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Adherence of Pharyngeal and Skin Strains of Group A Streptococci to Human Skin and Oral Epithelial Cells

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Group A streptococci isolated from skin adhere in greater numbers to human skin epithelial cells than to cells obtained from buccal mucosa, whereas streptococci isolated from a throat tend to adhere in greater numbers to buccal epithelial cells than to skin epithelial cells in vitro. M protein-producing strains of group A streptococci did not adhere in significantly greater numbers than M-negative strains. Lipoteichoic acid inhibited binding of streptococci to skin epithelial cells as well as was previously shown for oral epithelial cells. Our results suggest that lipoteichoic acid is more centrally involved than M protein in binding streptococci to skin and mucosal surfaces.

The strains of group A streptococci associated with pyoderma appear to be distinct from those associated with acute pharyngitis (5, 6, 10, 14, 15, 17). It has been suggested that the capacity of a given microorganism to colonize a particular epithelial surface is proportional to the ability of the organism to adhere to that surface (8). The virulence properties that determine the predilection of a given streptococcal strain for skin versus pharyngeal sites, however, have not been identified. It is widely accepted that virulence is proportional to the ability of the organisms to produce M protein and hyaluronic acid capsules on their surfaces (16). Ellen and Gibbons suggested that production of M protein is associated with adherence (7). Recently, however, we demonstrated that although M protein renders the organisms resistant to phagocytosis, it is not essential for adherence to oral epithelial cells (3). Adherence to oral epithelial cells appears to be mediated by surface lipoteichoic acid (LTA) rather than M protein (1, 3, 13). Whether or not LTA is involved in adherence of streptococci to epithelial cells of skin has not been previously investigated.

In the present study, we exposed M-typable and non-M-typable strains of streptococci to oral epithelial cells and compared their adherence with their resistance to phagocytosis. In addition, streptococci isolated from skin or throats of infected patients were exposed to epithelial cells from the oral cavity, from the palm of the hand, and from skin of the back. Our data support the hypothesis that M protein is not involved in the adherence process and indicate that adherence to skin epithelial cells is mediated by LTA. Moreover, skin strains adhere in greater numbers to skin epithelial cells, and pharyngeal strains adhere in greater numbers to oral epithelial cells.

Adherence experiments were performed by a modification (12) of the method of Gibbons and van Houte (9), using scrapings from buccal mucosa, the palm of the hand, and skin of the back as previously described (3). Cells obtained from three healthy donors were pooled. The same donors were used in all experiments. Repeated throat cultures of these subjects failed to show the presence of beta-hemolytic streptococci. The diameter of epithelial cells of different origins was measured with an ocular micrometer under the microscope. Statistical evaluations of all results were performed by use of the t statistic for two means.

Group A streptococci isolated from patients participating in another study (11) were cultured on 5% sheep blood agar plates, subcultured in 20% rabbit serum in Todd-Hewitt broth, and stored frozen at −70°C or lyophilized. For each experiment, the streptococci were grown overnight in Todd-Hewitt broth, washed twice, and suspended in 0.02 M phosphate–0.15 M NaCl, pH 7.4. To break long chains of streptococci, the suspensions were vigorously shaken (Vortex Genie Mixer, Scientific Products, Evanston, Ill.) for 5 min and then brought to an optical density of 0.3 at 530 nm; 0.5 ml of the bacterial suspension was incubated for 30 min at 25°C with 0.5 ml of 0.02 M phosphate–0.15 M NaCl, pH 7.4, containing 1×10^5 epithelial cells. Nonadherent bacteria were removed by repeated differential centrifugation at $200 \times g$ (12). Smears were stained with crystal violet, and the number of bacteria adherent to 50 epithelial cells was de-

termined by microscopy as previously described (12, 13). Results were expressed as the mean number of adherent bacteria per epithelial cell. T and M typing were performed with specific antisera obtained from the Center for Disease Control, Atlanta, Ga. Resistance to phagocytosis was determined as previously described (2, 4).

Thirteen laboratory strains of group A streptococci were exposed to oral epithelial cells. Adherence ranged between 171 and 276 bacteria per cell (Table 1). No significant difference in adherence was observed between M-typable and non-M-typable strains. Neither was there a difference in adherence between the strains resistant to phagocytosis and the strains that were readily phagocytosed, or between T-typable and non-T-typable strains, indicating that M and T proteins are not involved in adherence.

We investigated the ability of 9 strains of streptococci isolated from throats and 30 strains isolated from skin to adhere to epithelial cells derived from buccal mucosa and skin (Table 2).Throat isolates tended to adhere better to cells from the oral cavity than to cells from skin (182 ± standard error [SE] of 38 versus 84 ± SE of 15 bacteria per cell). Streptococci isolated from skin adhered better to skin than oral epithelial cells (112 ± 12 versus 76 ± 10).

One skin isolate (T3/13/B3264) was exposed to epithelial cells derived from skin that had been preincubated with 1 mg of LTA per ml prepared from lab strain 1RP41 (T28, M13) as previously described (13). Adherence was diminished by 76% (Table 3), indicating that LTA competes for streptococcal binding sites on skin epithelial cells similar to the phenomenon described for oral epithelial cells (1, 13).

TABLE 1. *Adherence of streptococci to oral epithelial cells compared with T type, M type, and resistance to phagocytosis*[a]

Strain	Type		Resistance to phagocytosis	Bacteria/cell
	T	M		
SF42	12	12	+	171 ± 25[b]
D24		30	+	157 ± 28
SF130-13	1	1	+	221 ± 21
"Vaughn"		24	+	222 ± 24
S43	6	6	+	212 ± 20
"Baker"	28/56	56	+	188 ± 23
492	12	NT[c]	+	253 ± 13
T2	2	2	+	276 ± 6
T1av	1	NT	−	158 ± 23
C203S	3/13	NT	−	229 ± 18
1RP41	28	NT	−	153 ± 23
414		NT	+	222 ± 23
122		NT	−	131 ± 26

[a] The difference between adherence of M-positive and M-negative streptotococci, as determined by precipitin reactions or resistance to phagocytosis, was not significant ($P < 0.8$ and $P < 0.6$, respectively).

[b] Plus or minus SE.

[c] NT, Not typable.

TABLE 2. *Binding of pharyngeal and pyoderma strains of streptococci to epithelial cells of oral cavity and skin*[a]

Streptococci isolated from:	No.	Epithelial cells	Mean no. of bacteria adherent/equivalent area[b] of epithelial cells ± SE	*P*
Throat	9	Oral	182 ± 38	
Throat	9	Skin	84 ± 15	<0.05
Skin	30	Oral	76 ± 10	
Skin	30	Skin	112 ± 12	<0.02

[a] Detailed data for each of the strains represented in this table are available upon request from the authors.

[b] Diameters of 50 epithelial cells (skin or oral) were measured by direct micrometry, using light microscopy. The areas were then calculated, and the values for adherence were adjusted to a value representing a surface area equivalent to that of the average oral epithelial cell. SE, Standard error of the mean.

TABLE 3. *Inhibition of adherence of pyoderma-associated type T3/13/B3264 M streptococcus to skin epithelial cells by streptococcal LTA*[a]

Skin epithelial cells preincubated with:	No. of bacteria adherent/epithelial cell
PBS	63
LTA, 1 mg/ml	15

[a] After preincubation of epithelial cells with LTA or buffer (PBS) the cells were washed and exposed to streptococci in the same ratio as in previous experiments.

In this survey of various group A streptococci, our data show that the presence or absence of M protein did not influence adherence to human oral epithelial cells. The methods used for detecting M proteins could have some inaccuracies, but the amount of M-negative bacteria in a culture of an M-positive strain that has not been subcultured or stored properly is small, as demonstrated by the absence of phagocytosis of organism in such cultures. This agrees with our previous study (3) in which we showed that streptococci from which M protein had been selectively removed did not lose their ability to adhere to epithelial surfaces. Therefore, the mere presence of M protein on the surface of streptococci that adhere well to epithelial cells does not indicate that it is centrally involved in the binding process, as has been suggested (7).

Our data on the affinity of group A streptococci for different epithelial surfaces demonstrate that strains that are known pathogens of the upper respiratory tract adhere better to cells

lining the oral cavity than to epithelial cells obtained from skin sites. In contrast, strains pathogenic for skin sites adhere more avidly to skin epithelial cells, indicating a degree of tissue tropism among these organisms.

The affinity of different strains of streptococci for epithelial surfaces could explain why skin and throat infections are caused by different serotypes of streptococci (5, 6, 10, 14, 15, 17). It should be noted, however, that so-called "skin strains" do adhere to oral epithelial cells, and "throat strains" adhere to skin epithelial cells. The finding is consistent with the observation that strains typically associated with pharyngitis can be isolated from skin and that those strains typically associated with pyoderma can be isolated from throats of asymptomatic carriers (5, 6, 11).

Our previous studies (1, 3, 13) suggested that LTA is centrally involved in adherence of streptococci to oral epithelial cells. In the present study, the adherence of streptococci to skin epithelium was blocked by LTA, suggesting that adherence to skin is mediated in a similar fashion. The molecular basis of the varying ability of different serotypes of group A streptococci to adhere to epithelial surfaces, however, remains unclear. The LTAs derived from various strains possess similar biological properties, including binding to human cell membranes (M. Alkan and E. H. Beachey, unpublished observations). Although this does not take into account the possible variation of the molecular configuration of LTA among other surface macromolecules, it is unlikely that structural differences in LTA itself account for the differences in the adhesive properties of throat and skin strains of group A streptococci.

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