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Antigenicity and Immunogenicity of a Synthetic Peptide Derived from a Glucan-Binding Domain of Mutans Streptococcal Glucosyltransferase

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The immunogenicity and antigenicity of a multiply antigenic peptide construct containing four copies of the synthetic peptide TGAQTIKGQKLYFKANGQQVKG were measured in rodents and humans, respectively. The composition of this peptide construct (termed GLU) was derived from a major repeating sequence in the C-terminal region of mutans streptococcal glucosyltransferases that synthesize water-insoluble glucan (GTF-I). The GLU peptide elicited high levels of serum immunoglobulin G antibody to GLU after subcutaneous injection into Sprague-Dawley rats. These antisera also reacted with intact GTF isozymes from *Streptococcus sobrinus* and *Streptococcus mutans* (by enzyme-linked immunosorbent assay [ELISA] and Western blot [immunoblot] analyses) and with an 87-kDa glucan-binding protein from *S. sobrinus* (by Western blot). The synthesis of filter-retained glucan by GTF-Sd of *S. sobrinus* could be inhibited (30%) by preincubation with anti-GLU rat serum. Splenic and lymph node lymphocytes from rats injected once with *S. sobrinus* GTF isozymes demonstrated significant proliferation after 5 days of culture with GLU. The GLU peptide reacted with 4 of 29 human parotid saliva samples and 5 of 29 human serum samples (by ELISA). These results suggest that the GLU peptide contains B- and T-cell epitopes that are similar to those of intact mutans streptococcal GTFs and possibly certain other glucan-binding proteins as well. Furthermore, since antibody to this epitope(s) appears to inhibit GTF function, sequences within this peptide construct may have value for inclusion in a synthetic dental caries vaccine.

Mutans group streptococci have been implicated in the initiation of dental caries in humans (12). The ability of these organisms to accumulate on the tooth surface has been associated, in part, with the extracellular synthesis of glucans from sucrose (2, 9). The glucan-mediated accumulation of cariogenic mutans streptococci in dental plaque occurs through its interaction with cell-associated glucan-binding proteins (GBPs). Mutans streptococcal glucans are synthesized by constitutively secreted glucosyltransferase enzymes (GTFs). Since these enzymes will bind to glucan (20), they can also be considered GBPs. Because of the central role that GTFs play in dental plaque formation, they have been considered as potential components of a dental caries vaccine (30). Experiments with animal models and with humans have supported this potential. For example, GTFs from *Streptococcus sobrinus* and *Streptococcus mutans* of the mutans streptococci have been demonstrated to elicit immune responses which are protective against experimental dental caries caused by infection with several mutans streptococcal species (24, 25, 29). The salivary immunoglobulin A (IgA) antibody response to oral and/or local administration of GTFs to humans has been significantly correlated with interference with reaccumulation of indigenous mutans streptococci (23, 27). Alternative routes of active immunization that elicited primarily an IgG antibody response to other mutans streptococcal antigens in the gingival crevicular fluid and passive oral administration of IgG antibody have also been shown to modify *S. mutans* colonization (reviewed by Michalek and Childers [17]).

Although the basis for experimental protection with GTF-based vaccines is unknown, it is likely to involve antibody-mediated inhibition of the catalytic and/or the glucan-binding activity of GTF (31, 32). Separate molecular domains appear to be responsible for these activities. A catalytic site, i.e., the transfer of glucose from sucrose to glucan, has been identified in the N-terminal third of the GTF molecule (18, 19). Structural (1, 6) and biochemical (20, 35) studies suggest that a separate glucan-binding region(s) exists in the C-terminal third of the GTF molecule. Analysis of the deduced amino acid sequences of GTFs from both *S. mutans* and *S. sobrinus* reveals multiple repeating sequences (8, 21). Tryptic fragments from this area of the GTF molecule can bind to glucan polymers (35). Furthermore, the principal GTF repeating C-terminal sequences have considerable homology with repeating sequences of a non-GTF streptococcal protein having glucan-binding characteristics (3, 34). Thus, these repeating sequences have been suggested to be associated with the glucan-binding properties of GTF.

Antibody directed to epitopes within catalytic or glucan-binding domains has the potential to disrupt the formation of mutans streptococcal plaques by several mechanisms. For example, inhibition of GTF enzyme activity would diminish the glucan-mediated accumulation of oral bacteria. In addition, mutans streptococci could be cleared from the oral cavity by antibody-induced aggregation through interaction with cell-associated GTF. Identifying such epitopes within GTF would permit informed strategies for genetically engineered- or synthetic peptide-based vaccine preparation. The aim of the present study was to design a synthetic peptide construct whose sequence was derived from highly conserved, duplicated regions within the putative C-terminal glucan-binding domain of GTF. The design also included synthesis of four copies of the peptide on a lysine backbone

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TABLE 1. Comparison of the synthetic peptide sequence with deduced sequences in the A repeating region of GTFs and in a GBP from oral streptococci

Protein	Streptococcal species (reference)	Deduced sequence	% Homology with synthetic peptide
GTF-I	*S. downei* (21)	1293 FYLGKDGAAV TGAQTIKGQKLYFKANGQQVKG DIV 1328	100
GTF-I	*S. sobrinus* (1)	1288 FYLGEDGAAV TGAQTIRGQKLYFKANGQQYEG DIV 1322	86
GTF-I	*S. salivarius* (7)	1373 YYAGANGKTV TGAQVINGQHLYFNADGSQVKG GVV 1407	77
GTF-S	*S. downei* (8)	1225 WGYRKDGQLL TGEQTIDGQKVFFQDNGVQVKG GTA 1260	68
GTF-I	*S. mutans* (21)	1279 FYFDNNGYAV TGARTINGQLLYFRANGVQVKG EFV 1313	77
GBP	*S. mutans* (3)	264 WFYLEDGKAA IGWRTIGKK.YYFDTNGVQVKG 295	50
Synthetic peptide		TGAQTIKGQKLYFKANGQQVKG	

(28) in order to enhance immunogenicity while avoiding the irrelevant responses that result from use of carrier proteins. The immunogenicity of the resulting peptide construct in rats was then evaluated with respect to antibody reactivity with and lymphocyte proliferation by the peptide, *S. sobrinus* GTF, and *S. mutans* GTF and with respect to functional inhibition of GTF activity.

MATERIALS AND METHODS

Antigens. (i) GLU synthetic peptide. Analyses of deduced GTF amino acid sequences and the ability of tryptic peptides from the C-terminal third of GTF to bind glucan suggested that a glucan-binding domain may be associated with repeating "A" sequences within this region (5, 18, 20). We selected a 22-mer peptide, TGAQTIKGQKLYFKANGQQVKG, that had complete homology with the derived sequence of the A repeat region (residues 1293 through 1328) of *Streptococcus downei* GTF-I (Table 1) (21). The GTF sequence associated with this peptide is modestly hydrophilic (4) and is very similar to sequences in the proposed glucan-binding regions of the *S. sobrinus* and *S. mutans* GTF-I molecules (Table 1). The peptide was synthesized (Applied Diagnostics, Foster City, Calif.) by the stepwise solid-phase method of Merrifield (16) on a core matrix of three lysines to yield a multiply antigenic peptide (MAP) macromolecule with four identical 22-mer peptides per molecule, by the method of Tam (28). Purity (>90%) was assessed by high-pressure liquid chromatography, amino acid analysis, and molecular weight determination by mass spectrometry. This MAP construct, referred to as GLU, was used for immunization and antibody analysis.

(ii) Poly-L-lysine and poly-DL-alanine–poly-L-lysine synthetic peptides. Poly-L-lysine (approximate mass, 10.5 kDa) and poly-DL-alanine–poly-L-lysine (approximate mass, 153 kDa) (Sigma Chemical Co., St. Louis, Mo.) were used as controls in various peptide analyses.

(iii) GTFs. GTFs from *S. sobrinus* 6715 and *S. mutans* JF were separated from glucose-grown defined-medium cultures by affinity chromatography on Sephadex G-100 (Pharmacia Fine Chemicals) with 3 M guanidine HCl as the eluting solvent. These GTF-rich pools were then subjected to fast protein liquid chromatography (FPLC) on Superose 6 (Pharmacia) with 6 M guanidine for elution as previously described (21). The gel filtration step removes non-GTF and other GBPs from GTF preparations of both mutans streptococcal species, as evidenced by the fact that only components with enzyme activity were observed after sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis. The *S. mutans* GTF preparation taken to this level of purification synthesized greater than 95% water-soluble glucan by both tube and filter assays (32) and thus was analogous to the *gtfD* gene product of *S. mutans* GS5 described by Hanada and Kuramitsu (10, 11, 22). This preparation was designated GTF_{sm} and was used for injection, enzyme-linked immunosorbent assay (ELISA), lymphocyte blastogenesis, and enzyme inhibition studies with human sera.

S. sobrinus GTF preparations obtained after gel filtration on Superose 6 contained a mixture of GTF-I (water-insoluble glucan product), GTF-Sd (primer-dependent water-soluble glucan product), and GTF-Si (primer-independent water-soluble glucan product) (13, 14). This preparation was designated GTF_{ss} and was used for injection, ELISA, and lymphocyte blastogenesis.

S. sobrinus GTFs used for measuring enzyme inhibition by rat antisera were obtained by ion-exchange chromatography of the Superose 6 pool on DEAE–Bio-Gel A (Bio-Rad) (32) by the method of McCabe et al. (13, 14). Enzymes were bound in 0.01 M sodium acetate (pH 5.5) and released in an NaCl gradient (0 to 1 M). The GTF-I eluted at a concentration of 0.44 M NaCl. Virtually all of the glucan product of GTF-I synthesis was water insoluble in both tube and filter assays (assay conditions reported in reference 32). Glucan synthesis by GTF-I was enhanced threefold in the presence of dextran primer. The quantitatively predominant GTF, eluting at a higher salt concentration, synthesized a water-soluble product from sucrose in tube assays. Enzyme activity was enhanced sixfold in the presence of dextran primer; thus, this GTF species was considered to be GTF-Sd as reported by McCabe et al. (14). In filter assays, a fraction of the glucan product of GTF-Sd synthesis was retained by the filter. The percentage of retained glucan varied from 8 to 30%, depending on the enzyme-substrate ratio. Thus, for the purposes of the inhibition assay (see Table 3) the enzyme activities of GTF-Sd were empirically described as water soluble (not retained by the filter) and water insoluble (retained by the filter).

(iv) GBPs. A GBP was purified from *S. sobrinus* 6715 by a combination of affinity chromatography on Sephadex G-100 (Pharmacia), FPLC on Superose 6 (Pharmacia), and ion-exchange chromatography on Mono-Q (Pharmacia). The first two steps have been previously described for GTF preparation (26). The GBP pool eluted at 56% of the bed volume of the Superose 6. This pool was then applied to a Mono-Q column in 0.02 M bis-Tris–6 M urea (pH 6.5) and eluted in this buffer with an NaCl gradient (0 to 1 M). The GBP eluted at a position of 0.15 M NaCl. This preparation revealed a principal band of 87 kDa and minor, lower-molecular-mass bands in SDS–7% polyacrylamide gel electrophoresis.

ELISA. Blood was taken from the antecubital sinuses of 29 healthy young adult male human subjects and the retroorbital sinuses of rats. Serum was then obtained from the coagulated specimen after centrifugation and stored frozen at −20°C until use. Human parotid saliva (27) and rat whole saliva (29) samples were collected as previously described. Serum and saliva samples were tested for antibody activity by a previously described biotin-avidin, alkaline phosphatase ELISA method (27). Polystyrene microtiter plates (Flow Laboratories, McLean, Va.) were coated with either 1 μg of polyalanine-polylysine or 0.5 μg of the GLU peptide per ml or with 0.5 μg of *S. sobrinus* 6715 GTF_{ss} (Superose 6 pool containing GTF-I, GTF-Sd, and GTF-Si) or *S. mutans* JF GTF_{sm} (Superose 6 pool containing GTF-S activity). Antibody activity was then measured by incubation with 1:50 to $1:10^6$ dilutions of serum or 1:4 dilutions of saliva. Plates were then developed for IgG or IgA antibodies with appropriate biotinylated affinity-purified goat anti-rat or anti-human gamma-chain or alpha-chain reagents (Zymed, South San Francisco, Calif.), followed in sequence by strepavidin-alkaline phosphatase (Zymed) and *p*-nitrophenylphosphate. Antibody activity was expressed either as the reciprocal serum dilution of the last well whose absorbance (A_{405}; Biotek Instruments, Winooski, Vt.) was above the reagent background or as ELISA units after comparison of experimental absorbancies (A_{405}) that fell within the linear portion of a reference standard curve constructed from dilutions of a reference serum or saliva that had previously been shown to contain high levels of antibody activity to the respective antigen.

Immunization. In the first experiment, 11 CD strain Sprague-Dawley 65-day-old rats (Charles River Breeding Laboratories, Kingston, N.Y.) were used for injection. Groups (n = 3) consisted of rats injected subcutaneously (s.c.) in the flanks and back with (i) 50 μg of the GLU synthetic peptide, (ii) 25 μg of *S. sobrinus* 6715 GTF_{ss} (Superose 6 pool containing GTF-I, GTF-Sd, and GTF-Si), (iii) 25 μg of poly-L-lysine, or (iv) buffer alone (sham injected; n = 2). All rats were injected once, and all antigens, including buffer, were administered with complete Freund adjuvant (CFA). Blood was drawn 21 days after injection, and spleen and lymph nodes were removed for lymphocyte proliferation assays. In the second experiment, 17 CD strain Sprague-Dawley 65-day-old male rats (mean weight, 279 g) (Charles River Breeding Laboratories) were used for injection. Groups of two or three rats were injected s.c. in the flanks and back with (i) 50 μg of the GLU synthetic peptide, (ii) 25 μg of *S. sobrinus* 6715 GTF_{ss}, (iii) 10 μg of *S. mutans* JF GTF_{sm} (Superose 6 pool containing GTF-S activity), or (iv) buffer alone (sham injected). The initial injection included CFA; three subsequent injections included incomplete Freund adjuvant. The additional injections were given at approximately 3-week intervals. Animals were bled prior to injection and biweekly throughout the injection period. Rats were exsanguinated and spleens and lymph nodes were removed 88 days after the first injection and 12 days after the final injection, for lymphocyte proliferation assays.

Antibody inhibition of glucan synthesis. Rat sera were evaluated by a filter assay for their ability to inhibit glucan synthesis of *S. sobrinus* GTF-I and GTF-Sd. Rat sera diluted 1:10 or 1:50 with 0.02 M sodium phosphate (pH 6.5)–0.2% sodium azide (PBA) were preincubated with the respective GTF for 1 h in 0.04 ml of PBA, and then 1.7 mg of sucrose and 44 nCi of [^{14}C-*glucose*]sucrose (approximately 100,000 cpm) were added in 0.2 ml of PBA in the presence of 37 μg of dextran T10 (Pharmacia). Incubation proceeded for 18 h at 37°C, after which water-insoluble glucan was collected on Whatman GF/F glass fiber filters and washed with PBA and radioactivity was counted as previously described (32). Glucan in the filtrate was precipitated with 70% ethanol in the presence of carrier dextran T10, centrifuged, and processed as for total glucan (32). Essentially all of the glucan formed by *S. sobrinus* GTF-I was captured on the filter. Under the conditions of the assay, approximately 70% of the glucan formed by *S. sobrinus* GTF-Sd could be found in the filtrate.

Lymphocyte blastogenesis. Single-cell suspensions were prepared by centrifugation on Ficoll-Histopaque. Lymphocytes (5×10^5) were examined for proliferative responses to polylysine peptide, GLU peptide, polyalanine-polylysine peptide, *S. sobrinus* GTF_{ss}, or *S. mutans* GTF_{sm}. Cells were cultured with or without additive in RPMI 1640, supplemented with glutamine, 5×10^{-5} M 2 mercaptoethanol, 12.5 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid), 10% fetal calf serum, penicillin (100 IU), and streptomycin (100 μg/ml) for 5 days in culture at 37°C in 95% CO_2. Tritiated thymidine (New England Nuclear; 0.5 mCi per well) was added for the final 16 h of culture.

RESULTS

Immunogenicity of the GLU synthetic peptide. (i) ELISA. The sera of rats injected once s.c. with the synthetic MAP construct (GLU) or with GTF_{ss} from *S. sobrinus* were tested by ELISA for IgG antibody activity. Antibody to the homologous antigen was detected in three of three rats injected with GLU (mean of 224 ELISA units) and in three of three rats injected with GTF (mean of 216 ELISA units). Only one of three rats injected with GTF_{ss} from *S. sobrinus* reacted with GLU after one injection (mean of 7 ELISA units).

In a separate experiment, groups of rats were injected four times s.c. with GLU in CFA, or with CFA alone. The synthetic peptide elicited high levels of serum IgG antibody after this course of injection (Fig. 1, right). Serum IgG antibody to the immunizing peptide was detected in all rats after the second injection and continued to rise thereafter. No salivary IgA antibody to peptide could be detected at day 88 in the peptide-injected rats, although significant salivary IgA anti-GTF activity was detected in the rats injected with Superose 6-purified GTF_{ss} or GTF_{sm} (data not shown).

Other groups of rats were injected in a similar fashion with Superose 6-purified *S. sobrinus* GTF_{ss} or *S. mutans* GTF_{sm}. The reciprocal dilutions of sera from all immunized rats, each tested in ELISA against GLU, Superose 6-purified *S. sobrinus* GTF (GTF_{ss}), or *S. mutans* GTF (GTF_{sm}) are shown in Fig. 1. Reaction with GLU as the coating antigen was detected only with the serum IgG antibody of rats injected with the GLU (Fig. 1, right). A significant degree of cross-reactivity was observed when intact GTFs were used as the coating antigen (Fig. 1, left and center). GTF from *S. sobrinus* elicited a serum IgG antibody response which reacted with the homologous antigen at reciprocal dilutions of greater than 10^5 (Fig. 1, center) and with the heterologous GTF at dilutions of greater than 10^4 (Fig. 1, left). With GTF from *S. mutans* as the immunogen, reactive (Fig. 1, left) and cross-reactive (Fig. 1, center) responses with both intact GTF enzymes were detected in the sera. Importantly, sera from rats injected with GLU also reacted with both intact GTF antigens, albeit at much lower dilutions (1:800 to 1:1,600). These cross-reactivities provide evidence that a common epitope may exist on GLU and intact GTF.

Western blot. The ability of sera from rats injected with

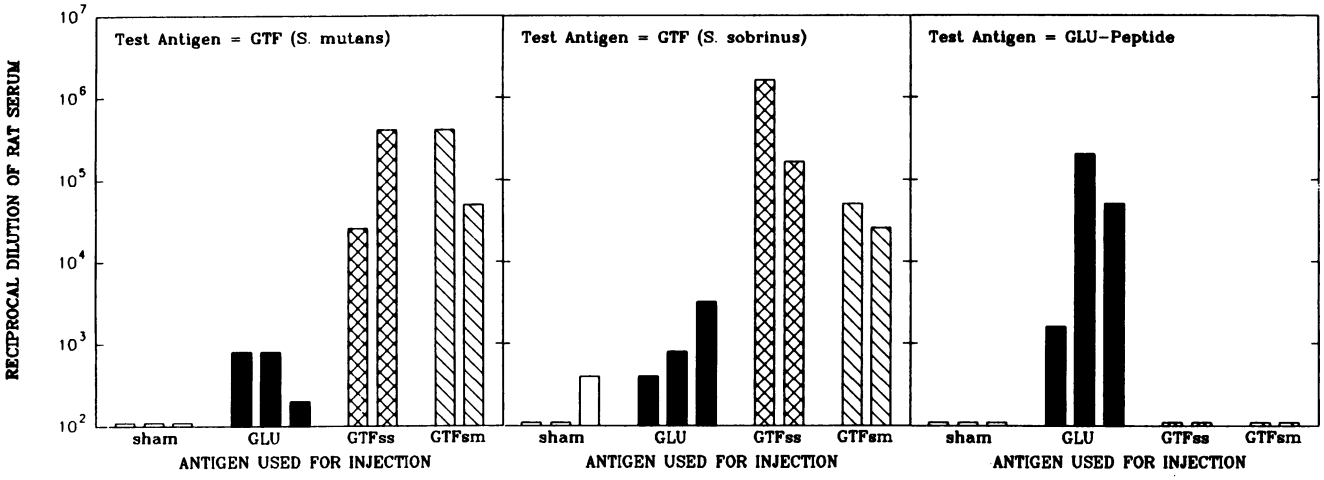

FIG. 1. IgG responses in sera of rats sham injected four times with CFA and incomplete Freund adjuvant (open bars) or injected four times with CFA and the Superose 6 pool of *S. sobrinus* GTF (GTF_{ss}, which contained GTF-I, GTF-Sd, and GTF-Si isozymes) (crosshatched bars), the Superose 6 pool of *S. mutans* GTF (GTF_{sm}, which contained GTF-S activity) (hatched bars), or the synthetic MAP construct (GLU) (solid bars). Bars within each panel represent the mean data of triplicate assays from individual rat sera. Antibody activity was measured by ELISA. Preimmune and injected rat sera were tested with each antigen simultaneously. Absorbance data obtained with preimmune sera were then subtracted from the absorbance data obtained with the sera from the respective injected rats at each serum dilution. The reciprocal dilution of the last subtracted A_{405} above background is presented for each injected serum-preimmune serum pair. The antigen used for injection of each rat and the test antigen used to coat the ELISA plates are indicated. Superose 6 pools of both *S. sobrinus* and *S. mutans* were used for injection and antibody analysis.

GLU to react with Sepharose-purified *S. sobrinus* or *S. mutans* GTFs or with GBP from *S. sobrinus* was also demonstrated by Western blot (immunoblot) (Fig. 2) analysis after polyacrylamide gel electrophoresis separation of the respective antigen and transfer to nitrocellulose. Rat antisera to the purified GTFs gave reactions with both heterologous and homologous antigens in the 150- to 165-kDa range (data not shown). Antisera to GLU reacted with Superose 6-purified *S. mutans* GTF_{sm} (Fig. 2, lane 1) and *S. sobrinus* GTF_{ss} (lane 2). Rat antisera to GLU also reacted with an 87-kDa GBP from *S. sobrinus* (lane 3), suggesting the possible presence of a similar epitope on GBP, GTF, and GLU.

Functional activity of antibody to GLU. Sera from rats injected four times with GLU or GTF were tested for their ability to inhibit the formation of glucan from [^{14}C-*glucose*-]sucrose by *S. sobrinus* isozymes GTF-I and GTF-Sd. A representative experiment is shown in Table 2. Serum from one of the three rats (R5) injected with the synthetic peptide significantly inhibited (30%) the GTF-Sd-catalyzed synthesis of the glucan retained on the filter. Formation of filter-retained glucan by either the GTF-I or GTF-Sd enzyme was nearly completely inhibited by antisera to the GTF_{ss} (i.e., rats injected with a mixture of GTF-I, GTF-Sd, and GTF-Si isozymes), which also inhibited 84% of the glucan synthesized by GTF Sd which passed through the filter.

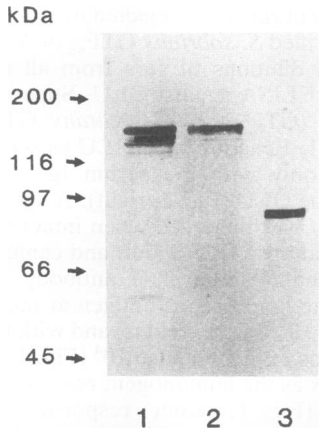

FIG. 2. Western blot profile of *S. mutans* GTF_{sm} (lane 1), *S. sobrinus* GTF-I (lane 2), and *S. sobrinus* GBP (lane 3) after exposure to rat antiserum against GLU (R5) and subsequent development with γ-chain-specific reagents as described in Materials and Methods. Migration of molecular mass standards is shown at the left of the blot.

Lymphocyte blastogenesis. The existence of T-cell epitopes on GLU was studied by measuring spleen and lymph node lymphocyte blastogenesis of tissues taken 21 days after rats had been given one s.c. injection of GLU, GTF from *S. sobrinus* 6715, or adjuvant alone. Cells (5×10^5) were cultured for 5 days with either no additive (control), GLU, or Superose 6 pools of *S. sobrinus* GTF (GTF_{ss}) or *S. mutans* GTF (GTF_{sm}). No significant proliferation above background values was detected after culture of lymph node or spleen cells from sham-injected rats with any antigen. The greatest proliferation of lymph node lymphocytes from rats injected with *S. sobrinus* GTF was with the homologous GTF antigen ($P < 0.001$) (Table 3). Significant proliferation was also observed when these lymph node cells were cultured with the synthetic peptide ($P < 0.01$) or GTF_{sm} ($P < 0.01$). Spleen cells from GTF_{ss}-injected rats also showed significant proliferation with the homologous GTF and the synthetic peptide. Significant proliferation occurred when lymph node and spleen cells from rats injected with GLU were cultured with the GLU (Table 3). Although no significant proliferation was observed when lymph node cells from these animals were cultured with either GTF preparation, spleen cells from two of three hyperimmunized rats (injected four times) showed significant ($P < 0.01$) proliferation with GTF_{ss} compared with background values.

TABLE 2. Inhibition of DEAE-cellulose-separated *S. sobrinus* GTF-I- or GTF-Sd-mediated glucan formation by rat antisera to GLU or to GTF_{ss}

Immunogen (rat serum)[a]	GTF-I		GTF-Sd			
			Filter-retained glucan		Glucan in filtrate	
	Filter-retained glucan (cpm)	% Inhibition[b]	cpm	% Inhibition	cpm	% Inhibition
Sham (R1)	8,375 ± 300[c]		2,883 ± 195		4,725 ± 0	
Sham (R2)	7,855 ± 97		2,889 ± 106		4,636 ± 26	
GLU (R3)	8,154 ± 76	0	2,942 ± 384	0	4,535 ± 188	3
GLU (R4)	8,213 ± 129	0	2,784 ± 298	4	4,543 ± 266	3
GLU (R5)	7,750 ± 135	5	2,075 ± 136[d]	30	4,774 ± 184	0
GTF_{ss}[e] (R6) (1:10)	267 ± 20[f]	97	157 ± 14[f]	95	759 ± 136	84[f]
GTF_{ss} (R6) (1:50)	346 ± 13[f]	96	408 ± 26[f]	86	Not tested	

[a] All antisera were tested at 1/10 dilutions unless otherwise indicated.
[b] Percent inhibition of GTF activity for the respective glucan product as measured in the filter assay. The mean incorporation of [^{14}C]glucose from [^{14}C-*glucose*]sucrose into the respective glucan in the presence of both sham-injected rat sera was taken as the 100% value.
[c] Mean ± standard deviation. Triplicate assays were done for sera R1, R2, R5, and R6; duplicate assays were done for sera R2 and R3.
[d] $P < 0.05$, compared with sera from sham-injected rats tested with the same GTF species.
[e] Superose 6 pool of *S. sobrinus* GTF, containing GTF-I, GTF-Sd, and GTF-Si.
[f] $P < 0.001$, compared with sera from sham-injected rats tested with the same GTF species.

Antigenicity of the GLU synthetic peptide. (i) ELISA. Subjects who were infected with mutans streptococci could, theoretically, respond immunologically to epitopes on the GTFs of indigenous microorganisms. Therefore, antibody to GTF epitopes which might react in common with GLU was sought in serum and parotid saliva samples by ELISA, using GLU, *S. sobrinus* GTF_{ss}, *S. mutans* GTF_{sm}, and polyalanine-polylysine as antigens. Figure 3 shows the serum IgG antibody reactions with GLU and with GTF from *S. sobrinus* 6715 for 29 subjects infected with mutans streptococci. Five serum samples (samples 9, 18, 23, 28, and 29) reacted with the synthetic peptide. However, the only significant correlation seen with serum IgG antibody was between the two mutans streptococcal GTF preparations ($r^2 = 0.7153$; $P < 0.001$ [data not shown]). Figure 4 shows the IgA antibody activity in parotid saliva samples from the same 29 subjects as analyzed for Figure 3. The IgA antibody levels in four of these saliva samples (samples 17, 27, 28, and 29) were elevated. The levels of salivary IgA antibody to *S. mutans* GTF were positively correlated with the levels of salivary antibody to *S. sobrinus* GTF ($r^2 = 0.4928$; $P < 0.01$). In addition, the levels of salivary IgA antibody to GLU were significantly correlated with levels of IgA antibody reactive with *S. sobrinus* GTF ($r^2 = 0.7067$; $P < 0.001$) or *S. mutans* GTF ($r^2 = 0.4947$, $P < 0.01$).

DISCUSSION

The structure of the MAP construct containing four copies of the sequence TGAQTIKGQKLYFKANGQQVKG (GLU) was based on the deduced amino acid sequence, from position 1303 to position 1324, of GTF-I of *S. downei* (21). This sequence was derived from a pattern of repeating sequences which have been identified in all GTFs from mutans streptococci whose amino acid sequences have been deduced from their nucleotide sequences (1, 6, 9, 11, 12). These major GTF repeating sequences, referred to as A repeats, are approximately 35 residues long and are located in the C-terminal third of GTF (Table 1). The significance of these sequences is thought to lie in their ability to bind glucan. The association of these sequences with glucan-binding function is based on the preservation of α1,6-glucan-binding ability (i) in large C-terminal tryptic fragments of GTF (18, 35) and (ii) in recombinant products containing some or all of the C-terminal third of GTF-I of *S. downei* (5) or *S. sobrinus* (1). The glucan-binding potential of these repeating GTF sequences is also supported by the observation that amino acid deletions in this region remove the glucan-binding activity (5) and that a non-GTF GBP of *S. mutans* contains reiterated sequences that are very similar to those found in GTF (3). In fact, nearly half of the sequence

TABLE 3. Proliferation of rat lymphocytes to GLU synthetic peptide and GTFs

Cells	Immunogen	Mean ^{3}H cpm ± SE with test antigen[a]			
		Control	GLU	GTF_{ss}	GTF_{sm}
Spleen	GLU	431 ± 19	1,253 ± 293[b]	514 ± 61	383 ± 34
	GTF_{ss}	452 ± 34	1,375 ± 425[b]	2,730 ± 933[b]	640 ± 124
	Sham	340 ± 41	354 ± 154	345 ± 72	550 ± 175
Lymph node	GLU	522 ± 67	3,808 ± 1441[b]	818 ± 228	931 ± 270
	GTF_{ss}	894 ± 109	3,526 ± 1021[b]	27,423 ± 6,811[c]	2,194 ± 55[b]
	Sham	160 ± 11	144 ± 14	172 ± 21	221 ± 40

[a] Three rats were tested per group.
[b] Level of significance compared with control, $P < 0.01$.
[c] Level of significance compared with control, $P < 0.001$.

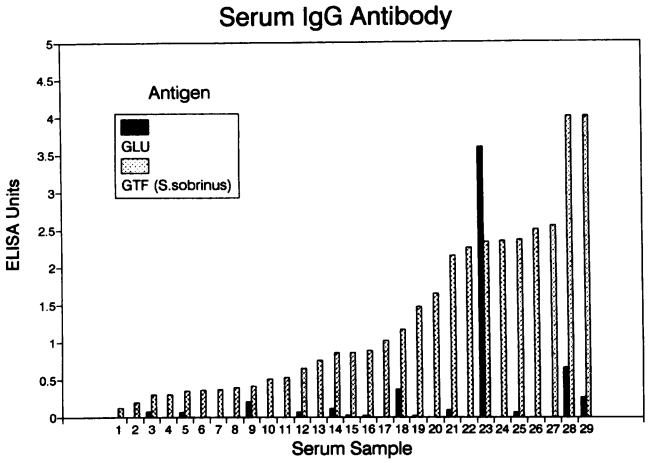

FIG. 3. ELISA measurement of adult human serum samples reacted with plates coated with GLU or with *S. sobrinus* 6715 GTF_{ss}. Antibody activity is reported as ELISA units. Serum samples were tested at dilutions of 1:100 and were developed for IgG antibody activity by using affinity-purified reagents as described in Materials and Methods.

of the synthetic GLU peptide is identical to the deduced GBP sequence of residues 274 through 295 of *S. mutans* (Table 1) reported by Banas and coworkers (3). In this regard, toxin A from *Clostridium difficile*, which has been postulated to bind carbohydrate on intestinal epithelium cells (33), also contains repeating sequences that are similar to those of the *S. mutans* GBP (36). These sequences include structural redundancies (G.....YF) that are shared among toxin A, GBP, GTF, and GLU. It has been suggested that the repetition of such aromatic acid-based motifs could be important in protein-carbohydrate complex formation (34, 36).

The similarities in repeating sequences of these putative GBPs are reflected in their immunological cross-reactions. Although the GLU synthetic peptide sequence was based on an *S. downei* sequence, rat antipeptide antibody reacted with GTFs from *S. sobrinus* 6715 (Fig. 1) and B13 (not shown) and *S. mutans* SJ (Fig. 1). These data suggest that an epitope that is shared among mutans streptococci exists in this region of the GTF molecule. A common epitope such as this could broaden the specificity of a potential synthetic vaccine against dental caries related to these organisms. Antisera to GLU also appeared to react with a GBP from *S. sobrinus*, presumably reflecting epitopes that are based on the similarities in sequence seen in Table 1. Interestingly, antisera to a decapeptide derived from a sequence of *C. difficile* that was similar to that of GLU also reacted with *S. mutans* GBP (36). Thus, this epitope may be shared among many carbohydrate-binding proteins.

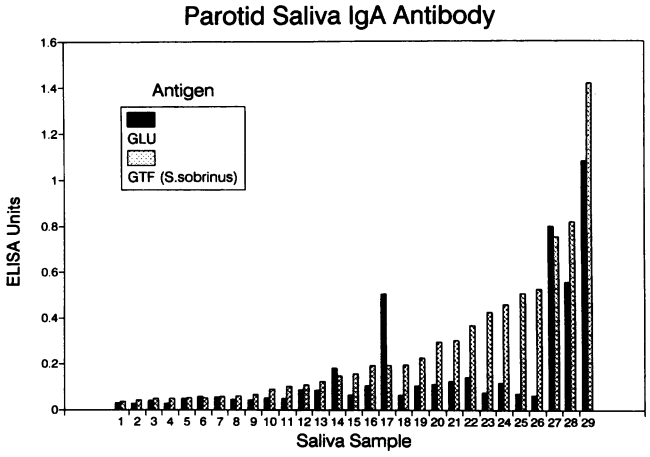

FIG. 4. ELISA measurement of adult human parotid saliva samples reacted with plates coated with GLU or with *S. sobrinus* 6715 GTF_{ss}. Antibody activity is reported as ELISA units. Saliva samples were tested at dilutions of 1:10 and were developed for IgA antibody activity by using affinity-purified reagents as described in Materials and Methods.

The ability of anti-GLU antibody to interfere with GTF function was indicated by the significant inhibition of *S. sobrinus* GTF-Sd activity (Table 2). The 30% inhibition of the formation of glucan retained by the filter reflected the overall loss of glucan (filter retained plus filtrate) synthesized by GTF-Sd and not simply a shift in glucan solubility. The significance of the preferential inhibition of the filter-retained glucan by the antibody is unclear, but it may be that the antibody interferes with aspects of glucan branching. Differences in antibody-mediated inhibition of insoluble and soluble glucans of GTF-I and GTF-S have been previously reported (32). The lack of detectable inhibition of *S. sobrinus* GTF-Sd by other rat antisera to GLU or of inhibition of GTF-I by any rat antiserum to GLU may be a function of antibody level or specificity. The basis for antibody-mediated inhibition of GTF activity is not clearly understood but may involve direct blocking of functional molecular domains and/or stearic hindrance of enzymatic activity by configurational modifications brought about by antibody binding to sites not associated with activity. The GLU sequence had been selected because of its association with a putative functional activity (glucan binding) of GTF. The analogous *S. sobrinus* GTF-I sequence (1) is 86% homologous with GLU (Table 1); the sequences of other *S. sobrinus* GTFs have yet to be described. The lack of complete sequence identity may have lowered the avidity of the polyclonal antipeptide antibody for the cross-reactive GTF epitope. Thus, under the conditions of the assay, the GTF-antibody interaction could be thermodynamically less stable than the GTF-glucan interaction and, therefore, favor the enzymatic rather than the inhibition consequence.

Antibody to GLU reacts with native GTF of both *S. sobrinus* and *S. mutans*. This antibody could also modify colonization of the oral cavity by mutans streptococci in ways other than by enzyme inhibition. Both GTF and GBPs can also be expressed on the surface of mutans streptococcal cells (15). Thus, antipeptide antibody that is reactive with GTF or GBP epitopes could agglutinate and result in clearance of these cariogenic microorganisms from the oral cavity by inhibition of adherence, colonization, or accumulation. Modification of the structure of the glucan synthesized in the presence of antipeptide antibody might also affect the plaque-forming ability of the mutans streptococci or the diffusability of organic acids within plaque. The potential for this synthetic MAP construct as a component of a caries vaccine is currently being tested in animals.

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