Immunological Characteristics of a Synthetic Peptide Associated with a Catalytic Domain of Mutans Streptococcal Glucosyltransferase

Daniel J. Smith, Martin A. Taubman, William F. King, Stephen Eida, Jonathan R. Powell, and Jean Eastcott

Department of Immunology, Forsyth Dental Center, Boston, Massachusetts 02115, and Unilever Dental Research, Colworth House, Sharnbrook, Bedford, MK44 1LQ United Kingdom

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The immunogenicity of a multiple antigenic peptide construct consisting of four copies of the synthetic 21-mer peptide DANFDSIRVDAVDNVDLLQ was measured. The composition of this peptide was derived from a sequence in the N-terminal region of mutans streptococcal glucosyltransferases (GTFs) containing an aspartic acid implicated in catalysis. The peptide (CAT) construct was synthesized as a tetramer on a lysine backbone and subcutaneously injected into Sprague-Dawley rats for polyclonal antibody formation or intraperitoneally injected into BALB/c mice, and then spleen cell fused with Sp2/OAg14 murine myeloma cells for monoclonal antibody formation. The resulting rat antisera and mouse monoclonal antibodies reacted with CAT and with native GTF isozymes from Streptococcus sobrinus and Streptococcus mutans (in enzyme-linked immunosorbent assay and Western blot [immunoblot] analyses). Functional inhibition of the water-insoluble glucan synthetic activity of S. sobrinus GTF-I was demonstrated with an immunoglobulin M anti-CAT monoclonal antibody (>80% inhibited) and with rat sera (approximately 17% inhibited). The monoclonal antibody preparation also modestly inhibited the water-soluble glucan synthetic activity of an S. mutans GTF mixture. These results suggest that the CAT peptide contains B-cell epitopes that are similar to those of intact mutans streptococcal GTFs and has the potential to elicit antibody that can inhibit GTF function. Thus, sequences within this peptide construct may have value for inclusion in a synthetic dental caries vaccine.

Dental caries in humans is associated primarily with the acidogenic and aciduric properties of the mutans streptococci (13). The colonization and accumulation of these cariogenic oral streptococci on the tooth surface have been associated with the ability of mutans streptococci to synthesize extracellular glucan from sucrose (9). Mutans streptococcal glucans are synthesized by glucosyltransferase enzymes (GTFs). The glucan-mediated accumulation of cariogenic mutants streptococci in dental plaque occurs through its interaction with cell-associated glucan-binding proteins (16). Immunologic interference with (25, 27) or genetic alteration of (14) glucan synthetic mechanisms has been associated with a reduction in mutans streptococcal cariogenicity. Thus, the catalytic activity of GTFs can be viewed as a virulence trait of mutants streptococci.

We have begun to explore the potential for development of subunit dental caries vaccines based on epitopes derived from GTF. Epitopes associated with enzyme function would theoretically be primary targets, provided that the relevant sequence(s) was located in molecular areas that are accessible to antibody. The location of a catalytic domain in the N-terminal third of GTF has been suggested by Mooser and coworkers (19, 20) on the basis of identification of an aspartic acid in this region that was found to be radiolabelled with glucose after pepsin digestion of GTF that had been rapidly denatured after incubation with radiolabelled sucrose. The aim of the present study was to synthesize and test a peptide whose sequence was derived from the highly conserved region containing this putative catalytic site within the GTF molecule. The synthetic design included four copies of this peptide (CAT) on a lysine backbone (26); this design may potentially enhance immunogenicity while avoiding irrelevant responses that result from the use of carrier proteins. The characteristics of polyclonal antibodies (rat) and monoclonal antibodies (MAbs; mouse) to the CAT construct were then evaluated with respect to reactivity with Streptococcus sobrinus and Streptococcus mutans GTF and by functional inhibition of GTF activity.

MATERIALS AND METHODS

Antigens. (i) CAT synthetic peptide. We selected for synthesis a 21-mer peptide, DANFDSIRVDAVDNVDLLQ, that had complete homology with the derived sequences of residues 444 to 464 of Streptococcus downei GTF-I (22) and residues 442 to 462 of S. mutans GTF-B (25) (Table 1). The sequences of these two mutants streptococcal GTFs contain the aspartic acid (residues 453 [S. downei] and 451 [S. mutans GTF-B]) that has been suggested by Mooser et al. (19) to be the residue to which glucose is covalently bound in the active site. This sequence is highly conserved among mutants streptococcal GTFs (1, 6, 9, 10, 14) (Table 1). The peptide was synthesized (Applied Diagnostics, Foster City, Calif.) by using the stepwise solid-phase method of Merrifield (17) on a core matrix of three lysines to yield a multiple antigenic peptide macromolecule with four identical 21-mer peptides per molecule, after the method of Tam (26). Purity (90%) was assessed by high-performance liquid chromatography, amino acid analysis, and molecular weight determination by mass spectrometry. This multiple antigenic peptide construct, referred to as CAT, was used for immunization and antibody analyses.

(ii) GTFs. GTFs from S. sobrinus 6715 and S. mutans SJ were obtained as described previously (24). Briefly, after bacterial growth in glucose-containing defined media, enzymes were isolated by chromatography on Sephadex G-100 (Pharmacia Fine Chemicals), with 3 M guanidine HCl used as the eluting solvent. These GTF-rich pools were then subjected to fast protein liquid chromatography on Superose 6 (Pharma-
TABLE 1. Comparison of the synthetic peptide sequence with deduced sequences of GTFs from oral streptococci containing a putative active-site aspartic acid

<table>
<thead>
<tr>
<th>Protein</th>
<th>Streptococcal species (reference)</th>
<th>Deduced sequence*</th>
<th>% Homology with synthetic peptide</th>
</tr>
</thead>
<tbody>
<tr>
<td>GTF-I</td>
<td>S. downei (8, 22)</td>
<td>444 DARFDSIRVDAVNDVADLDLQ 464</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>S. sobrinus (1)</td>
<td>438 G</td>
<td>91</td>
</tr>
<tr>
<td></td>
<td>S. salivarius (?)</td>
<td>464 S</td>
<td>91</td>
</tr>
<tr>
<td>GTF-S</td>
<td>S. downei (8)</td>
<td>428 G</td>
<td>86</td>
</tr>
<tr>
<td>GTF-B</td>
<td>S. mutans (23)</td>
<td>442 G</td>
<td>100</td>
</tr>
<tr>
<td>GTF-C</td>
<td>S. mutans (10, 29)</td>
<td>487 G</td>
<td>100</td>
</tr>
<tr>
<td>GTF-D</td>
<td>S. mutans (11, 12)</td>
<td>455 G</td>
<td>81</td>
</tr>
<tr>
<td>CAT synthetic peptide</td>
<td></td>
<td>DARFDSIRVDAVNDVADLDLQ</td>
<td></td>
</tr>
</tbody>
</table>

* ψ, indicates the aspartic acid putatively associated with the catalytic activity of GTF by Mooers and coworkers (19, 20).

cia), with 6 M guanidine used for elution. The gel filtration step removes non-GTF and other glucan-binding proteins from GTF preparations of S. mutans and S. sobrinus, as evidenced by the fact that the protein bands observed after sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) were all associated with enzymatic activity after incubation of duplicate gels in sucrose. The S. mutans GTF preparation taken to this level of enrichment synthesized more than 95% of the water-soluble glucan by both tube and filter assays (27) and thus was analogous to the gtfD gene product of S. mutans GSS (12, 13). This preparation was designated GTF\textsubscript{Sd} and used for injection and enzyme-linked immunosorbent assay (ELISA).

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) (15, 16). This preparation was designated GTF\textsubscript{Sd} and used for injection and ELISA.

S. sobrinus GTF-I and GTF-Sd, used for measuring enzyme inhibition by rat antisera, were obtained by anion-exchange chromatography of the Superose 6 pool on MONO-Q (Pharmacia) as described previously (24, 28). Virtually all of the glucan product of GTF-I synthesis was water insoluble when tested by either tube or filter assays (conditions reported in reference 24). Glucan synthesis by GTF-I was enhanced threefold in the presence of dextran T10 primer (Pharmacia). The quantitatively predominant GTF-Sd (16) synthesized a water-soluble product from sucrose in tube assays, which was enhanced sixfold in the presence of dextran primer. In filter assays, depending on the enzyme-substrate concentration, 8 to 30% of the glucan product of GTF-Sd synthesis was retained by the filter. Thus, for the purposes of the inhibition assay (see Tables 3 and 4), the enzyme activities of GTF-Sd were described empirically as water soluble (not retained by the filter) and water insoluble (retained by the filter).

Immunization. Sprague-Dawley strain CD 65-day-old male rats (Charles River Laboratories) were used for injection. Rats were injected subcutaneously in the flanks and back with (i) 50 µg of the CAT construct (n = 3), (ii) 25 µg of S. sobrinus 6715 GTF\textsubscript{Sd} (n = 2), or (iii) buffer alone (sham immunization; n = 3). The initial injection included complete Freund adjuvant (Difco); 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 88 days after the first injection (12 days after the final injection), and serum was prepared.

An additional 21 Sprague-Dawley strain CD rats were injected under a separate protocol. Two immunizations were given at 25 and 35 days of age. Rats were injected subcutaneously in the salivary gland region with either 10 µg of the CAT synthetic peptide construct (n = 7), 15 µg of GTF\textsubscript{Sd} (n = 7), or buffer alone (n = 7). The initial injection included complete Freund adjuvant, and the second injection included incomplete Freund adjuvant. Animals were bled 10 days after the second injection, and serum was prepared.

MAb to the CAT peptide (immunoglobulin M [IgM] MAb\textsubscript{CAT}) was prepared by intraperitoneal injection of male BALB/c mice with the CAT construct, which was bound to nitrocellulose. Other mice were injected intraperitoneally with mixtures of culture supernatants of laboratory strains of S. sobrinus and S. mutans (SB105 and SB1) that had been precipitated with ammonium sulfate at 50% saturation (IgG MAb\textsubscript{GTF}). A third group of mice were injected with GTF (and other glucan-binding proteins) adsorbed onto Sephadex G-50 from 50% ammonium sulfate-precipitated S. sobrinus SB105 culture supernatant (IgM MAb\textsubscript{GTF}). Spleen cells from mice with elevated serum antibody to the respective antigens were fused with Sp2/OAg14 murine myeloma cells (ICN Biomedicals, Inc., Costa Mesa, Calif.). Hybridomas producing antibodies to GTF or CAT were detected in an ELISA using the peptide construct or purified S. sobrinus GTF. Positive hybridomas were cloned by limiting dilution. MAbs 4721.2 (IgM MAb\textsubscript{CAT}) and 4631.2 (IgM MAb\textsubscript{GTF}) were prepared with concanavalin A (30) from clones expanded in hollow fibers (Tecmouse; Integra Biosciences, Inc., Woburn, Mass.). The IgG MAb 4688.1 (IgG MAb\textsubscript{GTF}) was prepared from mouse ascites by protein A affinity chromatography.

ELISA. Blood was taken from the retroorbital sinus of each rat. Serum, obtained from the coagulated specimen after centrifugation, was stored frozen at −20°C until use. Rat sera and mouse MAb reagents were tested for antibody activity by a previously described biotin-avidin, alkaline phosphatase ELISA method (25). Polystyrene microtiter plates (Flow Laboratories, McLean, Va.) were coated with either 1 µg of poly-DL-alanine-poly-L-lysine (approximate molecular mass, 153 kDa; Sigma Chemical Co., St. Louis, Mo.) per ml, 0.5 µg of the CAT peptide per ml, 0.5 µg of S. sobrinus 6715 GTF\textsubscript{Sd} (Superose 6 pool containing GTF-I, GTF-Sd, and GTF-Si), or S. mutans SJ GTF\textsubscript{Sd} (Superose 6 pool containing GTF-S activity (24). Antibody activity was then measured by incubation with 1:50 to 1:100 dilutions of sera. Plates were then developed for IgG or IgM antibody with the appropriate biotinylated avidin-purified goat anti-rat or anti-mouse gamma-chain or mu-chain reagents (Zymed Laboratories, South San Francisco, Calif.) and then with streptavidin-alkaline phosphatase (Zymed) and p-nitrophenylphosphate, in that order. Reactivity was then recorded as absorbance (A\textsubscript{405}) with
a microplate reader (Biotek Instruments, Winooski, Vt.). Each immune serum or MAb reagent was assayed simultaneously with either the respective preimmune serum or an irrelevant MAb of the appropriate isotype. These preimmune serum or irrelevant MAb control absorbances were then subtracted from the values for similarly diluted immune sera. The end point of the assay was taken as the dilution of the subtracted immune serum absorbance that was at least three standard deviations above that of the reagent background.

**Antibody inhibition of glucan synthesis.** Rat sera and MAb mouse reagents were evaluated for their ability to inhibit glucan synthesis of *S. sobrinus* GTFsa, *S. sobrinus* GTF-I and GTF-Sd, or *S. mutans* GTF by a filter assay. Ten to 20-μl volumes of sera (diluted from 1:10 to 1:8,000 with 0.02 M sodium phosphate-buffered saline and 0.02% sodium azide [PBSA; pH 6.5]) from rats injected four times with antigen, IgG or IgM MAb (tested at concentrations ranging from 15 to 370 μg/ml), or mouse IgG MAb (MOPC 21; Sigma) or IgM MAb (TEPC 183; Sigma) were preincubated with the respective GTF for 1 h at 37°C in a total volume of 0.04 ml of PBSA. Then, 1.7 mg of sucrose and 44 nCi of [14C]-glucose (sucrose (approximately 100,000 cpm) were added to 0.2 ml of PBSA 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, the filters were washed with PBSA, and radioactivity was determined as described previously (28). Essentially, all of the glucan formed by *S. sobrinus* GTF-I was captured on the filter. Under the conditions of the assay, approximately 70 and 95% of the glucan formed by *S. sobrinus* GTF-Sd and *S. mutans* GTF, respectively, could be found in the filtrate (referred to as water-soluble glucan). Sera from rats injected twice were tested with *S. sobrinus* or *S. mutans* GTF in a slightly modified assay. The serum (1 μl) and the enzyme were combined in a final volume of 100 μl in PBSA and incubated for 2 h at 37°C. Thereafter, 100 μl of PBSA containing 0.85 mg of sucrose and 22 nCi of [14C]-glucose (sucrose (approximately 50,000 cpm) was added and incubated for 2 h at 37°C. Water-insoluble glucan was collected on GF/F glass fiber filters, the filters were washed with PBSA, and radioactivity was determined. Water-soluble glucan in the filtrate was precipitated with 70% ethanol after the addition of 4 ml of carrier dextran T10 and centrifuged, and radioactivity was determined as described (28).

**Inhibition with IgM monomers.** In other experiments, IgM MAb monomeric subunits were tested for their ability to inhibit the synthetic capacity of *S. sobrinus* GTF-I. Subunits were prepared from the control TEPC 183 IgM MAb and the IgM MAbCAT after the method of Miller and Metzger (18). Approximately 200 μg of IgM in 0.2 M Tris-NaCl (pH 8.0) was incubated with 0.05 M cysteine for 40 min at 25°C. The reduced subunits were then alkylated with a 10% molar excess of iodoacetamide in 0.2 M Tris-NaCl (pH 7.3) for 15 min at 25°C. The reduced and alkylated subunits were then dialyzed extensively at 25°C in 0.2 M Tris-NaCl (pH 8.5). Under these conditions, essentially all of the pentameric IgM was converted to the monomer as indicated by the elution positions of dialyzed preparations after chromatography on Superox 6 (Pharmacia). Monomeric IgM fractions were then pooled and used in the inhibition assays as described above.

**Competitive inhibition with CAT construct.** In some experiments, the CAT construct was preincubated with polyclonal rat anti-GTF sera or anti-CAT MAb reagents to assess the ability of the peptide to remove antibody-mediated inhibitory activity. In these experiments, immune reagents were diluted initially to give approximately 50% inhibition of *S. sobrinus* GTF-I activity. CAT peptide, in a thousandfold range of concentrations, was then preincubated with immune and control reagents for 0.5 h at 37°C. After incubation of peptide, the assay for inhibition was conducted as described above.

**RESULTS**

**Immunogenicity of the CAT synthetic peptide.** (i) ELISA. IgG antibody to the CAT construct appeared in all rats after two subcutaneous injections of CAT (0.1 mg/mouse of GTF, 106 X 105) (Table 2) and continued to rise thereafter (not shown). GTF from *S. sobrinus* (GTFsa) elicited a serum IgG antibody response reactive with the homologous antigen at reciprocal dilutions greater than 105 and at dilutions greater than 106 with *S. mutans* GTF (Table 2). Sera from rats injected with CAT also reacted with both intact *S. sobrinus* and *S. mutans* GTF antigens, albeit at much lower dilutions than the anti-GTF sera, suggesting that a common epitope may exist on CAT and intact GTF.

Mouse MAb to the CAT construct or to GTF-containing preparations were also tested for activity to these antigens in an ELISA (Table 2). Of these MAbs, only the IgG anti-CAT MAb (IgM MAbCAT) showed a significant reaction with the CAT peptide. Each MAb preparation reacted with both *S. sobrinus* and *S. mutans* GTFs. The reactivity of the anti-CAT clone (IgM MAbCAT) with GTF at reciprocal dilutions greater than 105 confirmed that common epitopes exist on CAT and native GTF.

(ii) Western blot (immunoblot) analysis of rat polyclonal sera. The specificity of IgG antibody in the sera of rats injected with CAT with *S. sobrinus* GTF-I was also demonstrated in Western blot analyses (Fig. 1). IgG antibody in rat antisera to *S. sobrinus* (lane 1) and *S. mutans* GTF (lane 2) reacted with *S. sobrinus* GTF-I. Most importantly, IgG antibody in sera from rats injected with CAT also reacted with *S. sobrinus* GTF-I (lane 3). No reaction was seen with serum from a control rat injected with adjuvant alone (lane 4).

**TABLE 2. Antibody activity of polyclonal antibodies and MAb in an ELISA**

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Geometric mean reciprocal endpoint dilution&lt;sup&gt;b&lt;/sup&gt; with ELISA coating antigen of:</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgM MAbCAT</td>
<td><em>S. sobrinus</em> GTF&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>3.2 × 10&lt;sup&gt;3&lt;/sup&gt;</td>
<td>3.2 × 10&lt;sup&gt;3&lt;/sup&gt;</td>
</tr>
<tr>
<td>IgG MAbGTF</td>
<td>5.1 × 10&lt;sup&gt;2&lt;/sup&gt;</td>
</tr>
<tr>
<td>IgM MAbGTF</td>
<td>3.2 × 10&lt;sup&gt;2&lt;/sup&gt;</td>
</tr>
<tr>
<td>Polyclonal rat IgG anti-CAT</td>
<td>3.9 × 10&lt;sup&gt;3&lt;/sup&gt;</td>
</tr>
<tr>
<td>Polyclonal rat IgG anti-GTF&lt;sub&gt;sa&lt;/sub&gt;</td>
<td>5.1 × 10&lt;sup&gt;3&lt;/sup&gt;</td>
</tr>
<tr>
<td>Polyclonal sham rat IgG sera</td>
<td>&lt;10&lt;sup&gt;2&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Immunogens included CAT (IgM MAb<sub>CAT</sub>, polyclonal rat anti-CAT), culture supernatants of *S. sobrinus* and *S. mutans* that had been precipitated with (NH₄)₂SO₄ (IgG MAb<sub>GTF</sub>), *S. sobrinus* GTF adsorbed onto Sephadex G-50 from culture supernatants of *S. sobrinus* (IgG MAb<sub>GT</sub>), and a Superox 6 pool containing a mixture of *S. sobrinus* GTF isoforms (polyclonal rat anti-GTF<sub>sa</sub>).

<sup>b</sup> The geometric mean reciprocal endpoint dilution of triplicate assays is given for each MAb or set of polyclonal rat sera (n = 2 to 3) after subtraction of corresponding preimmune or irrelevant MAb absorbances. The absorbance of the endpoint of the assay was taken as the dilution of the subtracted immune absorbance that was at least three standard deviations above that of the reagent background.

<sup>c</sup> Superox 6 pool of *S. sobrinus* GTF containing GTF-I, GTF-Sd, and GTF-Si (GTF<sub>sa</sub>).

<sup>d</sup> Superox 6 pool of *S. mutans* GTF.
Inhibition of GTF functional activity by antibody to CAT.
Sera from a group of rats, injected twice with CAT, were tested for their ability to inhibit the formation of water-soluble or -insoluble glucan from \(^{14}C\)-glucose/sucrose by *S. sobrinus*

**TABLE 3. Inhibition of *S. sobrinus* GTF \(S_d\)-mediated glucan formation by rat polyclonal antiserum to CAT or GTF \(S_d\)**

<table>
<thead>
<tr>
<th>Antigen injected</th>
<th>% Inhibition of WIG (mean ± SE)</th>
<th>(P^a)</th>
<th>% Inhibition of WSG (mean ± SE)</th>
<th>(P^a)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAT (n = 7)</td>
<td>17.0 ± 4.6</td>
<td>&lt;0.01</td>
<td>10.0 ± 3.2</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td><em>S. sobrinus</em> GTF (S_d) (n = 6)</td>
<td>49.6 ± 2.6</td>
<td>&lt;0.001</td>
<td>14.5 ± 2.8</td>
<td>&lt;0.002</td>
</tr>
</tbody>
</table>

\(^a\) Female Sprague-Dawley rats were injected twice 1 week apart with the indicated antigen incorporated in complete Freund adjuvant (first injection) or incomplete Freund adjuvant (second injection). Serum was collected 1 week after the second injection and tested at a level of 1 \(\mu\)l per assay.

\(^b\) Incorporation of \(^{14}C\)-glucose into water-insoluble glucan (WIG) or water-soluble glucan (WSG), synthesized from \(^{14}C\)-glucose/sucrose in the absence of primer, was measured after a 1-h preincubation with the respective serum. Under these conditions, *S. sobrinus* GTF \(S_d\), synthesized 1.976 ± 35 cpm of \(^{14}C\)-total glucan in the absence of inhibitor, approximately three-fourths of which was associated with water-insoluble glucan. Percentage inhibition was expressed relative to the mean counts per minute incorporated into water-insoluble or water-soluble glucan synthesized by GTF after preincubation with eight sham-injected rat serum samples as follows: mean ± standard deviation of \(1 - (\text{mean replicate cpm of an individual rat anti-CAT or anti-GTF serum sample/mean cpm of the eight sham-injected rat serum samples}) \times 100\).

\(^c\) Expression of significance is based on a comparison of counts per minute of GTF preincubated with immune sera with counts per minute of GTF preincubated with sham sera by using one-way analysis of variance and the Tukey-Kramer multiple comparisons test.

GTF (Table 3). These rat sera gave relatively low but significant inhibition of both water-insoluble (17.0% ± 4.2% [mean ± standard error]) and water-soluble (10.0% ± 3.0%) glucan formation by GTF \(S_d\).

**TABLE 4. Inhibition of *S. sobrinus* GTF \(S_d\)-mediated formation of water-insoluble glucan (WIG) or water-soluble glucan (WSG) by mouse MAbs to CAT or GTF**

<table>
<thead>
<tr>
<th>MAb (^a)</th>
<th>WIG</th>
<th>WSG</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgM MAb (\text{CAT}^a)</td>
<td>1,342 ± 9 (35)</td>
<td>4,125 ± 25 (35)</td>
</tr>
<tr>
<td>IgM MAb TEPC 183</td>
<td>2,063 ± 3</td>
<td>6,450 ± 100</td>
</tr>
<tr>
<td>IgG MAb (\text{GTF}^a)</td>
<td>426 ± 23 (44)</td>
<td>4,136 ± 10</td>
</tr>
<tr>
<td>IgG MAb MOPC 21</td>
<td>760 ± 15</td>
<td>4,102 ± 100</td>
</tr>
<tr>
<td>IgM MAb (\text{GTF}^a)</td>
<td>1,670 ± 106 (19)</td>
<td>6,450 ± 50</td>
</tr>
<tr>
<td>IgM MAb TEPC 183</td>
<td>2,063 ± 3</td>
<td>6,211 ± 261</td>
</tr>
</tbody>
</table>

\(^a\) MAbs were each tested at approximately 150 \(\mu\)g/ml (approximately 1.5 to 3.0 \(\mu\)g per assay).

**TABLE 5. Inhibition of *S. sobrinus* GTF-I-mediated formation of water-insoluble glucan (WIG) or *S. mutans* GTF-mediated formation of water-soluble glucan (WSG) by mouse MAbs to CAT or GTF**

<table>
<thead>
<tr>
<th>MAb (^a)</th>
<th>WIG</th>
<th>WSG</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgM MAb (\text{CAT}^a)</td>
<td>4,050 ± 173 (49)</td>
<td>2,812 ± 74 (13)</td>
</tr>
<tr>
<td>IgM MAb TEPC 183</td>
<td>7,069 ± 240</td>
<td>3,213 ± 25</td>
</tr>
<tr>
<td>IgG MAb (\text{GTF}^a)</td>
<td>2,970 ± 25 (18)</td>
<td>1,479 ± 10 (20)</td>
</tr>
<tr>
<td>IgG MAb MOPC 21</td>
<td>3,602 ± 20</td>
<td>1,825 ± 25</td>
</tr>
<tr>
<td>IgM MAb (\text{GTF}^a)</td>
<td>3,459 ± 239 (51)</td>
<td>3,350 ± 50</td>
</tr>
<tr>
<td>IgM MAb TEPC 183</td>
<td>7,069 ± 240</td>
<td>3,213 ± 25</td>
</tr>
</tbody>
</table>

\(^a\) MAbs were each tested at approximately 150 \(\mu\)g/ml (approximately 1.5 to 3.0 \(\mu\)g per assay).

\(\text{[14C]}\)-glucan values are means ± standard deviations. Percentage inhibition of the formation of water-insoluble glucan or water-soluble glucan was expressed as \(1 - (\text{mean cpm of the CAT or GTF MAb/mean cpm of the irrelevant MAb}) \times 100\). The indicated percentages of inhibition were significant at the \(P\) of <0.05 to <0.01 level.
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control IgM monomer. Although lower than the inhibition with a similar concentration of intact IgM MAb<sub>CAT</sub> (approximately 25%) (Fig. 2A), the presence of monomer-based inhibition indicated that the IgM-mediated effects were not simply a function of the pentameric configuration and size.

IgM MAb<sub>GTF</sub> and IgG MAb<sub>GTF</sub> reactive with epitopes on intact GTF also inhibited the formation of filter-retained glucan by both <i>S. sobrinus</i> GTF-I and GTF-Sd activity (Tables 4 and 5). However, these IgG MAbs showed little or no inhibition of water-soluble glucan formation by <i>S. sobrinus</i> GTF-Sd or <i>S. mutans</i> GTF.

The specificity of the reaction of the immune sera and MAbs was further tested by preincubating the rat antiserum to GTF<sub>Ss</sub>, and the IgM and IgG MAb reagents with increasing concentrations of the CAT construct (Fig. 2B). This pretreatment removed detectable GTF-inhibitory activity from the IgM anti-CAT MAb. No significant reduction in <i>S. sobrinus</i> GTF-I inhibition was observed by pretreatment of a rat antiserum to GTF<sub>Ss</sub> with the CAT construct at concentrations as high as 1 mg/ml.

**DISCUSSION**

Molecular targets for potential immunological interference with GTF activity should be conformationally accessible to permit binding to antibody. The sequence DANFDSIRV-DAVDNVADDLQ, selected for construct preparation and for immunization in this study, apparently shares an accessible epitope(s) with native GTF since antisera from rats injected with CAT, and MAb derived from similarly immunized mice, showed a substantial reaction with <i>S. sobrinus</i> GTF (Fig. 1; Table 2).

Another consideration in the selection of sequences for a potential subunit vaccine to achieve the broadest protective effect would be that target epitopes are shared on GTFs from all mutans streptococcal species that infect humans. Much of the N-terminal sequence of mutants streptococcal GTF is highly conserved (1, 6, 7, 9, 11, 25). In fact, the sequence reported in Table 1 differs by no more than two residues from the sequences of GTF-I's from mutants streptococci or <i>Streptococcus salivarius</i>. Thus, it could be expected that if the CAT peptide sequence includes an epitope(s) that is also sequentially defined on GTF, then a similar epitope(s) could be expected to be found on the GTF-I sequence of several species. Support for this hypothesis is seen in the reactions of both the rat polyclonal antibodies and mouse MAb's to CAT with <i>S. sobrinus</i> and <i>S. mutans</i> GTF (Fig. 1; Table 2).

Although antibody binding to functionally important domains may not be necessary theoretically to achieve immunological protection, binding to catalytic domains should result in a loss of enzyme activity. The sequence DANFDSIRVDAVD NVADDLQ contains an aspartic acid that has been implicated in an early catalytic step of glucan synthesis (19, 20). Rat serum antibody (Table 3) and mouse IgM MAb (Tables 4 and 5) to the CAT construct both significantly inhibited the ability of <i>S. sobrinus</i> GTF to synthesize glucan. The mouse IgM anti-CAT MAb (IgM MAb<sub>CAT</sub>) was shown to inhibit GTF-I activity at concentrations as low as 50 ng per assay (Fig. 2). The pentameric structure was not required for inhibition by the

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**FIG. 2.** (A) Percentage inhibition of GTF-I-mediated water-insoluble glucan formation by anti-CAT mouse IgM MAb (closed circles) or serum from a rat injected four times with <i>S. sobrinus</i> GTF (closed triangles) as described in Materials and Methods. Various amounts (micrograms per assay per 100) of IgM MAB to CAT or control IgM MAb (TEPC 183) were assayed for the ability to inhibit [14C]glucose-labelled water-insoluble glucose formation from [14C]glucose by <i>S. sobrinus</i> GTF-I. Various amounts (noliters of serum per assay) of sera from <i>S. sobrinus</i> GTF-immunized (closed triangles) or sham-immunized (open triangles) rats were also assayed for the ability to inhibit water-insoluble glucan formation. (B) Assay of the ability of the CAT peptide to competitively inhibit mouse anti-CAT IgM MAb reagent, TEPC 183 IgM MAB of irrelevant specificity, CAT polyclonal rat anti-GTF<sub>Ss</sub> serum, or sham-injected rat serum. Each of the above serum samples or MAb reagents were preincubated with the four indicated concentrations of the CAT construct prior to incubation with <i>S. sobrinus</i> GTF-I and then assayed for water-insoluble [14C]glucan formation. The mouse IgM MAb<sub>CAT</sub> and rat anti-GTF<sub>Ss</sub> serum inhibited 54 and 45% of the activity of <i>S. sobrinus</i> GTF-I, respectively, in the absence of CAT. No significant inhibition was observed with either the control mouse MAB (TEPC 183) or the rat polyclonal (sham serum) reagent at any CAT concentration. Each datum point in the figure represents the mean of at least one assay performed in duplicate. Individual serum replicates varied by no more than 4.7% from the mean (mean variation, 1.9%).
IgM MAb. The water-insoluble glucan product of *S. sobrinus* GTF-Sd, whose activity is also dependent on the presence of dextran primer, was also inhibited by the MAb. The multiple specificities in polyclonal rat antibody to *S. sobrinus* GTF<sub>sa</sub> gave the broadest range of inhibition, although the synthesis of water-soluble glucan by GTF-Sd was least affected (data not shown).

We have recently shown that antibody to a repeated sequence in the C-terminal third of GTF (GLU) could also inhibit GTF activity (24). This sequence has been associated with glucan-binding properties of GTF (21, 22). The pattern of GTF inhibition observed with the GLU differed from that by antibody to GTF-C. Only the formation of water-insoluble glucan by *S. sobrinus* GTF-Sd was inhibited by rat anti-GLU antibody under the conditions of the assay. In contrast, the formation of water-insoluble glucan by both *S. sobrinus* GTF-I and GTF-Sd were the principal activities inhibited by the mouse IgM MAb to CAT. Rat antiserum to CAT modestly inhibited the formation of water-soluble and water-insoluble glucan from *S. sobrinus* GTF mixtures. The mouse IgM MAPCAT also inhibited *S. mutans* GTF, although the level of inhibition was low. This interspecies inhibition pattern by the anti-CAT antibody may be due to the fact that the sequences corresponding to the CAT peptide are more highly conserved than those sequences corresponding to the GLU peptide. However, Chia et al. (2) found that MAbs directed to a C-terminal sequences more than to the N-terminal third of *S. mutans* GTF inhibited the GTF-C but not the GTF-D isozyme, despite sequence identity in the respective regions of these two enzymes. These observations suggest that sequence homology is only one of several structural attributes that dictate epitope structure among mutans streptococcal GTFs.

Cope and Mooser (4) have reported that rabbit IgG antibody to two 22-mer peptides reacted only weakly with native GTF and did not inhibit *S. sobrinus* GTF-I or GTF-S, although their primary sequences were nearly identical to that of CAT. They suggested that these 22-mer peptides may not lie on the surface of GTF, thus limiting access of potentially inhibitory antibody to the eliciting peptide. Although this may be the case, these authors (4) used a monovalent peptide, unbound to carrier, for immunization, which may have limited the immunogenicity of the peptide. In the present study, the CAT peptide was synthesized as a quatrivalent construct on a lysine backbone. The construct used in our studies resulted in the formation of rat antibody that reacted with native *S. sobrinus* and *S. mutans* GTF in an ELISA, and some of the more-high-titered sera significantly inhibited *S. sobrinus* GTF activity. The peptide configurations adopted by the monovalent (4) and quatrivalent multiple antigenic peptide constructs, and the epitopes created by these configurations, are likely to differ in solution and give rise to antibody that differs in amount, specificity, and/or affinity. The genetic backgrounds of rats, mice, and rabbits may also contribute to variations in immune response parameters that lead to the formation of antibody.

Thus, antibody to epitopes present in the N-terminal (CAT) and C-terminal (GLU) regions of GTF apparently can interfere with enzyme function. In addition, Dertzbaugh and Macrina (5) have demonstrated inhibition of GTF activity with antibody to a chimeric peptide that included a sequence closer to the N terminus (residues 342 to 356) than the CAT sequence (residues 487 to 507) in *S. mutans* GTF-C (12, 29). Also, Chia and coworkers (3) have synthesized a 19-mer peptide, homologous to a sequence nearly adjacent to the CAT region, that contained an epitope able to remove some of the GTF-inhibitory activity of polyclonal antibodies directed to *S. mutans* GTF-B and GTF-C. These observations suggest that inhibition of GTF activity can be achieved by the binding of antibody to sequences that may not necessarily be associated with function. Although the anti-peptide antibodies are intended to be directed to sequential epitopes, antibody binding potentially could modify enzyme function, not only by directly blocking access of substrate to functional domains, but also through conformational changes that affect catalytic activity.

These studies suggest that subunit vaccines that are based on GTF sequences have the potential to modify dental caries caused by mutans streptococci. Antibody-mediated inhibition of the caries process could be achieved by interference with GTF catalytic activity, by modification of the nature of the glucan product, or by agglutination of microorganisms through bacterial cell surface GTF epitopes. The success of such a vaccine strategy is predicated on the incorporation of an appropriate peptide(s) into a carrier construct that results in an effective immune response.

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