Inhibition of Streptococcal Attachment to Receptors on Human Buccal Epithelial Cells by Antigenically Similar Salivary Glycoproteins

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Preparations of salivary glycoproteins inhibited the attachment of certain indigenous oral streptococci to human buccal epithelial cells and fostered the desorption of previously attached bacteria. The adherence-inhibiting and desorptive activities of the glycoproteins correlated with their ability to aggregate these organisms. Pretreatment of glycoprotein preparations with certain blood group antisera impaired their adherence-inhibiting effect, suggesting that components with blood group substance reactivity were involved. Pretreatment of buccal epithelial cells with certain blood group antisera or concanavalin A masked the receptors associated with the attachment of *Streptococcus sanguis* SG1. The association of blood-group-reactive substances with the receptors involved in bacterial attachment may provide a basis for understanding the distinct specificities that bacteria exhibit for attaching to different tissues, organs, and hosts. Antiserum raised in rabbits to human epithelial cells also exhibited receptor-masking activity, and absorption of this serum with homologous salivary glycoproteins removed the antibodies responsible. These observations indicate that some salivary glycoproteins are antigenically similar to components on the epithelial cell surfaces they bathe. It is suggested that by mimicking the receptors present on epithelial cells, the mucinous glycoproteins of secretions may competitively inhibit the sorption of infectious agents and facilitate their removal after they are attached. These activities help to explain how mucinous glycoproteins augment the cleansing action of secretions.

The secretions that bathe the mucosal surfaces of man and animals contain a prominant group of high-molecular-weight glycoproteins that historically have been termed "mucins" (13, 29). These mucinous glycoproteins have been ascribed a lubricating and cleansing function on the surfaces they bathe (8). Mucinous glycoproteins exhibit diverse microheterogeneity, particularly in their carbohydrate moieties, and it is not clear how this contributes to their functional abilities, nor is it clear how they facilitate the removal of noxious substances and microorganisms from the surfaces they bathe.

We reported earlier that antibodies in secretions can bind to specific bacterial surface antigens and impair the attachment of the organisms to epithelial surfaces (30). Inhibition of bacterial attachment logically should impair the colonization of affected bacteria on bathed epithelial surfaces. The glycoproteins in secretions have also been found to bind to and agglutinate certain bacterial species with a high degree of specificity (10, 14). It therefore seemed possible that glycoprotein interactions with

bacterial surface components could also impede bacterial attachment and colonization. Moreover, the mucinous glycoproteins in secretions are well known to exhibit blood group substance reactivity, and they possess these antigenic determinants in common with surface components of several body cell types, the range of which includes erythrocytes and epithelial cells (13, 23). This report presents data suggesting that antigenic components, including those with blood group substance reactivity, are in some way associated with the receptors on buccal epithelial cells that are involved in the attachment of certain indigenous streptococci, and that antigenically similar glycoproteins of saliva may competitively inhibit bacterial attachment.

MATERIALS AND METHODS

Cultures and cultural conditions. Fresh strains of *Streptococcus salivarius, S. sanguis*, and *S. miteor* were isolated from human tongue or tooth surfaces by using Mitis-Salivarius agar (Difco). The streptococcal strains were identified by using characteristics defined by Carlsson (4). Cultures were maintained by

weekly transfer in Trypticase soy broth (BBL). All cultures were incubated aerobically at 35 C. It was observed that strains maintained for several months by laboratory transfer tended to lose their ability to attach to epithelial cells; this necessitated isolating fresh strains during the course of the investigation.

For testing, cell suspensions of each organism were prepared from overnight Trypticase soy broth cultures. The streptococci were harvested by centrifugation, washed twice with 0.067 M phosphate buffer, pH 6.0, and resuspended in buffer at a concentration of 2 × 10⁸ cells per ml.

Salivary glycoprotein preparations. Paraffin-stimulated whole saliva was collected from individual donors and heated at 60 C for 30 min to inactivate degradative enzymes. Except when noted otherwise, samples from two to four donors were pooled together. The saliva was clarified by centrifugation, dialyzed against distilled water for 24 h, and lyophilized. The material was dissolved in 0.01 M phosphate-buffered saline (PBS), pH 6.0, containing 10^{-3} M $CaCl_2$, and the high-molecular-weight mucinous glycoproteins were isolated by chromatography on Bio-Gel A-15 equilibrated with PBS as described by Hay et al. (14). The glycoprotein fractions were dialyzed against distilled water for 24 h and lyophilized. For all studies reported, the glycoproteins were dissolved in PBS to give a concentration of 0.5 mg of protein per ml. Such solutions contained approximately 0.25 mg of anthrone-reactive carbohydrate per ml. Previous studies have partially characterized these salivary glycoprotein fractions and have shown that almost all of the material traveled in a single peak when analyzed by analytical ultracentrifugation and had an isoelectric point below pH 3 (14). The glycoprotein preparations used did not contain detectable levels of immunoglobulin (Ig) A, IgG, or IgM when tested by immunodiffusion using monospecific rabbit antisera to these human immunoglobulins (Behring Diagnostics, New York). Moreover, the glycoprotein preparations used were well separated from free salivary immunoglobulins in the chromatographic procedure used for their isolation (14, 30).

Effect of salivary glycoproteins on bacterial aggregation and attachment to epithelial cells. Standardized suspensions of strains of the *Streptococcus* species studied were screened for agglutination with pooled preparations of salivary glycoprotein as previously described (10, 14). Strains of each species that were or were not agglutinated were selected for adherence inhibition studies. Washed streptococcal suspensions (1 ml) were incubated for 1 h at 35 C with an equal volume of glycoprotein solution. Control bacteria were incubated with PBS. The streptococci were then washed by centrifugation and resuspended in 1 ml of PBS, and their ability to attach to pooled human buccal epithelial cells was determined.

The possible involvement of glycoproteins possessing blood group substance reactivity in bacterial aggregation and inhibition of attachment was studied. Equal volumes (0.2 ml) of pooled glycoprotein solution and blood group antiserum or lectin were incubated together for 1 h at 35 C. Control glycopro-

tein solutions were incubated with normal rabbit serum, PBS, or a goat antiserum to human IgG-IgM-IgA (Hoechst Pharmaceutials, Kansas City, Mo.). The mixtures were then incubated for 1 h at 35 C with 0.4 ml of a standardized suspension of *S. sanguis* strain SG1 or *S. miteor* strain 26. The suspensions were examined for agglutination, after which the streptococci were washed by centrifugation, resuspended in PBS, and assayed for their ability to attach to pooled buccal epithelial cells.

Effect of salivary glycoprotein on the desorption of bacteria. Standardized buffered suspensions (1 ml) of *S. salivarius* strains were incubated with 1 ml of washed epithelial cells collected from a single donor for 1 h at 35 C as previously described (11). The epithelial cells were then washed free of unattached bacteria by using membrane filters and resuspended in 1 ml of glycoprotein solution prepared from the saliva of the same individual who donated the epithelial cells. Epithelial cells suspended in PBS served as controls. The mixtures were incubated for 1 h at 35 C, after which the epithelial cells were again washed free of unattached bacteria. The number of organisms remaining attached was determined by direct microscopic counts of 25 epithelial cells per sample.

Effect of pretreatment of epithelial cells with antisera and concanavalin A on bacterial attachment. Membrane-washed suspensions of buccal epithelial cells were prepared from one individual (of blood group A) as previously described (11). Suspensions containing 10⁵ epithelial cells per ml were incubated with an equal volume (0.2 ml) of antiserum or lectin for 1 h at 35 C. Control epithelial cells were pretreated with normal rabbit serum. For pretreatment with the hemagglutinin concanavalin A (Con A) (Miles Laboratories, Kankakee, Ill.), washed epithelial cell suspensions were incubated with approximately 2.5 mg of Con A dissolved in 0.17 M succinate buffer, pH 6.0, containing 0.2 M NaCl and 1 mM $CaCl_2$ for ·30 min at 35 C. Epithelial cells incubated in the succinate-NaCl buffer with $CaCl_2$ served as controls. After incubation with either antiserum or Con A, the epithelial cells were washed by centrifugation, resuspended in PBS, and studied for bacterial attachment.

Blood group antisera. Blood group antisera and anti-H lectin were obtained from Hyland Laboratories, Los Angeles, Calif. These had been diluted and standardized as received for use as blood-typing reagents. Consequently, to judge the immunological specificities of the sera, their total protein content was determined from their adsorption at 280 and 260 nm (28).

Preparation of anti-epithelial cell serum. Buccal epithelial cells were collected from a single individual (blood group A). The cells were washed by membrane filtration and suspended in saline at a concentration of 10⁵ cells/ml. A vaccine consisting of equal volumes of the epithelial cell suspension and Freund complete adjuvant was prepared. A rabbit was immunized with 0.1 ml of the vaccine in each footpad. The animal was boosted 3 weeks later and bled the following week. Antibody development was assessed by slide agglutination of erythrocytes obtained from the same indi-

vidual who donated the epithelial cells. Serum obtained from the rabbit before immunization served as a control.

Glycoprotein-absorbed anti-epithelial cell serum was prepared by incubating equal volumes of anti-epithelial cell serum and a glycoprotein solution prepared from saliva obtained from the donor of the epithelial cells for 2 h at 35 C. Glycoprotein-antibody complexes and excess glycoprotein were removed by ultracentrifugation (2 h at 100,000 × g) (14). Normal rabbit serum to be used for controls was absorbed with the salivary glycoprotein solution in a similar manner.

Analytical methods. Glycoprotein preparations were assayed for total protein by the method of Itzhaki (16), using bovine serum albumin as a standard, and for total anthrone-reactive carbohydrate by the procedure of Van Handel (26).

Bacterial attachment to epithelial cells was determined by using an in vitro system previously described (11). Unless otherwise specified, the assays used buccal epithelial cells pooled from four to six donors. The mean number of bacteria attached per epithelial cell was determined by direct microscopic enumeration of 25 epithelial cells. Counts of control epithelial cells that had not been incubated with bacteria were performed for each experiment to determine the number of bacteria already attached to the cells at the time of their collection. These values (usually 8 to 12 bacteria/cell) were subtracted to obtain the net number of adherent cells of the test organism.

RESULTS

Effect of salivary glycoproteins on streptococcal attachment to buccal epithelial cells. Previous studies have shown that strains of S. salivarius, S. sanguis, and S. miteor attach to human buccal epithelial cells (11, 19). When washed cells of S. salivarius strain SS1 and S. sanguis strain SG3 were incubated with a preparation of pooled salivary glycoprotein, the streptococci were not aggregated, and their ability to attach to epithelial cells was not significantly impaired as compared with buffer-treated controls (Table 1). However, S. salivarius SS3, S. sanguis strains SG2 and SG1, and S. miteor 26 were aggregated by the glycoprotein preparation, and this pretreatment significantly inhibited their ability to attach to epithelial cells (Table 1). The inhibition of bacterial attachment by the salivary glycoprotein preparation was therefore strain specific and it correlated with agglutinating activity.

Desorption of streptococci by salivary glycoproteins. When epithelial cells containing sorbed cells of S. salivarius strains SS3 and CM6 were incubated in salivary glycoprotein solution obtained from the epithelial cell donor, a significant number of streptococci became desorbed as compared with epithelial cells suspended in PBS (Table 2) (strain SS3 $P < 0.010$; strain CM6 $P < 0.005$). The glycoprotein preparation did not foster the desorption of cells of S. salivarius strain HH-1. On the basis of the few strains tested, the desorptive effects of the glycoprotein solution correlated with its agglutination activity (Table 2).

Effect of pretreating pooled salivary glycoproteins with blood group antisera on bacterial agglutination and inhibition of

TABLE 1. *Effect of pooled salivary glycoproteins on agglutination of streptococci and their attachment to pooled buccal epithelial cells*

Organism	Treatment	Agglutination	Mean net no. of bacteria attached per epithelial cell (± standard error)	% Attachment compared with buffer controls
S. salivarius SS1	Buffer	−	78 ± 25	100
	Salivary glycoprotein	−	66 ± 23	85
S. salivarius SS3	Buffer	−	111 ± 21	100
	Salivary glycoprotein	+	52 ± 14	47
S. sanguis SG3	Buffer	−	33 ± 12	100
	Salivary glycoprotein	−	41 ± 15	124
S. sanguis SG2	Buffer	−	72 ± 11	100
	Salivary glycoprotein	+	15 ± 11	21
S. sanguis SG1	Buffer	−	47 ± 6	100
	Salivary glycoprotein	+	6 ± 4	13
S. miteor 26	Buffer	−	88 ± 19	100
	Salivary glycoprotein	+	32 ± 9	36

attachment. Prior incubation of the pooled salivary glycoprotein preparation with certain blood group antisera had no effect upon its ability to agglutinate or to inhibit the attachment of *S. miteor* 26 to epithelial cells (Table 3). However, after incubation with anti-Fyᵃ, anti-M, or anti-I serum, the salivary glycoprotein preparation no longer aggregated *S. sanguis* SG1, and its ability to inhibit the attachment of this strain to epithelial cells was significantly reduced (Table 3). Preincubation of the glyco-

protein preparation with anti-A, -B, -K, or -N sera or anti-H lectin had little or no effect. Although immunoglobulins were not detected in the salivary glycoprotein preparations by immunodiffusion against monospecific rabbit antisera to human IgG, IgA, or IgM, it was nevertheless of interest to determine whether an antiserum to human immunoglobulins could affect their adherence-inhibiting activity. However, preincubation with antiserum to human IgG-IgM-IgA had no effect upon the agglutinat-

TABLE 2. *Effect of salivary glycoproteins on the desorption of streptococci attached to homologous buccal epithelial cells*

Organism	Treatment	Agglutination	Mean net no. of bacteria attached per epithelial cell (± standard error)	% Attached compared with buffer controls
S. salvarius HH-1	Buffer desorption	−	56 ± 10	100
	Glycoprotein desorption	−	61 ± 13	109
S. salivarius SS3	Buffer desorption	−	135 ± 20	100
	Glycoprotein desorption	+	67 ± 15	49
S. salivarius CM6	Buffer desorption	−	84 ± 20	100
	Glycoprotein desorption	+	15 ± 5	18

TABLE 3. *Effect of pretreating pooled salivary glycoprotein with blood group antisera and lectins on bacterial agglutination and inhibition of attachment to pooled buccal epithelial cells*

Organism	Glycoprotein pretreatment	Agglutination	Mean net no. of bacteria attached per epithelial cell (± standard error)	% Bacterial attachment compared with control
S. sanguis SG1	Buffer control	−	149 ± 21	100
	Salivary glycoprotein + PBS	+	42 ± 12	28
	Salivary glycoprotein + anti Fyᵃ serum	−	93 ± 13ᵃ	62
	Salivary glycoprotein + anti-M serum	−	90 ± 19ᵇ	60
	Salivary glycoprotein + anti-I serum	−	139 ± 24ᵃ	93
	Salivary glycoprotein + anti-A serum	+	60 ± 16	40
	Salivary glycoprotein + anti-B serum	+	43 ± 12	29
	Salivary glycoprotein + anti-H lectin	+	50 ± 15	34
	Salivary glycoprotein + anti-K serum	+	65 ± 17	44
	Salivary glycoprotein + anti-N serum	+	56 ± 21	38
	Salivary glycoprotein + anti-IgG-IgM-IgA serum	+	48 ± 14	32
	Salivary glycoprotein + normal rabbit serum	+	42 ± 12	28
S. miteor 26	Buffer control	−	88 ± 19	100
	Salivary glycoprotein + PBS	+	30 ± 12	34
	Salivary glycoprotein + anti-Fyᵃ serum	+	12 ± 6	14
	Salivary glycoprotein + anti-I serum	+	20 ± 6	23
	Salivary glycoprotein + anti-M serum	+	15 ± 6	17
	Salivary glycoprotein + anti-A serum	+	5 ± 4	6
	Salivary glycoprotein + anti-B serum	+	16 ± 12	18
	Salivary glycoprotein + anti-H lectin	+	25 ± 8	28
	Salivary glycoprotein + anti-K serum	+	8 ± 4	9
	Salivary glycoprotein + anti-IgG-IgM-IgA serum	+	15 ± 5	17
	Salivary glycoprotein + normal rabbit serum	+	32 ± 9	36

ᵃ Statistically significant at $P < 0.01$ level (Student's t test).
ᵇ Statistically significant at $P < 0.05$ level (Student's t test).

ing activity or the adherence-inhibiting activity of the glycoprotein preparation (Table 3). This observation further minimizes the possibility that the effects observed were due to traces of contaminating antibody in the glycoprotein preparation.

The ability of certain blood group antisera to inhibit the agglutinating and adherence-inhibiting activities of the salivary glycoproteins affecting S. sanguis SG1, but not those affecting S. miteor 26, indicates that different glycoprotein components of saliva affect each organism. The specificity observed is consistent with previous reports (10, 14, 15, 17). The data also imply that salivary glycoproteins with blood group substance activity are involved in the aggregation and adherence inhibition of S. sanguis.

Effect of pretreating epithelial cells collected from a single donor (blood type A) with antisera and Con A on adherence of S. sanguis SG1. Because salivary glycoproteins with blood group substance reactivity appeared to be involved in the agglutination and adherence inhibition of S. sanguis SG1, it was of interest to determine whether blood group antisera could affect the epithelial cell receptors involved in the attachment of this organism. In these experiments, epithelial cells were obtained from a single individual of blood type A. It was found that pretreatment of the epithelial cells with antisera to the A, I, or N blood group antigens significantly reduced the number of cells of S. sanguis SG1 that attached (Table 4). Pretreatment with antisera to AB (O), Fy^a, and M blood cell antigens and anti-H lectin also reduced the number of streptococci that attached, but the differences were not statistically significant (Student's t test). Antisera to A$_1$, B, Rh (CDE), K, and S antigens had no effect (Table 4). The adherence-inhibiting effect of these sera was not related to their protein content (Table 4), indicating the specificity of the observed effects. The observations therefore suggest that epithelial cell surface components with blood group antigen activity are either identical with or in close proximity to components serving as receptors for attachment of S. sanguis SG1. Pretreatment of epithelial cells with Con A also masked the receptors involved in the attachment of this organism (Table 4), suggesting that carbohydrate moieties are associated with the receptors.

Antigenic similarities of epithelial cell surface components and salivary glycoproteins. Because components with blood group antigen reactivity were associated with both the salivary glycoproteins and the epithelial cell receptors affecting streptococcal adherence, it was of

TABLE 4. *Effect of pretreating buccal epithelial cells from a single donor (blood type A) with blood group sera and Con A on the attachment of S. sanguis SG1*

Epithelial cell pretreatment	Total protein content (mg/ml) of antiserum tested	Mean net no. of bacteria per epithelial cell (± standard error)	% Bacterial attachment compared with buffer control
Buffer		129 ± 16	100
Normal rabbit serum	70.0	135 ± 20	105
Anti-A serum	36.0	74 ± 12^a	57
Anti-I serum	8.8	61 ± 14^a	47
Anti-N serum	5.5	57 ± 14^a	44
Anti-AB (O) serum	31.3	98 ± 13	76
Anti-Fy^a serum	33.0	96 ± 21	74
Anti-H lectin	0.04	97 ± 18	75
Anti-M serum	0.12	85 ± 19	66
Anti-A$_1$ serum	0.42	152 ± 22	118
Anti-B serum	39.0	139 ± 19	108
Anti-Rh (CDE) serum	80.0	130 ± 19	101
Anti-K serum	44.5	140 ± 19	109
Anti-S serum	19.5	143 ± 22	111
Con A	12.0	18 ± 5^a	14

^{a} Statistically significant at $P < 0.01$ level (Student's t test).

interest to determine whether these components were similar within a given individual. Antiserum raised in a rabbit immunized with epithelial cells obtained from a single donor was found to aggregate homologous erythrocytes when tested by slide agglutination whereas preimmunization serum did not. When homologous epithelial cells were pretreated with the anti-epithelial cell serum, the receptors required for the attachment of S. sanguis strain HSG and S. salivarius strain SS3 were strongly masked (Table 5). Epithelial cells pretreated with normal rabbit serum behaved comparably to buffer controls. When the anti-epithelial cell serum was absorbed with salivary glycoprotein obtained from the epithelial cell donor, antibodies responsible for masking the epithelial cell receptors were removed (Table 5). These observations indicate that the antigenic components associated with the epithelial cell receptors involved in the attachment of these Streptococcus strains are similar to those possessed by the mucinous glycoproteins in the secretions that bathe them.

DISCUSSION

Indigenous oral bacteria have been found to attach to various surfaces of the mouth with surprising specificity (9, 11, 18, 19, 27). Patho-

TABLE 5. *Effect of pretreating homologous buccal epithelial cells with anti-epithelial cell serum or glycoprotein-absorbed anti-epithelial cell serum on streptococcal attachment*

Organism	Epithelial cell pretreatment	Mean net no. of bacteria attached per epithelial cell (± standard error)	% Bacterial attachment compared with buffer control
S. sanguis HSG	PBS	98 ± 11	100
	Anti-epithelial cell serum	22 ± 4[a]	22
	Glycoprotein absorbed Anti-epithelial cell serum	77 ± 8	79
	Normal rabbit serum	89 ± 12	91
	Glycoprotein absorbed Normal rabbit serum	82 ± 12	84
S. salivarius SS3	PBS	82 ± 13	100
	Anti-epithelial cell serum	16 ± 5[a]	20
	Glycoprotein absorbed Anti-epithelial cell serum	59 ± 9	72
	Normal rabbit serum	69 ± 11	84
	Glycoprotein absorbed Normal rabbit serum	72 ± 18	88

[a] Statistically significant at $P < 0.01$ level (Student's t test).

genic strains of *S. pyogenes* and *Escherichia coli* also attach selectively to human buccal and pharyngeal epithelial cells and to the surfaces of various tissues of rodents (6, 7). In addition, certain *Streptococcus* species attach differently to human and to rodent tongues (Gibbons and Spinell, unpublished data). On bathed mucosal surfaces, bacteria must attach to colonize; otherwise they are washed away. It has been found that the relative ability of a bacterial species to attach to a given surface correlates with the degree or frequency with which that species naturally colonizes the surface (9, 11, 18, 19, 27). Because of epithelial cell desquamation, the progeny of colonizing bacteria must reattach for colonization of the surface to continue. The cyclical nature of these events serves to multiply differences in the innate adherence abilities between bacterial species and thus accentuate the resulting ecological effects. Differences in the extent to which bacteria may attach therefore may be presumed to influence the susceptibility of tissues, organs, and host species to bacterial colonization and infection.

It is apparent from these studies that the surface components of mucosal cells from different body tissues and from various mammalian species must possess subtle differences that significantly affect the adherence of bacteria. Likewise, the surface constituents of bacteria must also differ among species to account for their differences in adherence to the same tissue. It is likely that a variety of bacterial and epithelial cell surface polymeric materials function in these adherent interactions, depending upon the bacterium and the tissue in question. In the present investigation, it was observed that Con A and antisera to certain blood group substances could mask the epithelial cell surface components involved in the attachment of *S. sanguis* SG1. This suggests that cell surface glycoproteins or glycolipids, some of which possess blood group antigen reactivity, were either identical with or in close proximity to the receptors required for the attachment of this streptococcus. Cell surface glycoproteins, including those with blood group substance reactivity, have also been reported to serve as receptors for attachment of certain viruses (1, 23) and *Mycoplasma* species (21).

It is well known that components with blood group antigen reactivity exhibit qualitative and quantitative differences in their distribution between various body cells and between different mammalian species (24, 25, 29). If they prove to be associated with the receptors used for the attachment of a wide range of bacteria, they could provide a framework for understanding the tissue and host specificities observed in bacterial attachment and colonization. Previ-

ous reports of piliated bacteria attaching to and agglutinating erythrocytes from some mammalian species but not those from others are conconsistent with this mechanism (2, 5). Since components with blood group antigenicity are genetically determined, their association with certain bacterial receptors on mucosal epithelial cells may explain why resistance or susceptibility to some infectious agents can correlate with the blood group type of individuals (3). It may also explain why certain infectious agents can readily colonize the mucosal surfaces of some mammalian species but not those of others.

The mucinous glycoproteins present in the secretions that bathe mucosal surfaces are well known to possess blood group substance reactivity, and they have been reported to bind to the surfaces of bacteria (10) and to induce their aggregation in a species- and strain-specific manner (10, 14, 17). The ability of certain blood group antisera to impair the agglutinating activity of preparations of pooled salivary glycoproteins for *S. sanguis* SG1 but not for *S. miteor* 26 (Table 3) provides further evidence that different molecular species of glycoproteins affect different streptococci. In addition, the salivary glycoprotein preparation used inhibited the attachment to epithelial cells of those *Streptococcus* species and strains which it aggregated, but it did not affect the attachment of other strains.

By virtue of possessing blood group substance reactivity, the mucinous glycoproteins of secretions would be expected to be similar, at least in these determinants, to the antigenic glycoproteins or glycolipids present on epithelial cell surfaces that are associated with certain bacterial and viral receptors. The present investigation provides data directly supporting this view, for salivary glycoproteins prepared from a single donor were found to effectively remove receptor masking antibodies present in antiserum raised against the same individuals epithelial cells. It would not appear to be by chance that the glycoproteins in secretions bear a close similarity to the epithelial cell surface receptors they bathe. Rather, their similarity would be expected to permit the secretions to competitively inhibit the adsorption of infectious agents, antigenic pollens, and other noxious substances to antigenically related receptors on mucosal surfaces. The specific bacterial adherence-inhibiting effects of salivary glycoprotein preparations, and their ability to foster the desorption of already attached bacteria, as observed in the present study, support this view. The well-known ability of mucinous glycoproteins to competitively inhibit influenza virus-induced agglutination of erythrocytes appears to be an analogous situation. These properties would contribute to and augment the cleansing action of secretions.

Components of saliva, including glycoproteins, are adsorbed in a selective manner to the enamel surface of teeth, forming an organic film termed the acquired pellicle (12). The presence of a salivary pellicle on hydroxyapatite or enamel surfaces has been found to influence the sorption of buffered suspensions of bacteria. The sorption of some species (i.e., *S. sanguis* and *S. miteor*) has been reported to be enhanced, whereas that of other species (i.e., *S. salivarius*) is either unaffected or reduced (15, 20). It has been recently reported that salivary glycoproteins with blood group substance reactivity and influenza virus hemagglutination inhibition activity are among the salivary components that selectively adsorb to teeth (22). The ability of such components to bind to the surfaces of certain bacteria (10) may explain why they increase the sorption of some species to teeth but have little effect on others. It has also been reported that use of saliva as a suspending fluid can impair the sorption of bacteria to saliva-coated hydroxyapatite surfaces (20). This effect would seem to be analogous to the glycoprotein-mediated inhibition of bacterial attachment to epithelial cell surfaces observed in this study.

The role of cell surface and secretory glycoproteins in host-parasite interactions suggested in this report may also help to explain associations between altered glycoprotein synthesis and increased susceptibility to infections as has been recognized in certain disease states. Moreover, the hypotheses advanced and the supporting data provide a teleological explanation of why the mucinous glycoproteins of secretions possess blood group substance reactivity, why they exhibit microheterogeneity, and why they closely mimic components of the mucosal surfaces they bathe.

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