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Effects of Local Immunization of Hamsters with Glucosyltransferase Antigens on Infection with *Streptococcus sanguis*

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The effects of immunization with antigens of the *Streptococcus mutans* glucosyltransferase (GTF) complex on oral challenge with two *Streptococcus sanguis* strains (H7PR3 and 34) in hamsters were studied. Antisera to *S. mutans* GTF complex were able to inhibit one-third (strain H7PR3) to one-half (strain 34) of the *S. sanguis* GTF activity which could be inhibited when these *S. sanguis* GTFs were incubated with antisera to *S. sanguis* GTF. Washed, intact cells of strains H7PR3 and 34 were able to remove a significant amount of enzyme inhibitory activity and immunoglobulin G antibody activity from antisera to *S. mutans* GTF. These results established the existence of an antigenic relationship between *S. sanguis* and *S. mutans* GTFs. The effect of injection of *S. mutans* strain 6715 GTF or phosphate-buffered saline, incorporated into complete Freund adjuvant, on oral challenge with *S. sanguis* was compared in 243 hamsters in nine experiments. Salivary and serum GTF inhibitory activity was present in all GTF-injected animals before challenge. After a 2- or 3-day challenge with *S. sanguis* H7PR3 (seven experiments) or 34 (two experiments), fewer bacteria were recovered from GTF-injected hamsters in every experiment. Significant differences were observed in six of the nine experiments. During the 7- to 21-day period after challenge, 33% of the phosphate-buffered saline-injected sham group (group I) still had *S. sanguis* recoverable from the molar surfaces, whereas only 19% of the *S. mutans* GTF-injected group (group II) remained infected with *S. sanguis* ($P < 0.01$). These results suggest that immunization with GTF from *S. mutans* may influence the colonization potential of *S. sanguis* in the oral cavity.

Glucan in dental plaque may mediate binding of individual *Streptococcus mutans* organisms to one another (homotypic attachment [13]) and binding of *S. mutans* to other organisms, such as *Streptococcus sanguis* (heterotypic attachment [14]). Interference with the amount of glucan formed by *S. mutans* and *S. sanguis* could conceivably affect the ability of *S. mutans* cells to accumulate on dental surfaces by interfering with glucan-mediated homotypic and heterotypic binding. Both streptococcal species synthesize glucosyltransferase (GTF), which is responsible for glucan formation (3, 9, 25). Thus, GTF has been identified as a potential antigen for use in a human caries vaccine.

Immunization of rodents with GTF from *S. mutans* interferes with infection and disease caused by *S. mutans* (18, 23). The molecular basis for this protection is not completely understood; however, immunized animals have salivary antibody to GTF which preferentially inhibits water-insoluble glucan formation in vitro (24). The extent of the protective response elicited by immunization with GTF is broad. Animals immunized with GTF from one serotype are protected from infection with *S. mutans* of other serotypes (17, 18).

S. sanguis is one of the first microbial species to be found in human dental plaque (1, 2). Since these oral streptococci synthesize GTF, it is of interest to understand the influence that antibody to *S. mutans* GTF may have on the presence of *S. sanguis* in the oral cavity. We have shown that serum and salivary antibody to *S. mutans* GTF demonstrates limited in vitro reactivity with GTF from *S. sanguis* strain 34 (20). The studies described here were designed to explore the in vitro and in vivo antigenic relationships between *S. mutans* and *S. sanguis* GTFs.

MATERIALS AND METHODS

Bacteria. *S. mutans* strain 6715 (serotype g) and *S. sanguis* strains 34 and H7PR3 were obtained from J. van Houte, Forsyth Dental Center, Boston, Mass. Strains 6715, 34R, and H7PR3 are resistant to streptomycin at concentrations of 2,000 μg/ml.

TABLE 1. Experiment protocol

Expt	Hamster strain	Group and antigen (n)[a]		S. sanguis infection[b]		Dietary streptomycin supplement
		I PBS	II GTF (6715)	H7PR3	34R	
A	NIH white	+ (16)	+ (16)	+ (3 d)		Yes
B	LHC/lac	+ (15)	+ (15)	+ (3 d)		Yes
C	NIH white	+ (13)	+ (33)	+ (3 d)		Yes
D	LHC/lac	+ (12)	+ (12)	+ (3 d)		Yes
E	NIH white	+ (15)	+ (14)	+ (2 d)		Yes
F	NIH white	+ (13)	+ (13)	+ (2 d)		No
G	LHC/lac	+ (14)	+ (14)	+ (2 d)		No
H	NIH white	+ (12)	+ (12)		+ (2 d)	No
I	LHC/lac	+ (14)	+ (14)		+ (2 d)	No

[a] Groups were injected with PBS or GTF or WIP (experiment E) from *S. mutans 6715* incorporated into complete Freund adjuvant.

[b] Groups were orally infected with streptomycin-resistant strains of *S. sanguis* H7PR3 or 34R for the number of days (d) indicated in parentheses, as described in Materials and Methods.

GTF preparations. GTF for immunization and antibody analyses was prepared as previously described for *S. mutans* (19). Briefly, *S. sanguis* 34 or *S. mutans* 6715 were grown anaerobically in a 10% CO_2–90% N_2 atmosphere for 24 h at 37°C in 6 to 10 liters of chemically defined media (21). Water-insoluble polysaccharide (WIP) was synthesized after the addition of 10% sucrose in 0.02% sodium azide to cell-free supernatants. Each streptococcal strain formed more than 90% of the total polysaccharide as the water-insoluble fraction. Hamsters in experiment E were injected with a portion of the washed WIP from *S. mutans* after four washes with phosphate-buffered saline (PBS), pH 7.5. GTF enzymes for the remaining experiments were then eluted from the remaining washed WIP by incubation with 1 volume of 6 M guanidine hydrochloride for 1 h at room temperature. Enzymatic activity was determined in the dialyzed eluate by the Somogyi (22) and Glucostat (Worthington Biochemicals, Freehold, N.J.) assays. Protein was also measured (12). Fructose was the principal sugar released (*S. mutans*, 78%; *S. sanguis*, 83%) after incubation of the enzyme preparations with sucrose for 2 h at 37°C. *S. sanguis* and *S. mutans* GTF antigens were enriched by gel filtration on a column containing 10% agarose (Bio-Rad Laboratories, Richmond, Calif.). Column fractions were monitored spectrophotometrically (280 nm) and by the chemical assays described above. GTF activity eluted in the void volume and was pooled for use as antigen. Antigen prepared in this fashion has been shown to contain GTF, glucan, and glucan-binding protein as determined by disc gel electrophoresis for protein and by activity, immunodiffusion, Lowry and phenol-H_2SO_4 assays (19).

Immunization experiments. NIH white hamsters (raised at the Forsyth Dental Center) (experiments A, C, E, F, and H) or LHC/*lac* cream hamsters (Charles River/Lakeview Breeding Laboratories, Wilmington, Mass.) (experiments B, D, G, and I) were used for immunization. Two hamster strains were used because of limited availability of the strains from their respective suppliers. Both hamster colonies are free of *S. mutans* and *S. sanguis*. The H7PR3 and 34R strains of *S. sanguis* were used for infection because the resistance of the organisms to streptomycin facilitated identification in plaque samples. The protocol for immunization and infection in all experiments is shown in Table 1. In the experiments, all hamsters were randomly distributed into two treatment groups: group I was sham immunized with 0.1 ml of PBS incorporated into 0.1 ml of complete Freund adjuvant, and group II was immunized with 0.1 ml of GTF or WIP (experiment E) from *S. mutans* strain 6715 incorporated into complete Freund adjuvant and infected with *S. sanguis* strain H7PR3 (experiments A, B, C, D, E, F, and G) or 34R (experiments H and I). Animals in groups I and II were first injected when they were 25 days old and two to five times subsequently at 7-day intervals in the vicinity of the parotid and submandibular salivary glands. Three injections gave high levels of inhibitory antibody. The additional injections were given to maintain elevated levels of salivary antibody when infection was delayed. Each dose of *S. mutans* GTF antigen contained 0.30 mIU of enzyme activity in approximately 30 μg of protein for all experiments.

All hamsters were maintained on pelleted Purina Mouse Chow from weaning until 3 days before infection. At this time, animals in experiments A, B, C, D, and E were given diet 2000 (8) containing streptomycin sulfate (1 g per 5.5 kg of diet 2000) to facilitate implantation of *S. sanguis* organisms. This diet was continued for approximately 2 weeks, at which time it was replaced with diet 2000 alone. A streptomycin-resistant flora appeared in the oral cavities of animals given this diet, making enumeration of the challenge organisms difficult. Therefore, animals in experiments F, G, H, and I were given only diet 2000 without streptomycin. Weights of animals were monitored during each experiment and were not significantly different among groups of hamsters in each experiment on any occasion.

Saliva and serum. Saliva and sera were collected, treated, and stored before antibody assay as described previously (23). Saliva for inhibition of [^{14}C]glucose incorporation assays was dialyzed first against PBS containing 0.001 M EDTA and then against PBS alone.

Assay for inhibition of GTF activity. The procedure for determining inhibition of GTF activity by serum and saliva is a modification (20) of the method of Evans and Genco (6). GTF activity was determined by [^{14}C]glucose incorporation from glucosyl-labeled sucrose into ethanol-insoluble polysaccharide as previ-

ously described (19). Briefly, 5 μl of serum was preincubated with 0.80 mIU of GTF in 100 μl of 0.02 M phosphate buffer (pH 6.8) for 1 h at 37°C in a shaking water bath. A total of 330 μg of primer dextran T10 (Pharmacia Fine Chemicals, Inc., Piscataway, N.J.) and 0.018 μg of [^{14}C]glucose-labeled sucrose (specific activity, 275 mCi/mmol) in 0.2 ml of 0.01 M sodium phosphate were then added. This mixture was incubated at 37°C for 2 h, and the reaction was stopped by precipitation with ethanol (final concentration, 75% [vol/vol]). After centrifugation of the mixture at 13,000 × *g*, the pellet was dissolved in distilled water and reprecipitated with ethanol. The centrifuged pellet was dissolved in 0.2 ml of distilled water and mixed with 4 ml of Ready-Solv Solution IV (Beckman Instruments, Inc., Palo Alto, Calif.) and counted in a liquid scintillation spectrometer. Salivary inhibition was assayed by incubating 20 μl of dialyzed, unconcentrated saliva with 20 μl of GTF and 10 μl of 1% bovine serum albumin for 1 h at 37°C. A 330-μg amount of primer dextran and 0.018 μg of labeled sucrose were then added in 50 μl of sodium phosphate and incubated for 5 h. Glucan was then precipitated, washed, and counted as described above. Inhibition by immune serum or saliva samples was determined in the following manner. The counts per minute (cpm) incorporated into precipitated polysaccharide by enzyme in the presence of immune fluid was first expressed as a percentage of that incorporated by enzyme in the presence of the appropriate control fluid and then subtracted from 100: percentage of inhibition = 100 − [(immune cpm/control cpm) × 100].

Adsorption of GTF inhibitory activity. Bacterial cells (*S. mutans* strains 6715 and Ingbritt; *S. sanguis* strains H7PR3 and 34) used for adsorption experiments were grown for 24 h in tryptic soy broth (supplemented with glucose to 1%) at 37°C. Cells were collected by centrifugation, washed, and resuspended in PBS containing 0.2% NaN_3 and 6 mM NaF. A 0.33-ml suspension containing 10^9 cells was incubated for 2 h at room temperature with 0.15 ml of a 1:1,200 dilution of an antiserum pool obtained by immunizing six hamsters with *S. mutans* 6715 WIP (Table 1). Sera were collected approximately 1 month after the final injection. Cells were removed by centrifugation, and the adsorbed, diluted sera were tested for inhibitory activity for *S. mutans* 6715 GTF as described above. Sera from hamsters sham immunized with PBS in complete Freund adjuvant were similarly adsorbed and used as controls. Results were expressed as the percentage of reduction in counts per minute of adsorbed immune sera compared with that of adsorbed sham-immunized sera.

Antibody analysis. Antibody in adsorbed and unadsorbed sera was determined by a modification of the indirect enzyme-linked immunosorbent assay (5). GTF antigens for analysis of antibody-containing fluids were as used for immunization. Antigen (1 to 3 μg of protein) in 0.1 M sodium carbonate buffer (pH 9.6) was added to wells of polystyrene microtiter plates (Linbro Scientific, McClean, Va.) and incubated for 3 h at 37°C. Antigen-coated plates were washed and then incubated with appropriate dilutions of hamster serum. The amount of bound antibody was determined by addition of the rabbit anti-hamster immunoglobulin G serum, followed by addition of goat anti-rabbit immunoglobulin G (Miles Laboratories, Inc., Elkhart, Ind.) conjugated to alkaline phosphatase (type VII; Sigma Chemical Co., St. Louis, Mo.). After overnight incubation at room temperature, the substrate (*p*-nitrophenyl phosphate; Sigma) was added and the degree of reaction was determined at 400 nm.

Infection. Hamsters in groups I and II were orally infected within 15 days after the last injection with 0.3 ml of concentrated cultures of *S. sanguis* strain H7PR3 or 34R (approximately 10^9 CFU). Hamsters were infected twice each day, within 15 days after the last injection with 0.3 ml of concentrated cultures of *S. sanguis* strain H7PR3 or 34R (approximately 10^9 CFU), on the following days: experiment A, days 68, 69 and 70; experiment B, days 47, 48, and 49; experiment C, days 62, 63, and 64; experiment D, days 76, 77, and 78; experiment E, days 68 and 69; experiment F, days 44 and 45; experiment G, days 151 and 152; experiment H, days 51 and 52; experiment I, days 151 and 152. Before infection, salivary and serum anti-GTF activity (inhibition activity) to the homologous antigen was present in all GTF-injected animals.

The flora of all animals was periodically monitored by systematic swabbing of molar surfaces with Calgiswabs (Inolex Corp., Glenwood, Ill.) (23). Swabs were vortexed in 2 ml of 1/4 strength Ringer solution, appropriately diluted, and plated in duplicate on mitis salivarius agar and mitis salivarius agar containing 400 μg of streptomycin per ml. CFU of sham (group I) and immune (group II) animals were compared by Mann-Whitney U analysis (16). Differences between the implantation of organisms between groups were analyzed by the Kolmogorov-Smirnov one-sample test (16). In all experiments, comparisons were made between sham-immunized hamsters in group I and GTF-immunized hamsters in group II.

RESULTS

Antigenic relationships between *S. sanguis* and *S. mutans* GTFs. Previous experiments have shown that an antigenic relationship exists between GTFs from *S. sanguis* and *S. mutans* (19). To extend these observations for the *S. sanguis* strains used for infection in the present experiments, the following experiments were performed. A pooled antiserum to *S. mutans* 6715 GTF (diluted 1:3,200) was preincubated with 10^9 intact *S. mutans* or *S. sanguis* cells. The loss of homologous GTF inhibitory activity (Table 2) and reduction of antibody activity to the *S. mutans* 6715 GTF preparation was then measured (Table 2). Adsorption with homologous 6715 cells removed 84% of the inhibitory activity. Adsorption with a similar number of *S. sanguis* H7PR3 or 34 cells removed 58 and 38% of the inhibitory activity, respectively. Similar relationships were observed when adsorbed sera were assayed against the *S. mutans* GTF antigen preparation in the enzyme-linked immunosorbent assay. These results suggest that these cells contain surface-bound GTF which cross-react with *S. mutans* GTF. Representative sera from hamsters injected with GTF from *S. mutans* 6715 or *S. sanguis* 34 were also tested for their

TABLE 2. Adsorption of antibody activity in a hamster antiserum pool (1:3,200 dilution) to WIP of *S. mutans* 6715 with homologous and heterologous cells

Adsorbent[a]	% Inhibition of GTF (6715)[b]	ELISA (OD_{400})[c]
None	45 ± 2	0.823
S. mutans 6715	7 ± 1	0.273
S. sanguis H7PR3	19 ± 1	0.447
S. sanguis 34	27 ± 2	0.512

[a] A total of 10^9 cells were grown in tryptic soy broth and incubated with 0.4 ml of a 1:3,200 dilution of a serum pool from six hamsters immunized with WIP of *S. mutans* 6715 twice in 14 days and bled 3 weeks later.

[b] Inhibition calculated as 100 − [(GTF-injected sera cpm/sham-injected sera cpm) × 100]. A pool of sera from hamsters injected with PBS plus complete Freund adjuvant and adsorbed with the respective cells was used to calculate inhibition. Results are shown as mean ± standard error.

[c] GTF (6715) bound to polystyrene plates used as antigen to assay amount of immunoglobulin G antibody remaining to the GTF complex. OD_{400}, Optical density at 400 nm.

ability to inhibit enzyme from *S. mutans* and *S. sanguis* H7PR3 and 34 (Table 3). Sera from hamsters injected with GTF from either *S. mutans* or *S. sanguis* showed significant inhibition of enzyme from *S. sanguis* H7PR3, although sera from *S. sanguis* GTF-injected hamsters gave low inhibition of *S. mutans* GTF, as reported previously (20). These experiments indicate that an antigenic relationship exists between GTF from *S. mutans* 6715 and that from the two *S. sanguis* strains used for infection.

Effect of immunization with GTF and WIP from *S. mutans* on challenge with *S. sanguis*. Saliva and sera taken before infection from hamsters in experiments A through I were tested for inhibitory activity of GTF from *S. mutans* 6715 (Table 4). Hamsters in group II, which had been immunized with GTF from *S. mutans*, showed high levels of serum and salivary inhibitory activity to the homologous GTF.

To investigate the effect of immunization of hamsters with *S. mutans* GTF on infection with *S. sanguis* H7PR3 or 34R, we infected immunized and sham immunized hamsters by the protocols described in Table 1. Results with *S. sanguis* recovered from molar surfaces after challenge with H7PR3 and 34R are shown in Tables 5 and 6, respectively. Hamsters immunized with GTF from *S. mutans* (experiments A, B, C, D, F, G, H, and I) or WIP from *S. mutans* (experiment E) had fewer *S. sanguis* organisms recovered from molar surfaces than sham (PBS)-immunized animals on virtually every swabbing occasion, regardless of the number of infections or the presence of streptomycin in the diet. These differences were statistically significant on five of seven occasions on day 1 after challenge with H7PR3 (Table 5), and one of two occasions on the first day after challenge with 34R (Table 6).

The numbers of CFU of *S. sanguis* in the dental plaque of hamsters continued to decrease after initial challenge in both sham and immune animals. The numbers of hamsters from which *S. sanguis* could be recovered immediately and greater than 7 days after challenge are shown in Tables 5 and 6. *S. sanguis* could be recovered from virtually all hamsters on the initial swabbing occasion. When data from all experiments were combined, 33% of sham-immunized hamsters were still infected with *S. sanguis* during the 1- to 3-week period after challenge, whereas only 19% of the *S. mutans* GTF-immunized hamsters were positive for *S. sanguis* (significant at the $P < 0.01$ level). Significant differences were seen between the number of infected

TABLE 3. Comparison of percentage of inhibition of GTF by sera from hamsters injected with GTF from *S. sanguis* 34 or *S. mutans* 6715

1:10 diluted sera from hamsters injected with:	% Inhibition with following GTF (*n*)[a]:		
	S. mutans 6715	*S. sanguis* 34	*S. sanguis* H7PR3
6715 GTF	71 ± 3 (23)	35 ± 3 (25)	20 ± 4 (17)
S34 GTF	4 ± 5 (17)	63 ± 3 (19)	56 ± 2 (14)

[a] Arithmetic mean of percent inhibition ± standard error of the respective GTF by immune sera calculated as 100 − [(GTF-injected sera cpm/sham-injected sera cpm) × 100].

TABLE 4. Percentage of inhibition of GTF by serum and saliva from GTF (6715)-injected hamsters

Expt	% Inhibition in indicated group (antigen) by[a]:			
	Serum (1:10)		Saliva (Undiluted)	
	I (PBS)	II (GTF 6715)	I (PBS)	II (GTF 6715)
A	1 ± 1	70 ± 2	0 ± 2	24 ± 3
B	3 ± 3	73 ± 1	7 ± 3	15 ± 2
C	4 ± 3	73 ± 2	5 ± 4	24 ± 2
D	4 ± 4	76 ± 1	0 ± 2	33 ± 3
E	0 ± 5	68 ± 4	4 ± 2	33 ± 2
F	2 ± 2	64 ± 14	NT	NT
G	0 ± 1	67 ± 5	1 ± 3	23 ± 3
H	0 ± 1	57 ± 8	NT	NT
I	1 ± 1	71 ± 6	1 ± 4	25 ± 4

[a] Arithmetic mean (± standard error) of inhibition compared with that obtained with buffer control for all animals in group. The enzyme used in the inhibition assay was GTF (6715) for both serum and saliva. NT, Not available for testing.

TABLE 5. Bacterial recoveries from molar surfaces of hamsters immunized with glucosyltransferase (GTF) or water-insoluble polysaccharide (WIP) from *S. mutans* 6715 after challenge with *S. sanguis* H7PR3

Expt	Immunization treatment	Group	CFU ($\times 10^2$) of *S. sanguis* recovered on day 1[a]	No. positive for *S. sanguis*/total no. of hamsters on day:	
				1[a]	>7[a]
A	PBS	I	493	15/16	6/16
	GTF (6715)	II	59 ($P < 0.01$)[b]	16/16	3/16
B	PBS	I	7	14/15	0/15
	GTF (6715)	II	3 ($P < 0.05$)	14/15	0/15
C	PBS	I	562	13/13	5/14[c]
	GTF (6715)	II	200 ($P < 0.01$)	33/33	5/27[c]
D	PBS	I	251	12/12	NT[d]
	GTF (6715)	II	199	12/12	
E	PBS	I	189	15/15	12/13[c]
	WIP (6715)	II	69 ($P < 0.01$)	14/14	6/15[c]
F	PBS	I	214	13/13	0/12
	GTF (6715)	II	68 ($P < 0.025$)	12/13	2/12
G	PBS	I	82	14/14	5/14
	GTF (6715)	II	63	14/14	3/14
	Total (%)				
	PBS			97/99 (98)	23/58[c] (33)
	GTF			115/119 (97)	19/99[c] (19)

[a] One day and more than 7 days after a 3-day challenge with *S. sanguis* H7PR3, except experiment F, in which animals were swabbed 5 days after challenge.

[b] Differences between CFU of *S. sanguis* recovered from sham- and GTF-injected groups were statistically significant using the Mann-Whitney U analysis.

[c] Differences between infected and noninfected groups I and II statistically significant using Kolmogorov-Smirnov one-sample test.

[d] NT, Not tested.

sham group I and GTF-injected group II hamsters in experiments C and E.

DISCUSSION

Immunization of hamsters with GTF from *S. mutans* 6715 was often associated with significant changes in the numbers of organisms of *S. sanguis* strains H7PR3 and 34R recovered from plaque after challenge. *S. mutans* GTF-injected hamsters all had significant levels of salivary antibody to 6715 (Table 4) which has been shown to cross-react with *S. sanguis* GTF (20). The ability of GTF antibody to interfere with *S. sanguis* infection in the oral cavity could occur by several mechanisms. *S. sanguis* has cell-associated GTF which, depending on the strain, can form large amounts of glucan (7). *S. sanguis* strain H7PR3 forms predominantly water-soluble glucan in the presence of sucrose, whereas strain 34R forms mostly water-insoluble glucan under these conditions (personal observation). Antibody to antigens associated with the *S. mutans* GTF complex, which recognizes antigenically cross-reactive sites on the *S. sanguis* cell-associated GTF, could promote clearance by agglutinating these organisms in saliva, thus diminishing recovery of these organisms from plaque. *S. sanguis* organisms colonize hard surfaces in the oral cavity earlier than do *S. mutans* organisms (1, 2). Stimulation of antibody to *S. mutans* in the oral cavity before *S. mutans* colonization, i.e., before the appearance of hard surfaces, would presumably enhance the effectiveness of a dental caries vaccine. The results of the present investigation suggest that the antibody-mediated clearance of *S. sanguis* by antibody to *S. mutans* GTF could affect the colonization potential of *S. sanguis*.

Initial attachment of *S. sanguis* to hard surfaces in the presence of saliva is reported to be mediated by glycoproteins (10). Thus, antibody to antigens in the GTF complex may not be expected to interfere with these interactions. However, Liljemark and colleagues (11) have

TABLE 6. Bacterial recoveries from molar surfaces of hamsters injected with GTF from *S. mutans* after challenge with *S. sanguis* 34R

Expt	Treatment	Group	CFU ($\times 10^2$) of *S. sanguis* 34R recovered on day 1[a]	No. positive for *S. sanguis*/total no. of hamsters on day:	
				1	>7[a]
H	PBS	I	16	11/12	NT[b]
	GTF (6715)	II	6	9/11	
I	PBS	I	75	14/14	5/14
	GTF (6715)	II	29 ($P < 0.01$)[c]	14/14	3/14

[a] One day or greater than 7 days after final challenge with *S. sanguis* 34R.
[b] NT, Not tested.
[c] Differences between CFU of *S. sanguis* recovered from sham- and GTF-injected groups were statistically significant using the Mann-Whitney U analysis.

reported that small aggregates of these organisms bind to saliva-coated hydroxyapatite more effectively than do single cells. Since glucan can mediate aggregate formation (14), antibody to GTF could limit aggregate size and thereby interfere with this aspect of colonization. *S. sanguis* can also form heterotypic bonds with *S. mutans* via glucan (14). In addition, *S. mutans* cells have the capacity to bind GTF from *S. sanguis* (7), thereby moderately increasing the ability of these cells to demonstrate sucrose-dependent adherence to glass surfaces. Both of these phenomena may potentially increase the plaque-forming ability of cariogenic *S. mutans*. These contributions of *S. sanguis* to the pathogenicity of *S. mutans* could also be expected to be diminished by GTF antibody either by inhibition of enzyme activity or by steric interference with heterotypic interactions. An understanding of the potential effects of immunization with GTF on microorganisms in the plaque is important for the formulation and application of an effective human caries vaccine.

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