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A Streptococcal Adhesion System for Salivary Pellicle and Platelets

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A *Streptococcus sanguis* 133-79 adhesin identified by the monoclonal antibody 1.1 (MAb 1.1) binds both saliva-coated hydroxylapatite (sHA) and platelets. The complementary binding site(s) for the adhesin was identified by the anti-idiotypical MAb 2.1. To learn if this adhesion system, marked by the antiadhesin MAb 1.1 and anti-binding site MAb 2.1, is commonly used by strains within the sanguis group and other viridans group streptococci, 42 strains from seven species were tested. Strains that bind to both sHA and platelets use the same adhesin and binding site epitopes. Strains that do not adhere to platelets rely on other adhesin specificities to bind to sHA.

Streptococcus sanguis 133-79, a prototype of many blood culture isolates from patients with infective endocarditis (11, 13, 15, 29), adheres to human platelets and induces them to aggregate in plasma (11–13, 28). Strain 133-79 also adheres to saliva-coated hydroxylapatite (sHA), an in vitro model of the salivary enamel pellicle (9). To characterize the adhesins of *S. sanguis* 133-79, monoclonal antibodies (MAbs) against whole bacterial cells were prepared. MAb 1.1 reacts with 87- and 150-kDa adhesins on strain 133-79 and, at maximal concentrations, partially inhibits adhesion to both platelets (10) and sHA (9). To probe specific binding sites for *S. sanguis* 133-79 on platelets, MAb 1.1 was used to develop the anti-idiotypical MAb 2.1. MAb 2.1 simulated the adhesin of strain 133-79 and identified 175- and 230-kDa platelet membrane proteins as potential binding sites for this strain. On salivary pellicle, MAb 2.1 recognizes an α-amylase-secretory immunoglobulin A (IgA) complex as a presumptive receptor for strain 133-79 (10a). Hence, MAb 1.1 and MAb 2.1 appear to define a specific adhesion system.

The sanguis group is not readily discriminated from other viridans streptococci based on their ability to adhere. For example, an occasional strain of *S. mutans* or *S. gordonii* would cluster with the sanguis group based on their reactions with platelets (11, 14). Within the sanguis group, however, *S. sanguis* biovars 1 and 3 may preferentially induce human platelets to aggregate (5). Given that there may be similarities in the mechanisms of binding to platelets and sHA, we sought to determine if oral streptococci commonly use the MAb 1.1-MAb 2.1 adhesion system. Since MAb 1.1 has been characterized as an adhesin-reactive antibody (9), it was used to screen streptococcal strains in an indirect enzyme-linked immunosorbent assay (ELISA). From the screen, the prevalence of MAb 1.1-positive and -negative strains was determined. For each strain, MAb 1.1 binding and the ability to adhere to platelets and to sHA were then compared. To demonstrate binding epitopes on platelets and sHA for MAb 1.1-positive strains, MAb 2.1 was preincubated with platelets or sHA to inhibit streptococcal adhesion. The results strongly suggest that most strains of oral streptococci use the MAb 1.1-MAb 2.1 adhesion system in binding to platelets or sHA.

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MATERIALS AND METHODS

Oral streptococcal strains and growth. *S. sanguis* 133-79 and 2017-78 were obtained from R. R. Facklam, Centers for Disease Control and Prevention, Atlanta, Ga.; strains E1219 and S1219 were naturally occurring erythromycin- and streptomycin-resistant variants derived from the parental strain, *S. sanguis* 133-79. DNA fingerprinting patterns were visually identical within the parent-variant lineages (25). Strain 10556 was originally obtained from the American Type Culture Collection (ATCC), and strains 12 and 12NA were obtained from B. McBride, University of British Columbia, Vancouver, British Columbia, Canada. The following strains were the kind gift of W. F. Liljemark, University of Minnesota, Minneapolis: *S. sanguis* L74, L59, L14, L52, L22, 4124, L13, L31, and 4123; *S. gordonii* 10558, S7, and M5; *S. mutans* GS-5 and BHT; and *S. parasanguis* FW 213. J. Rudney, University of Minnesota, kindly provided *S. sanguis* 804 and HPC1; *S. gordonii* 12396, 33399, and Blackburn; *S. parasanguis* 15911, 15912, and MGH145; *S. oralis* 10557, 9811, and CR834; *S. crista* 51100 and 49999; and *S. mitis* 903. *S. gordonii* V288 was from L. Tao, University of Missouri, Kansas City; *S. mutans* JBP was provided by N. Ganeshkumar, Forsyth Dental Center, Boston, Mass.; and *S. mutans* 25175, 33402, 33535, and Ingbritt were gifts from P. R. Erickson, University of Minnesota.

All strains were stored in skim milk at −80°C. For ELISA and amylase binding and adhesion assays, bacterial cells were transferred from frozen stocks onto mitis salivarius plates and incubated for 48 h at 37°C in 5% CO_2. A single colony was picked, inoculated into Todd-Hewitt broth (THB), and allowed to grow overnight at 37°C in 5% CO_2. The cells were washed three times in 0.01 M sodium phosphate buffer, pH 7.4, with 0.9% sodium chloride (PBS). For assay of sialidase activity, the bacterial cells were grown on Columbia agar supplemented with 5% sterile defibrinated sheep blood (MicroPure, White Bear Lake, Minn.). All the broth and agar for bacterial growth were obtained from Difco, Detroit, Mich.

Phenotypical analysis. Our library of strains of the viridans group streptococci including clinical isolates from human dental plaque (L74, L59, L14, L52, L22, 4124, L13, L31, and 4123), had been phenotyped according to the scheme of Facklam (7) as described previously (14). To further characterize these strains, each was assayed for amylase binding (3, 19) and sialidase activity (1, 31).

To screen for amylase binding, 15-ml aliquots of THB cultures of streptococci were harvested by centrifugation at 1,400 × *g* for 15 min, washed twice in PBS, resuspended in 50 μl of clarified saliva, and allowed to incubate at 37°C for 30 min. Streptococci were then removed by centrifugation and the saliva-containing supernatant (10 μl) was added into wells punched in starch agarose (medium EEO; Fisher, Fair Lawn, N.J.) plates and allowed to incubate at 37°C for 3 h. The 1% (wt/vol) starch agarose was prepared in PBS containing 1% starch (Sigma, St. Louis, Mo.). After incubation, plates were stained by covering the surface with Lugol's iodine. Amylase activity remained in saliva after incubation with amylase binding-negative strains, as indicated by clear or unstained rings around each well. Strains that completely removed the amylase activity from saliva (amylase binding positive) showed no clear or unstained ring.

Sialidase activity was determined as described by Whiley et al. (31). The fluorogenic substrate 2′-(4-methylumbelliferyl)-α-D-*N*-acetylneuraminic acid, dimethyl sulfoxide, and *N*-trismethyl-2-aminoethanesulfonic acid buffer (TES buffer, pH 7.5) were purchased from Sigma. The substrate was dissolved in a minimum volume of dimethyl sulfoxide and diluted in 50 mM TES buffer to a

TABLE 1. Phenotypic characteristics of streptococcal strains

Organism	Fermentation[a] of:		Hydrolysis[a] of:		Amylase binding	Sialidase activity
	Inulin	Raffinose	Arginine	Esculin		
S. sanguis						
133-79[b]	+	−	+	+	−	−
S1219	+	−	+	+	−	−
E1219	+	−	+	+	+	−
L14	+	+	+	+	−	−
L52	+	+	+	+	−	−
L4123	+	−	−	+	−	−
L4124	+	+	+	+	−	−
L22	+	+	+	+	−	−
L59	+	−	+	+	−	−
L13	−	+	−	−	−	+
L31	+	−	+	+	+	−
L74	+	+	+	+	−	−
ATCC 10556	+	−	+	+	−	−
2017-78[b]					−	−
S. gordonii						
M5	+	−	+	+	+	−
ATCC 10558	+	−	+	+	+	−
V288[c]					+	−
S. oralis						
ATCC 10557					−	+
ATCC 9811[d]					+	+
S. mitis ATCC 903					+	−

[a] Data reported originally by Herzberg et al. (11).
[b] Biotyped by R. R. Facklam (5). Strain 133-79 was also ribotyped as consistent with a *S. sanguis* genotype (24). By DNA fingerprinting, the parent strain, 133-79, and variants S1219 and E1219 showed the same lineage (25).
[c] Obtained from Lin Tao, University of Missouri. This strain was initially isolated and identified as a strain of *S. challis* by Don Clewell, University of Michigan. Subsequently, the strain was named V288 and classified as *S. gordonii* by Francis L. Macrina, Virginia Commonwealth University, Richmond (22a). Strain V288 is catalogued by the ATCC as *S. sanguis* 35105.
[d] Genotype identified as *S. oralis* by ribotyping (24, 26).

final concentration of 100 μg/ml. Bacterial colonies were removed with sterile swabs and suspended in TES buffer. The suspension was adjusted to an optical density of 0.1 at 620 nm. Substrate solution (20 μl) was mixed with 50 μl of bacterial suspension in wells of a flat-bottomed, clear microdilution plate and incubated at 37°C for 3 h. Degradation of substrates (release of 4-methylumbelliferyl) was visualized by viewing the plates under a long-wave-length UV lamp; sialidase-positive strains produced blue fluorescence.

Preparation of MAbs. MAbs were prepared as reported previously (9, 10). Briefly, MAbs against *S. sanguis* adhesin (MAb 1.1) were raised by immunizing BALB/c mice intraperitoneally with live cells of strain 133-79. MAb 1.1 was screened against both adhesive and nonadhesive strains and tested for the ability to inhibit adhesion of *S. sanguis* to both platelets and sHA. To produce anti-idiotypic MAbs, the hybridoma producing MAb 1.1 was injected intraperitoneally into BALB/c mice as described previously (10). The enlarged spleens were harvested and MAb 2.1 hybridomas were prepared. MAb 2.1 was screened in indirect ELISA for reaction with rabbit polyclonal IgG antibodies against the 87-kDa adhesin antigen and also tested for inhibition of *S. sanguis* adhesion to platelets and sHA.

Indirect ELISA. Streptococcal strains were screened by ELISA for reaction with MAb 1.1 as described by Elder and Fives-Taylor (6), with slight modifications (10). The bacteria were washed in PBS, resuspended in sodium carbonate buffer (pH 9.6), added to 96-well flat-bottom microtiter plates (Costar, Cambridge, Mass.) at 5×10^6 cells/well, and allowed to incubate overnight at 4°C. The plates were dried at room temperature (RT) and stored at 4°C. Immediately before use in ELISA, the plates were washed three times with PBS with 0.05% Tween 20 (PBST) and blocked with 1% bovine serum albumin (Sigma) for 1 h at RT. Next, 100 μl of a 1:4 dilution of MAb 1.1 culture supernatant (containing about 1 μg of IgG1/ml) was added to each well and incubated for 3 h at RT. After three additional washes with PBST, a 1:3,000 dilution of goat anti-murine IgG conjugated with alkaline phosphatase (Bio-Rad, Richmond, Calif.) was added at 100 μl/well. After 2 h of incubation at RT, the wells were washed and the substrate was added at 150 μl/well. The plates were allowed to stand at RT for 1 h and then read at 405 nm. Reaction of nonspecific mouse IgG (0.25 μg/ml, 100 μl/well) with each strain served as the negative control (background).

Adhesion assays. All procedures for the platelet-bacterium adhesion assay were performed as described previously (11). In brief, platelets from outdated platelet-rich plasma (PRP) (American Red Cross Blood Center, St. Paul, Minn.) were washed with PBS. Washed platelets and washed streptococcal cells were incubated together or alone (controls) in microwells; the small clusters of adhering platelets and bacteria were separated from noninteracting particles by centrifugation. The sedimentation of adhering mixtures relative to controls was quantitated by the following formula: percent adhesion = $100 \times \{1 - [\text{mixture } A_{620}/(\text{bacterium } A_{620} + \text{washed-platelet } A_{620})/2]\}$. Based on previous studies of the variability of the method (14), only adhesion scores of ≥20% were considered positive.

Adhesion of streptococcal cells to sHA was assayed by a modification of the method used by Liljemark and coworkers (21, 22) and Tellefson and Germaine (30). The assay was performed in 1.5-ml polypropylene microcentrifuge tubes

TABLE 2. Adhesion phenotype

Organism	% Adhesion to:		Reaction with MAb 1.1 (A_{405})[c]
	sHA[a]	Platelets[b]	
S. sanguis			
133-79	24.0 ± 0.9	59.0 ± 1.3	0.177 ± 0.036
S1219	27.4 ± 3.5	63.2 ± 4.5	0.205 ± 0.011
E1219	1.1 ± 0.02	7.5 ± 2.0	0
L74	16.0 ± 3.4	24.6 ± 10.3	0.104 ± 0.016
L59	18.5 ± 2.9	42.2 ± 1.3	0.135 ± 0.012
L14	22.3 ± 2.0	53.4 ± 2.1	0.165 ± 0.027
L52	19.0 ± 0.9	46.6 ± 13.8	0.167 ± 0.038
L22	20.7 ± 0.8	46.3 ± 4.7	0.127 ± 0.027
4124	23.5 ± 1.3	56.8 ± 3.0	0.229 ± 0.007
2017-78	3.8 ± 0.9	10.1 ± 3.9	0.075 ± 0.005
4123	0.5 ± 0.02	11.0 ± 3.0	0.043 ± 0.014
12	3.2 ± 0.4	16.7 ± 1.3	0.063 ± 0.018
12NA	4.4 ± 0.8	15.4 ± 0.2	0.070 ± 0.014
ATCC 10556	2.2 ± 0.2	2.8 ± 0.9	0.006 ± 0.006
804	1.4 ± 0.1	3.9 ± 1.1	0
HPC1	2.0 ± 0.2	2.9 ± 2.1	0
S. crista			
ATCC 51100	7.3 ± 0.9	11.0 ± 1.1	0.063 ± 0.017
ATCC 49999	6.6 ± 1.0	2.8 ± 0.4	0.037 ± 0.004
S. gordonii			
V288	14.2 ± 1.5	49.6 ± 4.9	0.163 ± 0.005
ATCC 10558	15.7 ± 1.6	36.5 ± 2.7	0.162 ± 0.025
ATCC 12396	22.0 ± 0.6	25.1 ± 4.2	0.109 ± 0.020
ATCC 33399	16.2 ± 1.0	3.0 ± 0.2	0.016 ± 0.005
Blackburn	26.1 ± 0.2	2.6 ± 0.1	0
S7	2.1 ± 0.1	4.5 ± 0.4	0
M5	4.1 ± 1.0	4.8 ± 0.6	0
L31	40.2 ± 8.2	14.9 ± 1.6	0.027 ± 0.026
S. mitis ATCC 903	0.7 ± 0.1	1.9 ± 0.4	0
S. mutans			
JBP	7.9 ± 0.6	34.1 ± 3.0	0.144 ± 0.023
Ingbritt	6.6 ± 1.3	5.8 ± 0.9	0
ATCC 25175	2.5 ± 0.03	7.6 ± 2.4	0.028 ± 0.026
ATCC 33402	0.2 ± 0.03	0	0.014 ± 0.007
ATCC 33535	2.5 ± 0.2	8.8 ± 2.6	0.058 ± 0.011
GS-5	0.4 ± 0.06	2.8 ± 1.8	0.015 ± 0.012
BHT	1.5 ± 0.1	4.4 ± 0.4	0.002 ± 0.026
S. oralis			
ATCC 10557	18.7 ± 0.7	21.6 ± 2.7	0.098 ± 0.014
9811	1.1 ± 0.1	5.5 ± 1.4	0
CR834	3.4 ± 0.1	3.4 ± 0.6	0.022 ± 0.02
L13	43.7 ± 7.6	17.1 ± 2.2	0.022 ± 0.014
S. parasanguis			
ATCC 15911	20.5 ± 5.4	5.6 ± 0.6	0.038 ± 0.011
ATCC 15912	0.7 ± 0.1	3.1 ± 1.0	0.003 ± 0.013
MGH145	0.4 ± 0.03	3.7 ± 0.6	0.016 ± 0.008
FW 213	1.3 ± 0.2	6.5 ± 1.4	0.077 ± 0.011

[a] Values are expressed as means ± standard deviations ($n = 3$).
[b] Means of duplicate determinations with each of two platelet samples.
[c] Values are expressed as means ± standard deviations ($n = 4$).

TABLE 3. Frequency of strain-specific streptococcal binding of 42 strains to sHA, platelets, and MAb 1.1

Adhesion[a]	No. of strains	
	MAb 1.1 positive[b]	MAb 1.1 negative
sHA positive and platelet positive	13	0
sHA negative and platelet negative	0	21
sHA positive and platelet negative	0	8

[a] sHA-positive strains showed >6.5% adhesion; platelet-positive strains showed >20% adhesion.
[b] MAb 1.1-positive strains showed an A_{405} of >0.098 in ELISA.

with 0.01 M phosphate buffer, pH 6.8 (PB), at RT in all experiments. Streptococci were grown overnight in THB with 10 μCi of [*methyl*-^{3}H]thymidine (Research Products International Corp., Mount Prospect, Ill.) per ml. Radiolabeled cells were washed three times with PB, sonicated three times for 3 s each to break the bacterial chains, and resuspended in the same buffer at 10^9 cells/ml. The specific activity for labeled streptococcal strains varied from 752 ± 22 (mean ± standard deviation) to 9,792 ± 152 bacteria per cpm. The data are presented as a percentage of total bacterial input (10^9 cells) adhering to 10 mg of sHA and were calculated as follows: [(radioactivity associated with sHA × specific activity)/10^9 cells] × 100. Given the sensitivity and reproducibility of the method, an adhesion score of ≥6.5% was considered positive.

To learn if the strains adhere to the same binding site, platelets (10^9/ml) or sHA (10 mg/ml) was preincubated for 30 min with 1% bovine serum albumin and then incubated for 1 h at RT with MAb 2.1 (2 nmol of IgG/10^9 platelets/100 μl or 33.3 pmol of IgG/10 mg of sHA). Nonbinding MAb 2.1 was removed by washing, and streptococcal adhesion to platelets or sHA was tested. The percent inhibition of streptococcal adhesion was calculated as follows: [(adhesion_{PB} − adhesion_{MAb})/adhesion_{PB}] × 100, where adhesion_{PB} occurs in the presence of buffer and adhesion_{MAb} occurs in the presence of added antibody.

RESULTS

We confirmed the phenotypes of representative strains from our panel (Table 1). Several strains were previously classified as *S. sanguis* (14) but based on additional criteria were considered for reassignment. Strain L31 (inulin fermentation positive and arginine and esculin hydrolysis positive) and E1219, an erythromycin-resistant variant of strain 133-79, were amylase binding positive. Based on its ability to bind amylase, strain L31 was reclassified as *S. gordonii*. Strain E1219, which was also amylase binding positive, was not reassigned because the parent strain was confirmed to be *S. sanguis*. Strain L13 (inulin fermentation negative, raffinose positive, and arginine and esculin hydrolysis negative) showed sialidase activity and was reassigned as *S. oralis*.

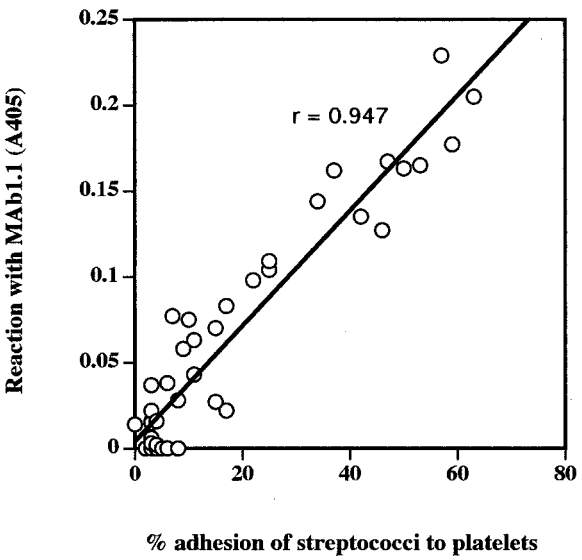

FIG. 1. Correlation between MAb 1.1 binding and platelet adhesion. Each point represents the mean values for MAb 1.1 binding (n = 4) and adhesion to platelets (n = 2) for a given strain of streptococcus.

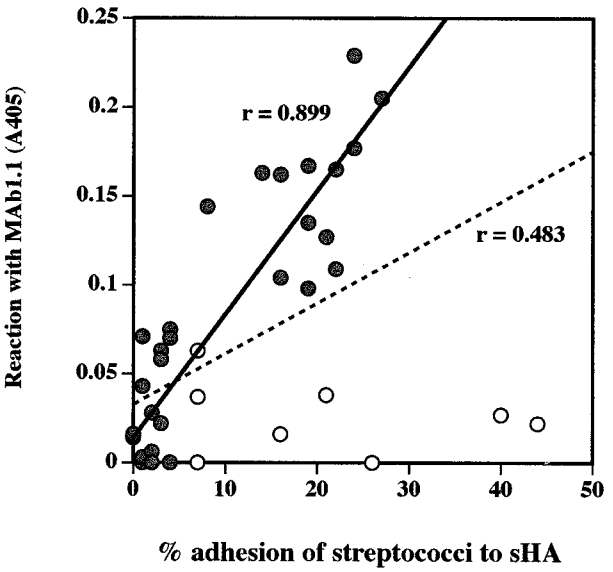

FIG. 2. Relationship among strains in their ability to adhere to sHA and bind with MAb 1.1. The broken line represents the relationship when all 42 strains (open and solid circles) were included in the analysis. For analysis excluding sHA adhesion-positive, platelet adhesion-negative strains (open circles), the solid line represents the relationship for all other strains (solid circles). Each point represents the mean values for sHA binding (n = 3) and MAb 1.1 binding (n = 4) for a given strain of streptococcus.

Of 16 *S. sanguis* strains, 50% were adhesion positive with both platelets (>20% adhesion) and sHA (>6.5% adhesion) (Table 2). Among the seven species surveyed, 13 of 42 strains (31%) adhered to platelets and 21 (50%) adhered to sHA. All strains that bound to platelets also adhered to sHA and could be identified as MAb 1.1 binding positive (Table 3). Strains that were MAb 1.1 binding negative did not adhere to platelets. Conversely, sHA adhesion-positive, platelet adhesion-negative strains also did not react with MAb 1.1.

Binding of MAb 1.1 by all strains was highly correlated with their ability to adhere to platelets (r = 0.949) (Fig. 1). When strains that only adhered to sHA were excluded from the analysis, the abilities of the remaining strains to adhere to sHA and bind with MAb 1.1 were strongly related (r = 0.897) (Fig. 2). When all strains were included in the analysis, the correlation between the ability to adhere to sHA and binding with MAb 1.1 was lower (r = 0.481).

For all streptococcal strains that bound MAb 1.1, adhesion to sHA and platelets could be inhibited by the anti-idiotypic MAb 2.1 (Table 4). Examples of MAb 1.1-positive strains inhibited in adhesion activities by MAb 2.1 were noted in most taxa of viridans group streptococci tested. Inhibition of streptococcal adhesion to sHA and platelets by MAb 2.1 was highly correlated with the ability of strains to bind MAb 1.1 (r = 0.832 and 0.777, respectively) (Fig. 3).

DISCUSSION

Of the *S. sanguis* strains and viridans group streptococci tested, every strain that bound to platelets also adhered to sHA. Adhesion to sHA and platelets was shown previously to be inhibited by MAb 1.1 in a dose-dependent manner (9, 10).

TABLE 4. Inhibition of streptococcal adhesion to sHA and platelets by MAb 2.1

Organism	% MAb 2.1 inhibition of adhesion to:	
	sHA[a]	Platelets[b]
S. sanguis		
133-79	57.5 ± 1.0	40.7 ± 1.7
S1219	65.0 ± 2.2	46.6 ± 5.9
L74	86.9 ± 2.8	56.5 ± 4.5
L59	64.9 ± 1.8	48.3 ± 5.3
L52	47.3 ± 1.2	31.2 ± 3.0
L22	56.0 ± 10.8	37.6 ± 6.1
L14	66.4 ± 1.9	42.9 ± 1.6
4124	48.9 ± 2.0	29.2 ± 6.5
4123	0	0
12	0	0
12NA	4.2 ± 0.8	4.5 ± 0
2017-78	13.2 ± 0.5	0
S. gordonii		
V288	66.1 ± 3.5	52.1 ± 5.5
ATCC 10558	56.6 ± 10.8	33.1 ± 1.3
ATCC 12396	51.1 ± 0.6	45.4 ± 1.7
ATCC 33399	0	0
Blackburn	0	0
L31	9.5 ± 6.5	0
S. mutans		
JBP	72.2 ± 0.6	34.0 ± 0.3
Ingbritt	0	0
ATCC 25175	0	13.8 ± 0.6
ATCC 33535	5.3 ± 1.0	15.1 ± 1.1
S. oralis		
ATCC 10557	46.4 ± 0.7	52.3 ± 0.8
CR834	0	0
L13	9.6 ± 5.0	0
S. parasanguis		
ATCC 15911	0	0
FW213	0	24.3 ± 1.1

[a] Values are means ± standard deviations (n = 3).
[b] Means of duplicate determinations with each of two platelet samples.

MAb 1.1 reacts with a two-domain adhesin on *S. sanguis* 133-79 (9); this adhesin is required for interactions with human platelets (10) and sHA (9). The adhesin-specific MAb 1.1 reacts with strains that adhere to both platelets and sHA (9, 10), suggesting that the same functional adhesin epitope is expressed. MAb 1.1, therefore, served in this study as a marker for this functional adhesin epitope. While strains vary in the amount of MAb 1.1 required for saturation, the current data show that the MAb 1.1-positive adhesin epitope is widely expressed among the viridans group streptococci. MAb 1.1-positive strains also adhere to these two disparate substrates. Other strains are MAb 1.1-negative. As expected based on work with *S. sanguis* 133-79, these strains failed to bind to platelets (10). For those MAb 1.1-negative strains that adhered well to sHA, an alternative adhesin specificity is implied to function.

The anti-idiotypical MAb 2.1 was prepared by immunization of syngeneic mice with MAb 1.1 (10). MAb 2.1 should mimic immunochemically the adhesin epitope recognized by MAb 1.1. Hence, MAb 2.1 should bind the immunochemically complementary binding sites for the *S. sanguis* adhesin marked by MAb 1.1 on adhesion substrates such as platelets and sHA. Indeed, MAb 2.1 bound to sHA and platelets to inhibit adhesion of MAb 1.1-positive strains. MAb 2.1 identifies, therefore, an immunochemically specific binding site for adhesion of MAb 1.1-positive viridans group streptococci. These platelet and sHA binding sites or receptors (identified by the anti-idiotype MAb 2.1) for streptococcal cells are likely to share essential structural features but to consist of very different proteins. Alternative binding sites on sHA for viridans group streptococci are also implied to function for MAb 1.1-negative strains.

The oral streptococci express a constellation of adhesins (17), several of which may be MAb 1.1 reactive (9). Among the better-characterized families of adhesins are P1 (2, 16), CshA and -B (23), and FimA (8, 20). In addition, *S. gordonii* expresses an amylase binding protein (27). While it is clear that the sanguis group expresses many protein adhesins, this report demonstrates a novel, epitope-specific, functional conservation.

The data indicate that MAb 1.1 and the corresponding anti-idiotypic MAb 2.1 mark a specific adhesion system for viridans group streptococci. The adhesion system is comprised of an adhesin epitope and its structurally complementary binding site or receptor. Of the 42 strains of viridans group streptococci tested, at least 13 employed this adhesion system for platelets and sHA. The ability to use the MAb 1.1-MAb 2.1 adhesion system was independent of taxon. To consider taxonomic restrictions on this trait, the classification of many of the strains in the panel was reevaluated on the basis of amylase binding (3, 4, 19) and sialidase activity (1). The original taxonomy for the strains in the panel employed the strategy of Kilian et al. (18). For several of the 16 strains for which taxonomic identity was reevaluated, the classification could be questioned. Strain L31, which produced acid from inulin, hydrolyzed arginine and esculin, and bound α-amylase, was reclassified from *S. sanguis* to *S. gordonii*. *S. sanguis*-like strains that bind amylase are generally classified as *S. gordonii* or other species (4, 19). In contrast, strain L13, which was negative for inulin fermentation, hydrolysis of arginine and esculin, and amylase binding, fermented

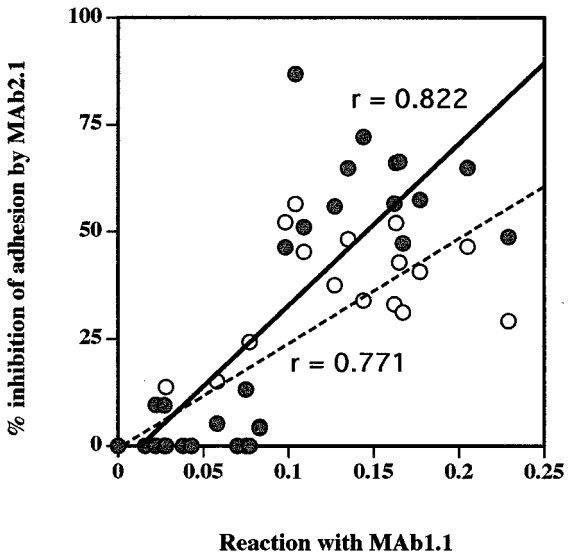

FIG. 3. Correlation between MAb 1.1 binding and MAb 2.1 inhibition of streptococcal adhesion to sHA and platelets. MAb 2.1 was preincubated with either sHA (solid circles) or platelets (open circles) and then tested for inhibition of adhesion with strains of viridans group streptococci. The relationship between MAb 2.1 inhibition of adhesion to sHA and the ability of strains to bind MAb 1.1 is shown by the solid line (solid circles). The relationship between MAb 2.1 inhibition of adhesion to platelets and MAb 1.1 binding is shown by the broken line (open circles). Each point represents the mean of at least three determinations.

raffinose and expressed sialidase activity. Based on the phenotypic characteristics, strain L13 was identified as *S. oralis* (1, 31). Strain E1219 was an erythromycin-resistant variant of *S. sanguis* 133-79 (14). It was not reclassified as *S. gordonii*, even though it binds amylase. The parent strain, 133-79, was confirmed to be *S. sanguis* by ribotyping (24) and the variant, which was selected after growth in the presence of erythromycin, was shown to be from the parental lineage by DNA fingerprinting (25). Further study of strain E1219 will be necessary to make a correct taxonomic assignment. Strain L4123 was classified as *S. sanguis*, even though it did not hydrolyze arginine. The assignment of this strain is clearly uncertain, but the limited scope of phenotypic characteristics examined precluded a decisively better assignment.

Hence, most of our *S. sanguis* strains remain assigned to that taxon, except for strains L13 and L31. It is clear, however, that expression of the MAb 1.1-MAb 2.1 adhesion system, which confers the ability of viridans group streptococci to adhere to both platelets and sHA, is independent of taxon and is common among the viridans group streptococci.

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