimbriae and the *S. gordonii* 62/60 molecules are one example. It has yet to be determined whether the fimbriae and 62/60 molecules interact with each other. The presence of multiple adhesins on bacteria is well established. Indeed, sanguis group streptococci, including *S. gordonii* may possess several adhesins for both salivary pellicle and *Actinomyces* (Hasty *et al.*, 1992), and *P. gingivalis* has more than one surface molecule capable of mediating hemagglutination (Boyd & McBride, 1984; Inoshita *et al.*, 1986; Mouton *et al.*, 1991; Okuda *et al.*, 1986; Progulske-Fox *et al.*, 1989).

Normal mouse serum also inhibited binding between *P. gingivalis* and *S. gordonii*. Maximum inhibition was about 20% and activity was lost at dilutions greater than 1 in 1000. These results are in agreement with earlier work showing inhibition of *P. gingivalis*-*S. gordonii* binding by normal rabbit serum (Stinson *et al.*, 1991). In the oral cavity, therefore, crevicular fluid, which is essentially a serum exudate, may help protect against colonization of *P. gingivalis* by inhibiting adherence to other bacteria.

The 170 kDa and related molecules were found to be antigenically related to the P1 surface molecule of *S. mutans*. The presence of lower molecular mass breakdown products of P1 has also been demonstrated in *S. mutans* and sanguis streptococci including *S. gordonii* (Wyatt *et al.*, 1988). Thus, the 170 kDa protein may be a P1 homologue in G9B. This is supported by studies with SSP-5, a P1-related molecule in *S. gordonii* M5 (Demuth *et al.*, 1990). This strain of *S. gordonii* adheres to *P. gingivalis* in a very similar manner to G9B (Lamont *et al.*, 1992). *E. faecalis* S161EB-5, that was transformed with the SSP-5 gene, and expresses the SSP-5 protein on the cell surface (Demuth *et al.*, 1989), demonstrated enhanced binding to *P. gingivalis*. Thus, SSP-5 can mediate binding to *P. gingivalis*. Furthermore, both M5 and the transformed *E. faecalis* produced the 62/60 kDa and 45 kDa proteins, which appear to possess the adherence activity. Thus the

170 kDa, 62/60 kDa and 45 kDa molecules may all be encoded by the same gene. This is consistent with the concept that the P1-like gene in some *S. gordonii* strains produces a high molecular mass molecule of at least 170 kDa that can be broken down into fragments of 62/60 and 45 kDa. These smaller fragments are then capable of binding to *P. gingivalis*.

The P1-related molecules are involved in a number of other adherence mechanisms. They interact with salivary agglutinin in the fluid phase to mediate bacterial aggregation, and they can react with agglutinin in the salivary enamel pellicle causing bacterial adherence (Demuth *et al.*, 1988, 1989; Lee *et al.*, 1989). In addition, a P1-like molecule in *S. gordonii* DL-1 can mediate binding to *A. naeslundii* (Jenkinson *et al.*, 1993), and P1 may effect binding of *S. mutans* to *S. gordonii* G9B (Lamont *et al.*, 1991). The functional domains involved in binding to salivary agglutinin have been delineated (Crowley *et al.*, 1993), but those involved in binding to other bacteria are as yet unknown.

In conclusion, we have identified proteins of 62/60 and 45 kDa in *S. gordonii* G9B that are involved in binding to *P. gingivalis* 33277. The 62/60 and 45 kDa molecules appear to be part of a larger molecule of 170 kDa that is related to the P1 molecule originally identified in *S. mutans*. The 170 kDa P1-like molecule may require processing to induce binding activity.

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REFERENCES

- Arroyo, R. & Alderete, J. F. (1989). *Trichomonas vaginalis* surface proteinase activity is necessary for parasite adherence to epithelial cells. *Infect Immun* **57**, 2991–2997.
- Boyd, J. & McBride, B. C. (1984). Fractionation of hemagglutinating and bacterial binding adhesins of *Bacteroides gingivalis*. *Infect Immun* **45**, 403–409.
- Cowman, R. A. & Baron, S. S. (1991). Studies on the subcellular localization of protease and arylaminopeptidase activities in *Streptococcus sanguis* ATCC 10556. *J Dent Res* **70**, 1508–1515.
- Crowley, P. J., Brady, L. J., Piacentini, D. A. & Bleiweis, A. S. (1993). Identification of a salivary agglutinin-binding domain within cell surface adhesin P1 of *Streptococcus mutans*. *Infect Immun* **61**, 1547–1552.
- Demuth, D. R., Davis, C. A., Corner, A. M., Lamont, R. J., Leboy, P. S. & Malamud, D. (1988). Cloning and expression of a *Streptococcus sanguis* surface antigen that interacts with human salivary agglutinin. *Infect Immun* **56**, 2484–2490.
- Demuth, D. R., Berthold, P., Leboy, P. S., Golub, E. E., Davis, C. A. & Malamud, D. (1989). Saliva-mediated aggregation of *Enterococcus faecalis* transformed with a *Streptococcus sanguis* gene encoding the SSP-5 surface antigen. *Infect Immun* **57**, 1470–1475.
- Demuth, D. R., Lammey, M. S., Huck, M., Lally, E. T. & Malamud, D. (1990). Comparison of *Streptococcus mutans* and *Streptococcus sanguis* receptors for human salivary agglutinin. *Microb Pathog* **9**, 199–211.

- Fives-Taylor, P. M., Macrina, F. L., Pritchard, T. J. & Peene, S. S. (1987). Expression of *Streptococcus sanguis* antigens in *Escherichia coli*: cloning of a structural gene for adhesion fimbriae. *Infect Immun* **55**, 123–128.
- Floders, E., Andersson, C., Linder, L. & Sund, M.-L. (1987). Aminopeptidase activity in strains of oral streptococci. *Oral Microbiol Immunol* **2**, 117–120.
- Ganeshkumar, N., Song, M. & McBride, B. C. (1988). Cloning of a *Streptococcus sanguis* adhesin which mediates binding to saliva-coated hydroxyapatite. *Infect Immun* **56**, 1150–1157.
- Gibbons, R. J. & Hay, D. I. (1988). Human salivary acidic proline-rich proteins and statherin promote the attachment of *Actinomyces viscosus* LY7 to apatitic surfaces. *Infect Immun* **56**, 439–445.
- Hasty, D. L., Ofek, I., Courtney, H. S. & Doyle, R. J. (1992). Multiple adhesins of streptococci. *Infect Immun* **60**, 2147–2152.
- Inoshita, E., Amano, A., Hanioka, T., Tamagawa, H., Shizukuishi, S. & Tsunemitsu, A. (1986). Isolation and some properties of exohemagglutinin from the culture medium of *Bacteroides gingivalis* 381. *Infect Immun* **52**, 421–427.
- Jenkinson, H. F., Terry, S. D., McNab, R. & Tannock, G. W. (1993). Inactivation of the gene encoding surface protein SspA in *Streptococcus gordonii* DL1 affects cell interactions with human salivary agglutinin and oral actinomycetes. *Infect Immun* **61**, 3199–3208.
- Kolenbrander, P. E. & Andersen, R. A. (1986). Multigeneric aggregations among oral bacteria: a network of independent cell-to-cell interactions. *J Bacteriol* **168**, 851–859.
- Kolenbrander, P. E. & Andersen, R. A. (1990). Characterization of *Streptococcus gordonii* (S. *sanguis*) PK488 adhesin-mediated coaggregation with *Actinomyces naeslundii* PK606. *Infect Immun* **58**, 3064–3072.
- Kolenbrander, P. E. & London, J. (1993). Adhere today, here tomorrow: oral bacterial adherence. *J Bacteriol* **175**, 3247–3253.
- Lamont, R. J. & Rosan, B. (1989). Characteristics of a protease of *Streptococcus sanguis* G9B which degrades the major salivary adhesin. *FEMS Microbiol Lett* **58**, 101–106.
- Lamont, R. J. & Rosan, B. (1990). Adherence of mutants streptococci to other oral bacteria. *Infect Immun* **58**, 1738–1743.
- Lamont, R. J., Rosan, B., Murphy, G. M. & Baker, C. T. (1988a). *Streptococcus sanguis* surface antigens and their interactions with saliva. *Infect Immun* **56**, 64–70.
- Lamont, R. J., Rosan, B., Baker, C. T. & Nelson, G. M. (1988b). Characterization of an adhesin antigen of *Streptococcus sanguis* G9B. *Infect Immun* **56**, 2417–2473.
- Lamont, R. J., Demuth, D. R., Davis, C. A., Malamud, D. & Rosan, B. (1991). Salivary-agglutinin-mediated adherence of *Streptococcus mutans* to early plaque bacteria. *Infect Immun* **59**, 3446–3450.
- Lamont, R. J., Hersey, S. G. & Rosan, B. (1992). Characterization of the adherence of *Porphyromonas (Bacteroides) gingivalis* to oral streptococci. *Oral Microbiol Immunol* **7**, 193–197.
- Lamont, R. J., Bevan, C. A., Gil, S., Persson, R. E. & Rosan, B. (1993). Involvement of *Porphyromonas gingivalis* fimbriae in adherence to *Streptococcus gordonii*. *Oral Microbiol Immunol* **8**, 272–276.
- Lee, S. F., Progulske-Fox, A., Erdos, G. W., Piacentini, D. A., Ayakawa, G. Y., Crowley, P. J. & Bleiweis, A. S. (1989). Construction and characterization of isogenic mutants of *Streptococcus mutans* deficient in major surface protein antigen P1 (I/II). *Infect Immun* **57**, 3306–3313.
- Li, J., Ellen, R. P., Hoover, C. I. & Felton, J. R. (1991). Association of proteases of *Porphyromonas (Bacteroides) gingivalis* with its adhesion to *Actinomyces viscosus*. *J Dent Res* **70**, 82–86.
- Listgarten, M. A., Mayo, H. E. & Tremblay, R. (1975). Development of dental plaque on epoxy resin crowns in man. *J Periodontol* **46**, 10–26.
- Mouton, C., Ni Eidhin, D., Deslauriers, M. & Lamy, L. (1991). The hemagglutinating adhesin HA-Ag2 of *Bacteroides gingivalis* is distinct from fimbriin. *Oral Microbiol Immunol* **6**, 6–11.
- Okuda, K., Yamamoto, A., Naito, Y., Takazoe, I., Slots, J. & Genco, R. J. (1986). Purification and properties of hemagglutinin from culture supernatant of *Bacteroides gingivalis*. *Infect Immun* **54**, 659–665.
- Progulske-Fox, A., Tumwasorn, S. & Holt, S. C. (1989). The expression and function of a *Bacteroides gingivalis* hemagglutinin gene in *Escherichia coli*. *Oral Microbiol Immunol* **4**, 121–131.
- Ray, T. L. & Payne, C. D. (1988). Scanning electron microscopy of epidermal adherence and cavitation in murine candidiasis: a role for *Candida* acid proteinase. *Infect Immun* **56**, 1942–1949.
- Rodgers, A. H., Zilm, P. S., Gully, N. J. & Pfennig, A. L. (1990). Some aspects of protease production by a strain of *Streptococcus sanguis*. *Oral Microbiol Immunol* **5**, 72–76.
- Rosan, B. (1973). Antigens of *Streptococcus sanguis*. *Infect Immun* **7**, 205–211.
- Rosan, B. (1976). Relationship of cell wall composition of group H streptococci and *Streptococcus sanguis* to their serological properties. *Infect Immun* **13**, 1144–1153.
- Schwarz, S., Ellen, R. P. & Grove, D. A. (1987). *Bacteroides gingivalis*–*Actinomyces viscosus* cohesive interactions as measured by a quantitative binding assay. *Infect Immun* **55**, 2391–2397.
- Slots, J. & Gibbons, R. J. (1978). Attachment of *Bacteroides melaninogenicus* subsp. *asaccharolyticus* to oral surfaces and its possible role in the colonization of the mouth and of periodontal pockets. *Infect Immun* **19**, 254–264.
- Slots, J. & Listgarten, M. A. (1988). *Bacteroides gingivalis*, *Bacteroides intermedius* and *Actinobacillus actinomycetemcomitans* in human periodontal diseases. *J Clin Periodontol* **15**, 85–93.
- Stinson, M. W., Safulko, K. & Levine, M. J. (1991). Adherence of *Porphyromonas (Bacteroides) gingivalis* to *Streptococcus sanguis* in vitro. *Infect Immun* **59**, 102–108.
- Stinson, M. W., Haraszthy, G. G., Zhang, X. L. & Levine, M. J. (1992). Inhibition of *Porphyromonas gingivalis* adhesion to *Streptococcus gordonii* by human submandibular-sublingual saliva. *Infect Immun* **60**, 2598–2604.
- Theilade, E., Wright, W. H., Jensen, S. B. & Loe, H. (1966). Experimental gingivitis in man. II. A longitudinal clinical and bacteriological investigation. *J Periodontol Res* **1**, 1–13.
- van Winkelhoff, A. J., van Steenberghe, T. J. M. & de Graaff, J. (1988). The role of black-pigmented *Bacteroides* in human oral infections. *J Clin Periodontol* **15**, 145–155.
- Wirth, R., An, F. Y. & Clewell, D. B. (1986). Highly efficient protoplast transformation system for *Streptococcus faecalis* and a new *Escherichia coli*-*S. faecalis* shuttle vector. *J Bacteriol* **165**, 831–836.
- Wyatt, J. E., Willcox, M. D. P., Russell, R. R. B. & Handley, P. S. (1988). Fibrillar strains of *Streptococcus sanguis* biotype 1 carry a surface protein which cross-reacts with antigen B from *Streptococcus mutans* Ingbritt. *Oral Microbiol Immunol* **3**, 162–168.

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