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Immunological Relationships Between Glucosyltransferases from *Streptococcus mutans* Serotypes

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Partially purified glucosyltransferase enzymes from *Streptococcus mutans* GS-5 (serotype *c*) have been utilized to prepare antibodies directed against the soluble glucan-synthesizing activity, GTF-B, and the insoluble-soluble glucan synthetic activity, GTF-A. Anti-GTF-A inhibited insoluble glucan formation catalyzed by the extracellular enzymes from strains GS-5 and FA-1 (serotype *b*) to a much greater extent than that of strain HS-6 (serotype *a*) or OMZ-176 (serotype *d*). This antibody fraction also inhibited both the cell-associated glucosyltransferase activities as well as the sucrose-mediated adherence of cells to glass surfaces by strains GS-5 and FA-1 but not that of strains HS-6 and OMZ-176. Anti-GTF-B inhibited soluble glucan formation catalyzed by the extracellular enzymes of strain GS-5 but not that of strain HS-6, FA-1, or OMZ-176. However, this antibody fraction did not strongly inhibit either the cell-associated glucosyltransferase activity or cellular adherence of any of the four strains. These results with both antibody fractions were also correlated with the ability of the antibodies to agglutinate the cells and form precipitin bands after immunodiffusion with the extracellular enzymes. Antibody prepared against the homogeneous soluble glucan-synthesizing enzyme demonstrated similar effects to the anti-GTF-B fraction. These results are discussed in terms of the antigenic relationships existing between the glucosyltransferases from different serotypes of *S. mutans*.

The important role of *Streptococcus mutans* in smooth surface dental caries formation has been implicated from both in vitro and in vivo experiments (6). The attachment of *S. mutans* to smooth surfaces is primarily a consequence of the ability of these organisms to synthesize insoluble glucan products from sucrose. Therefore, the enzymes involved in this conversion, glucosyltransferases (EC 2.4.1.5) (GTF), play a key role in the initiation of dental plaque formation and subsequent caries development. Previously, attempts have been made to induce immunity against caries in experimental animals utilizing crude GTF preparations as antigens (6). The results from these earlier experiments were quite variable. However, more recent investigations utilizing whole cell *S. mutans* antigens in monkeys (11) and rats (15, 18) as well as partially purified GTF preparations in rats (H. J. Schick, R. Vollerthun, and O. Zwisler, Abstr. Int. Assoc. Dent. Res., p. 81, 1976; M. A. Taubman, D. J. Smith, and D. S. Cox, Abstr. Int. Assoc. Dent. Res., p. 82, 1976) indicate that it is possible to immunize experimental animals against subsequent monoinfection with organisms from a single serotype of *S. mutans*. However, the situation in the human oral cavity is complicated not only by the presence of other microorganisms but also by the presence of organisms from more than one serotype of *S. mutans* in one individual. Therefore, to rationally develop a potentially effective anticaries vaccine acting in humans, it will be necessary to evaluate the effects of antibodies directed against *S. mutans* whole cells or GTF antigens on organisms from the different serotypes of *S. mutans* normally found in the human oral cavity.

The insoluble glucans synthesized by *S. mutans* are highly branched polysaccharides containing both α-1,3- and α-1,6-glucose linkages (2). This property suggested that at least two GTF enzymes with different specificities are produced by these organisms. GTF activities synthesizing soluble α-1,6-linked glucans, GTF-B, have been highly purified from strains HS-6 (4), 6715 (12), and GS-5 (9). The GTF complex synthesizing insoluble glucans, GTF-A, has also been purified extensively from HS-6 (17) and GS-5 (9). However, these latter preparations are not homogeneous and contain variable amounts of non-GTF contaminants. Anti-

bodies prepared against the GTF-A enzyme complex from HS-6 (13) and GS-5 (10) have been demonstrated to inhibit the sucrose-dependent adherence of *S. mutans* to glass surfaces. The anti-GTF-A preparation from strain HS-6 (serotype *a*) also inhibited both insoluble glucan formation and cellular adherence to glass surfaces of representative strains from *S. mutans* serotypes *a*, *b*, *c*, and *d* (13). However, antibody prepared against partially purified GTF-B from strain GS-5 was not nearly as effective as anti-GTF-A in inhibiting cellular adherence (10).

The present investigation was initiated to examine the serotype specificity of antibody preparations directed against both the GTF-A and GTF-B fractions produced by strain GS-5 (serotype *c*). These antibody preparations were also examined for their effects on the cellular adherence of representative strains of serotypes *a*, *b*, *c*, and *d* to smooth surfaces in the presence of sucrose.

MATERIALS AND METHODS

Organisms. The organisms from the four serotypes of *S. mutans* were maintained and routinely grown in Todd-Hewitt (Difco)-glucose medium as previously described (7). Strain GS-5 (serotype *c*) was obtained from R. J. Gibbons (Forsyth Dental Center, Boston, Mass.), while strains HS-6 (serotype *a*), FA-1 (serotype *b*), B-13 (serotype *d*), and OMZ-176 (serotype *d*) were supplied by H. D. Slade (Northwestern University Medical School).

Preparation of antibody. GTF-A and GTF-B utilized as antigens were isolated after gel-filtration chromatography on Bio-Gel A-15 columns (9). The GTF-A fractions, purified over 200-fold, incorporated 56 to 85% of the glucose from sucrose into insoluble polysaccharide and the remainder into soluble products. The GTF-B fraction, purified eightfold, synthesized soluble glucan products exclusively (9). Female New Zealand rabbits were immunized with the appropriate antigen, and the gamma globulin fractions were isolated from the sera as previously described (10). Anti-GTF-A inhibited both soluble and insoluble glucan synthesis catalyzed by crude extracellular preparations of strain GS-5, whereas anti-GTF-B inhibited only soluble glucan formation (10). The sera from nonimmunized rabbits was used to prepare control gamma globulin fractions.

Homogeneous soluble glucan-synthesizing activity, HTP-GTF-B, was isolated as previously described (9). The purified enzyme (80 μg) was mixed with an equal volume of Freund complete adjuvant (Difco) and injected subcutaneously into multiple sites on the backs of rabbits. After 1 month, 70-μg portions of the enzyme were injected intravenously into the rabbits at monthly intervals, and trial bleedings commenced 1 week after each injection. After the first intravenous injection, anti-HTP-GTF-B levels were detected in the sera. The gamma globulin fraction was isolated from the pooled immune sera as previously described (1).

Immunodiffusion. Gel diffusion was carried out in petri dishes (50 by 12 mm) containing 1% agarose (Sigma), 0.9% NaCl, and 0.02% sodium azide. The interaction of the antibody fractions with the enzyme preparations was allowed to proceed initially at 37°C for 18 h, and the plates were then stored at 7°C for several days until precipitin bands were fully developed.

Enzyme assays. Extracellular enzyme preparations were prepared after precipitation of the culture supernatant fluids with ammonium sulfate as previously described (9). Total, soluble, and insoluble glucan formation by enzyme fractions or cell suspensions utilizing [^{14}C]glucose-sucrose (New England Nuclear Corp.) was determined as previously described (9). Exogenous primer dextran T10 (Pharmacia) was included in the assay mixtures where indicated. When the effects of the antibodies on GTF activity were determined, the enzyme preparations or cell suspensions were preincubated with the antibody fractions for 30 min at 37°C prior to the addition of the standard assay components. Approximately equivalent activities of the different serotype preparations were incubated with the antibodies for comparison.

Viable cell adherence. The ability of viable cell suspensions of *S. mutans* to adhere to glass surfaces was assessed as previously described (8). The assay components were added in the following order: cells, gamma globulin fractions where indicated, 0.9% NaCl-0.02% sodium azide, and 2% sucrose.

Cellular agglutination. The ability of gamma globulin fractions to agglutinate cells was determined under a dissecting microscope after mixing of cells and antibody. Cells (approximately 3.8×10^8 colony-forming units) and appropriate portions of antibody were mixed together on microscope slides in a total volume of 0.20 ml and immediately monitored for visual agglutination. The presence (+) or absence (−) of visible clumping was evaluated by comparison with similar mixtures containing equal volumes of the gamma globulin fraction from nonimmune sera. All positive agglutination reactions occurred within a few minutes of mixing at 22°C. Mixtures exhibiting no visible clumping were continually monitored for up to 20 min.

RESULTS

Effects of anti-HTP-GTF-B on glucosyltransferase activity. Recent results utilizing antibody directed against partially purified GTF-B from strain GS-5 (10) demonstrated that this antibody preparation inhibited soluble but not insoluble glucan formation. It was therefore of interest to determine whether similar effects could be demonstrated with antibody directed against the homogeneous soluble glucan-synthesizing enzyme. The serum obtained from animals immunized with HTP-GTF-B markedly inhibited glucan synthesis by this purified enzyme preparation (Fig. 1). Since this enzyme fraction synthesizes soluble glucan exclusively

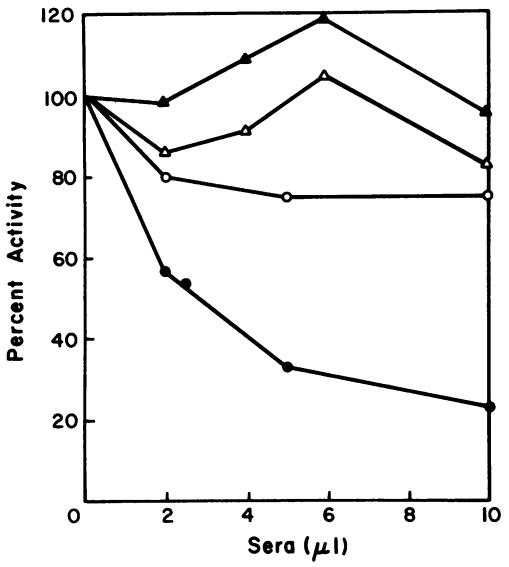

FIG. 1. *Effects of anti-HTP-GTF-B on soluble and insoluble glucan formation. Soluble glucan formation in the presence of primer dextran T10 catalyzed by HTP-GTF-B (3.63 U/mg) was measured in the presence of nonimmune sera (○) or anti-HTP-GTF-B sera (●). The activities are expressed relative to the activity in the absence of sera (0.5 mU). Insoluble glucan synthesis catalyzed by GTF-A (0.39 U/mg) was determined in the presence of nonimmune sera (△) and anti-HTP-GTF-B sera (▲). The activities are expressed relative to the activity in the absence of sera (0.48 mU).*

(9), the antisera specifically inhibits GTF activity producing soluble glucan products. Conversely, the antisera did not inhibit insoluble glucan formation catalyzed by the aggregated GTF complex, GTF-A, synthesizing these products. In fact, there is a small but consistent stimulation of insoluble glucan synthesis in the presence of the antisera.

Immunodiffusion on Ouchterlony plates (Fig. 2) revealed that the interaction of HTP-GTF-B with either anti-GTF-B or anti-HTP-GTF-B resulted in apparent single visible precipitin bands with a pattern of identity. Thus, both anti-GTF-B and anti-HTP-GTF-B are capable of interacting with and precipitating homogeneous HTP-GTF-B preparations. The single precipitin bands also indicate the relative homogeneity of the antibody preparations.

Serotype specificity of anti-GTF-A on insoluble glucan synthesis. Anti-GTF-A directed against the insoluble glucan-synthesizing enzyme complex from strain GS-5 was utilized to determine whether this antibody fraction could inhibit insoluble glucan synthesis catalyzed by crude GTF preparations from organisms representing the different serotypes of *S. mutans*. The results (Fig. 3) indicated that the enzymes displayed a wide range of sensitivities to inhibition by anti-GTF-A depending on the serotype examined. Insoluble glucan synthesis by the extracellular enzymes of strain GS-5 (type *c*) was inhibited approximately 80% by saturating levels of the antibody, whereas that of strain

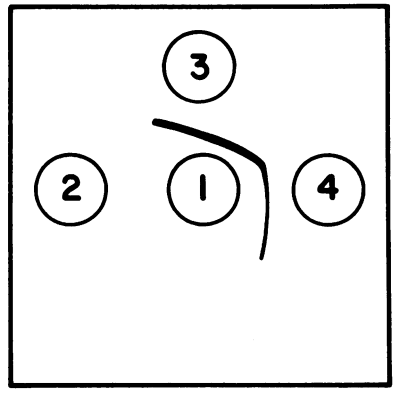

FIG. 2. *Gel immunodiffusion of HTP-GTF-B with anti-HTP-GTF-B and anti-GTF-B. The wells contained: (1) purified HTP-GTF-B (21 μg); (2) nonimmune sera (0.10 ml); (3) anti-GTF-B sera (0.10 ml); (4) anti-HTP-GTF-B sera (0.10 ml).*

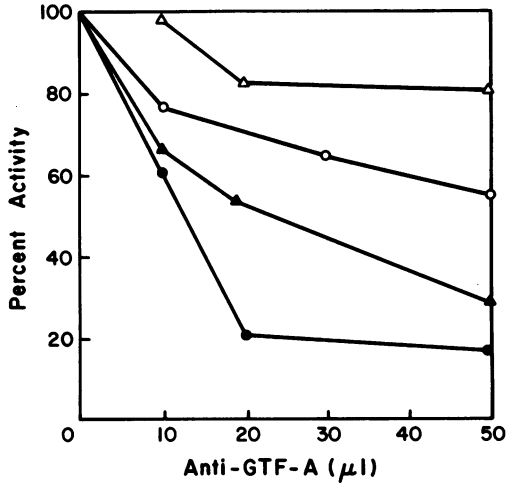

FIG. 3. *Serotype specificity of anti-GTF-A on insoluble glucan formation by extracellular enzyme preparations. Dialyzed ammonium sulfate-precipitated enzyme samples from the culture fluids of strains OMZ-176 (△), HS-6 (○), FA-1 (▲), and GS-5 (●) were assayed for insoluble glucan formation in the presence of the indicated amounts of anti-GTF-A gamma globulin (22.8 mg/ml). Activities are expressed relative to those determined in the presence of equivalent amounts of the gamma globulin fraction from nonimmune sera.*

FA-1 (type *b*) was also strongly inhibited but not to the same extent as GS-5. The activity of strain HS-6 (type *a*) was moderately inhibited, whereas that of OMZ-176 (type *d*) was only slightly inhibited in the presence of antibody. This latter effect was not restricted to strain OMZ-176, since similar results were also obtained with another serotype *d* strain (B-13; results not shown).

When the crude extracellular enzymes were examined for their capacity to form precipitable complexes with anti-GTF-A by immunodiffusion (Fig. 4A), precipitin bands were only detected after the interaction of the antibody preparation with the GS-5 and FA-1 extracellular enzymes. In the former case two distinct bands were obtained, whereas only a single band was detected with the latter enzyme preparation utilizing a level of antibody which precipitates maximal amounts of the GS-5 enzymes. When these two extracellular enzyme preparations were placed in adjacent wells, the single precipitin band of FA-1 showed complete identity with one of the two bands of GS-5 (results not shown).

Serotype specificity of anti-GTF-B on total glucan formation. Total glucan synthesis by the crude extracellular enzyme preparation from strain GS-5 was inhibited over 60% by anti-GTF-B (Fig. 5). These enzyme preparations incorporated approximately 72% of the glucose from sucrose into insoluble products and 28% into soluble polysaccharides in the presence of exogenous primer dextran T10. In contrast, the comparable enzyme fractions from strains of *S. mutans* representing the three other serotypes were either partially stimulated (strain FA-1) or only slightly inhibited (strains OMZ-176 and HS-6). Furthermore, as was observed for the enzymes from GS-5 (10), anti-GTF-B did not inhibit insoluble glucan formation catalyzed by the extracellular enzymes from strains HS-6, FA-1, and OMZ-176. Similar results were also obtained when anti-HTP-GTF-B was substituted for anti-GTF-B. In addition, the presence or absence of exogenous primer dextran T10 had little effect on these results. The enzyme preparations were also examined for their ability to form precipitable complexes with anti-GTF-B. Immunodiffusion analysis (Fig. 4B) indicated that such complexes could only be demonstrated upon the interaction of the antibody with the GS-5 extracellular enzymes and not with the extracellular enzymes from the other three serotypes.

Serotype specificity of anti-GTF-A on cell-associated glucosyltransferase activity. Previous results have indicated that the cell-associated GTF activity of strain GS-5 is identical in

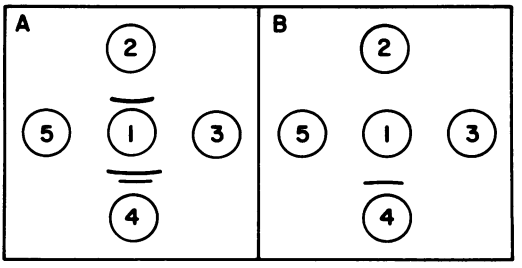

FIG. 4. *(A) Gel immunodiffusion of anti-GTF-A with extracellular enzyme preparations: (1) anti-GTF-A gamma globulin (0.20 ml); (2) FA-1 enzyme (0.10 ml); (3) HS-6 enzyme (0.10 ml); (4) GS-5 enzyme (0.10 ml); (5) OMZ-176 enzyme (0.10 ml). (B) Gel immunodiffusion of anti-GTF-B with extracellular enzyme preparations: (1) anti-GTF-B gamma globulin (0.20 ml); (2) FA-1 enzyme (0.10 ml); (3) HS-6 enzyme (0.10 ml); (4) GS-5 enzyme (0.10 ml); (5) OMZ-176 enzyme (0.10 ml).*

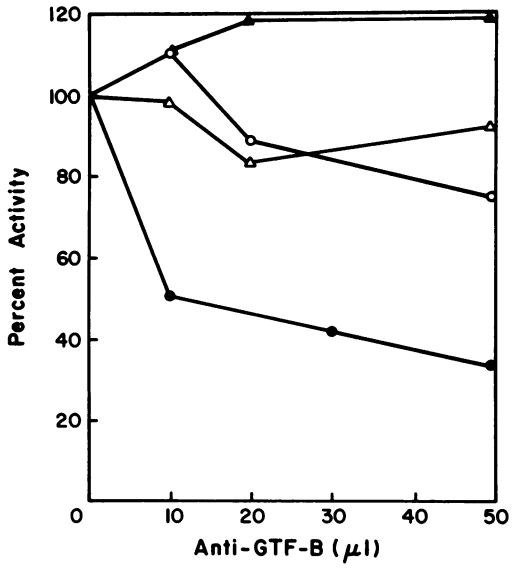

FIG. 5. *Serotype specificity of anti-GTF-B on total glucan formation by extracellular enzyme preparations. The enzyme fractions were assayed for total glucan formation in the presence of primer dextran T10 and the indicated levels of anti-GTF-B gamma globulin (19.0 mg/ml). Percentage of activity relative to the activities determined in the presence of equivalent amounts of the nonimmune gamma globulin fraction was measured for the enzymes of strains FA-1 (▲), HS-6 (○), OMZ-176 (△), and GS-5 (●).*

many properties to the extracellular activity (8). It was therefore of interest to determine whether both enzyme fractions from the four serotypes of *S. mutans* exhibited similar serotype sensitivities to the anti-GTF preparations. Anti-GTF-A strongly inhibited the cell-associated total glucan synthetic activities of strains

GS-5 and FA-1 to a similar extent (Fig. 6). However, the comparable activity of strain HS-6 was only moderately inhibited, whereas that of OMZ-176 was slightly inhibited. Only total glucan synthetic activity was measured with whole cell suspensions, since soluble polysaccharide products adhering to the cells would interfere with accurate measurements of insoluble glucan synthesis.

Serotype specificity of anti-GTF-B on cell-associated glucosyltransferase activity. When the cell-associated GTF activities from the four strains of *S. mutans* were assayed in the presence and absence of anti-GTF-B (Fig. 7), little or no inhibition of activity could be demonstrated. This was not surprising for strains HS-6, FA-1, and OMZ-176 in view of the relative insensitivities of the extracellular enzymes from these strains to anti-GTF-B (Fig. 5). However, the cell-associated activity of strain GS-5 was also relatively insensitive to inhibition by anti-GTF-B. Preincubation of the cells with the antibodies for 18 h at 7°C yielded results similar to the standard 30-min preincubation at 37°C. Furthermore, when the cell-associated GTF activity of GS-5 was extracted and solubilized with hypertonic NaCl solutions (8), the resultant total glucan synthetic activity was inhibited 48% by 50 μl of anti-GTF-A but was relatively insensitive to the same amount of anti-GTF-B.

Serotype specificites of anti-GTF-A and anti-GTF-B on cellular adherence. Since anti-GTF-A exhibited differential effects on insoluble glucan synthesized by the four strains of *S. mutans* (Fig. 3), it was of interest to determine the effects of this antibody preparation on cellular adherence. When washed cell suspensions of each strain were examined for sucrose-dependent adherence in the presence and absence of anti-GTF-A (Fig. 8), the observed adherence inhibition patterns paralleled the antibody sensitivities of the respective insoluble glucan-synthesizing activities from each strain. Cellular adherence of strains GS-5 and FA-1 was markedly inhibited in the presence of the anti-GTF-A, whereas that of strain HS-6 was only weakly inhibited. The adherence of strain OMZ-176 and of another serotype *d* strain, B-13 (results not shown), was not significantly inhibited in the presence of the antibody.

Anti-GTF-B did not produce marked inhibition of cellular adherence with any of the four strains examined (Fig. 9). Only strain GS-5 showed significant inhibition of cellular adherence amounting to approximately 20% in the presence of saturating levels of the antibody preparation. Similar results were also obtained when anti-HTP-GTF-B was utilized in these experiments. It was not possible to accurately measure the adherence of strain FA-1 at the higher concentrations of gamma globulin (>20 μl), since the control gamma globulin fraction

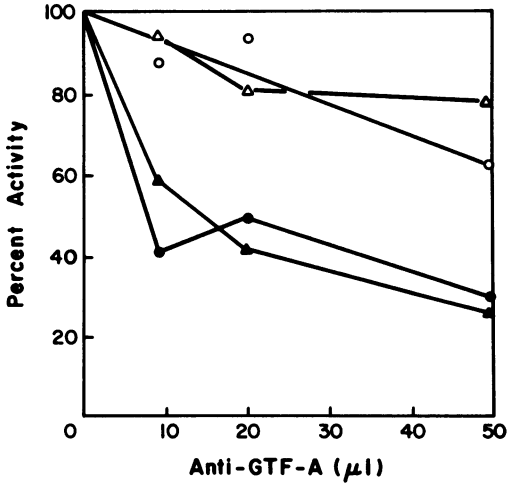

FIG. 6. *Serotype specificity of anti-GTF-A on cell-associated GTF activities. Cell suspensions of strains HS-6 (○), OMZ-176 (△), FA-1 (▲), and GS-5 (●) were assayed in the presence of the indicated levels of antibody. The activities are expressed relative to those determined in the presence of equivalent levels of control gamma globulin.*

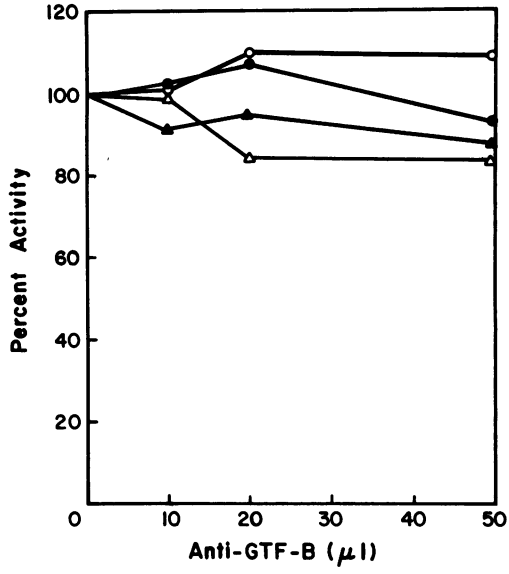

FIG. 7. *Serotype specificity of anti-GTF-B on cell-associated GTF activities. Enzyme activities were determined as described under Fig. 6, except that the indicated levels of anti-GTF-B were utilized.*

produced marked inhibition of adherence above this level.

Cellular agglutination in the presence of anti-GTF-A and anti-GTF-B. The ability of the four strains of *S. mutans* to agglutinate in the presence of the antibody fractions was also examined as another indication of the differential sensitivities of the cells to the antibodies (Table 1). Strains FA-1 and GS-5 showed rapid agglutination in the presence of anti-GTF-A but no visible agglutination when anti-GTF-B was added to the cell suspensions. On the other hand, strains OMZ-176 and HS-6 did not demonstrate visible agglutination in the presence of either anti-GTF-A or anti-GTF-B.

DISCUSSION

Previous results (10) have demonstrated that antibody directed against partially purified GTF-B did not inhibit insoluble glucan formation in strain GS-5. The present results also indicate that antibody produced against a much more highly purified soluble glucan synthesizing fraction, HTP-GTF-B, inhibited soluble but not insoluble glucan formation (Fig. 1). These results are therefore consistent with the proposal that at least two different structural genes code for GTF activity in *S. mutans*. It is also of interest that both anti-GTF-B (10) and anti-HTP-GTF-B produce a small but consistent stimulation of insoluble glucan production. The stimulation of glucan synthesis by anti-GTF preparations has also been noted previously (13). However, it is not yet clear whether this results from the specific interaction of anti-GTF antibodies with the enzymes or from other nonspecific effects.

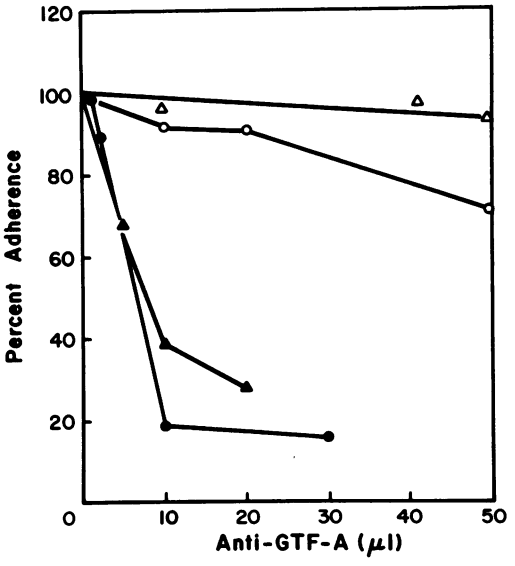

FIG. 8. *Serotype specificity of anti-GTF-A on cellular adherence. Viable cell suspensions of strains HS-6 (○), OMZ-176 (△), FA-1 (▲), and GS-5 (●) were incubated and treated as described previously (8) in the presence of the indicated levels of anti-GTF-A gamma globulin. Adherence is expressed relative to that measured in the presence of equivalent levels of control gamma globulin.*

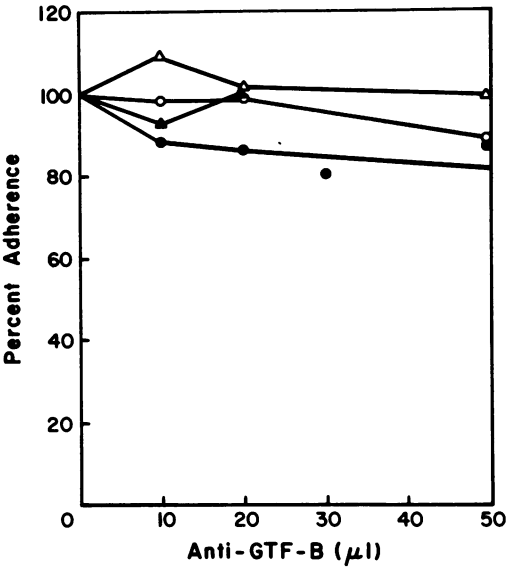

FIG. 9. *Serotype specificity of anti-GTF-B on cellular adherence. Adherence was measured as described under Fig. 8, except that the indicated levels of anti-GTF-B were utilized.*

TABLE 1. *Cellular agglutination in the presence of anti-GTF-A and anti-GTF-B*[a]

Organism	Anti-GTF-A	Anti-GTF-B
HS-6 (serotype *a*)	−	−
FA-1 (serotype *b*)	+	−
GS-5 (serotype *c*)	+	−
OMZ-176 (serotype *d*)	−	−

[a] Cellular agglutination was determined as described under Materials and Methods. Symbols: +, Visible agglutination; −, no visible agglutination compared to the control gamma globulin fraction.

Anti-GTF-A, directed against the insoluble glucan-synthesizing complex from strain GS-5, inhibited insoluble glucan formation catalyzed by the extracellular enzymes from the four strains of *S. mutans* in the following order of sensitivities: GS-5 > FA-1 > HS-6 > OMZ-176 (Fig. 3). Insoluble glucan synthesis by the enzymes from the two serotype *d* strains, OMZ-176 and B-13, was only slightly inhibited in the presence of anti-GTF-A. Furthermore, visible precipitation bands could be detected following

the interaction of a fixed concentration of anti-GTF-A with the crude extracellular enzymes from strains GS-5 and FA-1 but not with those of HS-6 and OMZ-176 (Fig. 4A). Thus, the enzyme(s) required for insoluble glucan synthesis in strain GS-5 is antigenically similar to the comparable enzymes from FA-1 (serotype *b*), somewhat less related to the enzymes from HS-6 (serotype *a*) and quite distinct from the enzymes of OMZ-176 (serotype *d*). Preliminary results from several other laboratories (J. E. Ciardi, G. J. Hageage, and W. H. Bowen, Abstr. Int. Assoc. Dent. Res., p. 80, 1976; D. J. Smith and M. A. Taubman, Abstr. Int. Assoc. Dent. Res., p. 81, 1976) have also revealed differential effects of monospecific anti-GTF preparations against enzyme fractions from different serotypes of *S. mutans*. However, recent results utilizing an anti-GTF-A preparation directed against a purified insoluble glucan-synthesizing complex from strain HS-6 indicated that this antibody preparation markedly inhibited insoluble glucan formation and cellular adherence to glass surfaces of serotypes *a*, *b*, *c*, and *d* (13). Thus the demonstration of cross-reactivity between the GTF enzyme preparations from different serotypes of *S. mutans* may be dependent on the serotype source used as the antigen, the method of purification of the GTF antigens, differences in antibody titers, or the routes of administration of the antigen. It is possible that alterations in any of these parameters may result in the production of antibodies directed primarily against different determinant groups on the enzymes.

Anti-GTF-B directed against the soluble glucan-synthesizing activity of strain GS-5 producing primarily α-1,6-glucans (9) exhibited marked inhibition of only the homologous enzyme preparation (Fig. 5). This antibody fraction was relatively ineffective in inhibiting glucan production by the enzymes from representative strains of serotypes *a*, *b*, and *d*. Furthermore, after immunodiffusion, precipitin bands were only detected upon the interaction of anti-GTF-B and the GS-5 enzymes. These latter results are apparently not due to differences in enzyme levels in the preparations, since comparable levels of enzyme activity are present in the samples from all four serotypes. Therefore, the antigenic structure of the GTF-B from strain GS-5 does not appear to be related to the comparable enzymes from the other three strains. Fukui et al. (5) have demonstrated that anti-GTF-B prepared against the homogenous enzyme from strain HS-6 cross-reacted with crude enzyme preparations from other strains of serotypes *a* and *d* but not with extracts from types *b* and *c*. Thus, the present results utilizing the anti-GTF-B from strain GS-5 are compatible with the results utilizing the HS-6 enzyme as an antigen.

The effects of anti-GTF-A on the cell-associated activities of the representative strains (Fig. 6) are consistent with the results utilizing the soluble enzyme fractions (Fig. 3). Furthermore, in view of the results utilizing the extracellular enzyme fractions (Fig. 5), it was not surprising that anti-GTF-B had little effect on the cell-associated activities of strains FA-1, HS-6, and OMZ-176 (Fig. 7). However, it was surprising that anti-GTF-B had little effect on the cell-associated activity of strain GS-5 while producing marked inhibition of the extracellular enzyme fraction (Fig. 5). In addition, anti-GTF-B did not cause visible agglutination of strain GS-5 (Table 1). Since previous results have indicated that the cell-associated and extracellular GTF fractions are composed of the same enzymes (8), these results suggest that either very little GTF-B is associated with the cell surface of strain GS-5 or that the enzyme present on the cell surface complex is inaccessible to the antibody. The former explanation is more likely in view of the insensitivity of the solubilized cell surface GTF activity to the action of anti-GTF-B. Therefore, most of the cell-associated GTF activity of strain GS-5 might consist primarily of the high-molecular-weight complex GTF-A, synthesizing insoluble glucan. Whether this indicates that the cell surface preferentially binds GTF-A relative to GTF-B or that the high-molecular-weight GTF-A complex cosediments with the cells upon centrifugation remains to be determined.

Cells of strains GS-5 and FA-1 were rapidly agglutinated in the presence of anti-GTF-A (Table 1), indicating that the determinant groups on the cell-surface enzymes are readily accessible for antibody cross-linking. In contrast, such groups were not available in sufficient concentration on the cell surfaces of strains HS-6 and OMZ-176 to produce visible agglutination with the concentration of antibody tested. The observation that anti-GTF-B did not induce visible agglutination with any of the four strains tested is consistent with the absence of significant inhibition of cell-associated GTF activity in the presence of this antibody fraction (Fig. 7).

The relative sucrose-dependent adherence properties of the four strains of *S. mutans* are affected by anti-GTF-A in parallel with the susceptibilities of their insoluble glucan synthetic activities to the antibody. Likewise, the recent study of Linzer and Slade (13) demon-

strated that the antisera directed against the insoluble glucan-synthesizing complex of strain HS-6 inhibited both insoluble glucan synthesis and sucrose-dependent adherence of cells from types *a*, *b*, *c*, and *d*.

Although the GTF-A preparation utilized as an antigen in the present study is not homogeneous (9), it is unlikely that the antibody effects observed resulted from the action of antibodies directed against non-GTF antigens. Mukasa and Slade have demonstrated that antibody directed against the purified cell-surface serotype *a–d* antigen inhibited the sucrose-dependent attachment of heat-killed cells of strain HS-6 to smooth surfaces (16). Therefore, it might be possible that the GTF-A preparation utilized as an antigen in the current investigation might be contaminated with the serotype *c* antigen. However, the GTF activities of both GS-5 (type *c*) and FA-1 (type *b*) were markedly inhibited by anti-GTF-A, and no cross-reactivity has been demonstrated between the serotype *c* and *d* antigens (12). In addition, the adherence assay utilized by Mukasa and Slade (16) involving heat-killed cells requires attachment of exogenous GTF enzymes to the cells. It is therefore likely that the anti-type *a–d* antibody mediates adherence by interfering with the attachment of GTF enzymes to the cell surfaces. In the present investigation, viable cell suspensions with preformed cell-surface GTF activity were utilized in the adherence assays. It is also unlikely that the anti-GTF-A preparation is acting as an anti-dextran preparation, since the interaction of anti-GTF-A with dextran T2000 (Pharmacia) after immunodiffusion yielded no detectable precipitin bands (results not shown). Furthermore, the presence or absence of exogenous primer dextran T10 did not change the relative sensitivities of the enzyme preparations to the action of either anti-GTF-A or anti-GTF-B. However, it will be necessary to further purify the GTF-A fraction to unequivocally exclude the possibility that the effects observed in this investigation were due to non-GTF antigens. Efforts in this regard are currently in progress in this laboratory.

The results from the present study suggest that any potential anticaries vaccine utilizing GTF preparations as antigens should contain primarily the GTF-A fraction. Furthermore, although organisms of serotype *c* are the predominant form of *S. mutans* isolated from the oral cavities of certain human populations (14), antibodies directed against the anti-GTF-A fraction from organisms of type *c* may not equally inhibit the sucrose-mediated attachment of organisms from all four major serotypes of *S. mutans* to smooth surfaces. Therefore, the GTF preparations from serotype *c* organisms alone may not be sufficient to provide maximum protection against caries in humans.

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ADDENDUM IN PROOF

Recent results utilizing an improved procedure for producing anti-GTF sera have demonstrated that higher-titer anti-GTF-A preparations will strongly inhibit both insoluble glucan synthesis and cellular adherence by strains HS-6 and OMZ-176.

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