Novel Epitope Region of Glucosyltransferase B from Streptococcus mutans

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In the development of a component vaccine against caries, the catalytic region (CAT) and glucan-binding domain (GBD) of glucosyltransferase B (GtfB) from Streptococcus mutans have been employed as target antigens. These regions were adopted as primary targets because they theoretically include epitopes associated with enzyme function. However, their antigenicities have not been fully evaluated. Although there are many reports about successful vaccination using these components, the principle has not yet been put to practical use. For these reasons, we came to doubt the effectiveness of the epitopes in vaccine production and reevaluated the antigenic region of GtfB by using in silico analyses combined with in vitro and in vivo experiments. The results suggested that the ca. 360-amino-acid variable region (VR) in the N terminus of GtfB is more reactive than CAT and GBD. This region is S. mutans and/or GtfB specific, nonconserved among other streptococcal Gtfs, and of unknown function. Immunization using an adeno virus vector-borne DNA vaccine confirmed that VR is an epitope that shows promise for the development of a caries vaccine.

Despite some success with preventive measures, dental caries continue to be prevalent and costly in many populations, and the development of a vaccine to prevent dental caries is still a desirable goal. Attempts to develop a vaccine against dental caries began with immunization using whole cells of the primary etiologic agent, Streptococcus mutans, in rhesus monkeys (23, 24). Although dental caries were successfully prevented in these studies, it was reported that the induced antibodies showed cross-reactivity with the human heart muscle (10, 48). Thereafter, components derived from S. mutans were investigated to avoid induction of antibodies that cross-react with human tissue. The molecules primarily focused on as target antigens are glucosyltransferase B (GtfB) (46) and antigen I/II (Ag I/II) (34).

GtfB was further truncated, and the catalytic region (CAT) (37) and the glucan-binding domain (GBD) (20) were employed as effective antigenic components. These components were combined with the cholera toxin B subunit (22), the saliva-binding region derived from Ag I/II (50), or both (30) to further enhance immunogenicity.

Despite these successful results reported more than 10 years ago, a GtfB-based vaccine against caries has not yet been put to practical use. One reason may be that induction of mucosal immunity is needed to produce the effective secretory IgA (S-IgA) antibodies that can prevent dental caries. Although encouraging results have been reported (3–6, 30, 38, 39), salivary S-IgA responses are often variable, transient, and of low magnitude compared with serum IgG responses. Another cause for the delay of the anticaries vaccine may be the antigenicity of the target antigens CAT and GBD, which were selected due to their association with enzyme function (40, 41). No study has addressed the immunogenicity of CAT and GBD by immunological, biochemical, or bioinformatic methods. It was recently suggested that effective screening for candidate vaccine antigens should include in silico analysis followed by in vitro and in vivo evaluations of antigenicity to reduce time and cost (29).

In this study, we reevaluated the immunogenicity of GtfB by using in silico approaches followed by in vitro and in vivo experiments to identify antigenic regions that may be employed in an effective anticaries vaccine. On the basis of our findings, we constructed a DNA vaccine using an adeno virus vector and confirmed its ability to induce specific antibodies contributing to immunity in mice.

MATERIALS AND METHODS

Bacterial strains and growth media. S. mutans MT8148 (serotype c) was routinely cultured in brain heart infusion (BHI) broth (Difco Laboratories, Detroit, MI) or on Mitis-Salivarius (MS) agar (Difco) at 37°C in air. Escherichia coli XL-2 (Stratagene Ltd., Cambridge, United Kingdom) was cultured aerobically in Luria-Bertani (LB) medium. When appropriate, erythromycin, kanamycin, and ampicillin (Wako Pure Chemicals, Osaka, Japan) were added to the LB medium to final concentrations of 500, 30, and 100 μg/ml, respectively.

Preparation of GtfB. S. mutans MT8148 was cultured in 5 liters of TTY medium (composed of Trypticase, tryptose, yeast extract, salts, and 1% glucose) at 37°C, and grown to an optical density of 0.8 at 550 nm. The bacteria were collected by centrifugation, and cell-associated Gtf (CA-Gtf) was extracted by treatment with 50 ml of 8 M urea at 25°C for 1 h. The extract was dialyzed against 10 mM sodium phosphate buffer (NaPB; pH 6.5) and adjusted to 60% saturation with ammonium sulfate. After centrifugation, the precipitate was dissolved in 10 mM NaPB (pH 7.5) and then dialyzed against the same buffer. The crude CA-Gtf sample was applied to a DEAE-Sepharose FF column (bed volume, 10 ml) and eluted with a linear gradient of 0 to 1.0 M NaCl in the same buffer. Active fractions, measured as described below, were pooled, concentrated by ammonium sulfate precipitation, dialyzed against 10 mM NaPB (pH 7.5), applied

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to a Bio-Scale CHT10-I column (bed volume, 10 ml; Bio-Rad Laboratories, Hercules, CA), and then eluted with a linear gradient of 10 to 500 mM potassium phosphate buffer (KPB) (15). To select the GtfB fractions, we performed a glcan synthesis assay (15), an enzyme-linked immunosorbent assay (ELISA) using anti-Ca-Gtf antibody, and a Western blot assay using anti-Cat-I (GtfB) and anti-Cat-SI (GtfC) monoclonal antibodies (47).

Glcan synthesis assay. Glcan-synthesizing activity was determined using [14C]glucose-sucrose as described previously (15). In brief, reaction mixtures composed of Gl and 10 mM [14C]glucose-sucrose (11.47 Gbq/mmol) in 20 ml of 50 mM KPB (pH 6.0) were incubated for 1 h at 37°C, spotted on a filter paper (1.0 by 1.5 cm), and air-dried. The filters were washed with methanol to remove nonincorporated [14C]glucose-sucrose and then immersed in scintillation fluid to estimate the amount of de novo-synthesized [14C]glcan.

Generation of anti-Ca-Gtf antiserum. Antiserum were prepared by repeatedly injecting rabbits intramuscularly with the emulsion made by mixing Gl-active fractions that were purified using a DEAE-Sepharose FF column and Freund's complete adjuvant (Difco) once, followed by Freund's incomplete adjuvant (Difco) twice. The antibodies were purified from rabbit antiserum by 3x precipitation with 33% ammonium sulfate.

SDS-PAGE and Western blotting. Conventional SDS-PAGE and Western blot analyses were carried out as described previously (12). In brief, Gtf samples and 2x SDS sample buffer carrying the recombinant plasmids were suspended in SDS gel loading buffer and boiled for 5 min. Proteins separated by SDS-PAGE were transferred onto a polyvinylidene fluoride (PVDF) membrane (Immobilon; Millipore, Billerica, MA). After blocking with 5% bovine serum albumin (BSA), the membrane was reacted with a primary antibody at 37°C for 1 h, and the bound antibody was subsequently detected using a solid-phase immunoassay employing goat anti-rabbit IgG antibody conjugated with horseradish peroxidase (HRP).

When NuPAGE 4 to 12% biss-Tris gels (Invitrogen by Life Technologies, Carlsbad, CA) were used, the samples were suspended in NuPAGE LDS sample buffer and NuPAGE sample reducing agent (Invitrogen) and incubated at 70°C for 10 min. Comassie brilliant blue (CBB) staining of the gels was performed using SimplyBlue SafeStain (Invitrogen). For immunoblotting, the proteins were transferred onto 0.2-μm PVDF membranes (Invitrogen) for 30 min at a constant voltage of 200 V. After blocking with 5% nonfat dry milk and 0.2% Tween 20 (PBST), the membranes were washed 5 times with PBST containing 0.2% Tween and then washed with secondary antibodies conjugated with HRP at a dilution of 1:3,000 in TBS containing 1% BSA at room temperature for 1 h. The membranes were washed 5 times with TBS containing 0.2% Tween and then incubated with secondary antibodies conjugated with HRP at a dilution of 1:1,000 in TBS containing 1% BSA at room temperature for 1 h. The membranes were then washed 5 times with TBS, and the signals were detected using a conventional solid-phase immunoassay.

ELISA. The ELISA was used to measure the amounts of GtfB or titers of antibody. The GtfB samples were applied to a 96-well plate, incubated at 37°C for 1 h, and washed 5 times with phosphate-buffered saline (PBS) containing 0.2% Tween 20 (PBST). The primary antibody at a dilution of 1:1,000 in PBST was applied onto each well, and the plates were incubated at 37°C for 1 h and then washed 5 times with PBS, followed by incubation with appropriate secondary antibody conjugated with HRP at a dilution of 1:10,000 to each well and allowed to react at 37°C for 1 h, and the plates were then washed 5 times. Subsequently, the GtfB amount or antibody titer was assessed using an alkaline phosphatase substrate kit according to the manufacturer's instructions (Bio-Rad).

Protein assay. The amounts of GtfB or protein in antiserum were estimated using a Pierce bicinchoninic acid protein assay kit according to the supplier's manual (Takara Bio Inc., Shiga, Japan).

PCRs. All PCR analyses in this study were performed using ExTag Hot Start version (Takara) according to the manufacturer's instructions as described previously (18). In brief, PCRs were performed using 30 cycles of a denaturing step at 98°C for 10 s and a primer-annealing and extension step at 60°C for 30 s or 1 min, according to the amplicon size.

Prediction of antigenic regions of GtfB in silico. The antigenic regions of GtfB were predicted using Kolaskar's method (21), available at http://imed.med.uc.es/Tools/antigenic.pl or Parker's method (33) and Welling's method (49) using ANTHEPORT 2000 version 6.0 software (7; http://antheport-phl.bcp.fr/index.php).

Preparation of recombinant CAT and GBD and generation of anti-CAT and anti-GBD antiserum. Based on the results with Kolaskar's method, PCR primers to amplify DNA fragments encoding CAT and GBD were designed (Table 1). The pSk6 plasmid that harbors the gtfB gene from S. mutans MT8148 (13) was used as a PCR template. Each amplified fragment was digested using the restriction enzymes that were added to the PCR primers and cloned into pGEX-6P-1 (GE Healthcare). The recombinant CAT and GBD were expressed as glutathione S-transferase (GST) fusion proteins. These recombinant proteins were digested by passing through PreScission protease (GE Healthcare), and their GST regions were removed using a GSTrap FF column (GE Healthcare). Recombinant proteins and the adjuvant were used to immunize the rabbits, and anti-CAT and anti-GBD antiserum were obtained as described above.

Construction of DNA vaccine plasmid and VR adenovirus. The variable region (VR) containing the antigenic regions predicted by Parker's method was amplified by PCR using the pSk6 plasmid as a template and the primers shown in Table 1. The amplicon digested by KpnI and PstI was cloned into pSecTag2B (Invitrogen), and pSecTag2B-VRGB was constructed as the DNA vaccine plasmid for VR (Fig. 1A). The expression cassette was cleaved by NruI and Spfl from pSecTag2B-VRGB, blunted, and used to construct the recombinant adenovirus according to methods described in a previous report (44). In brief, the blunt-end expression cassette was ligated with SwaI-digested pALC3 vector and introduced into E. coli DH10B by using an in vitro λ phosphage packaging kit (Nippon Gene). The structure of the resulting cosmids, pALC3-VRGB, is illustrated in Fig. 1B. To generate infectious recombinant adenoviral vectors, the pALC3-VRGB cosmids and pOG44 plasmid (Invitrogen) for the expression of FLP recombinase were cotransfected into HEK 293 cells (293 cell) seeded on a gelatin-coated well by using Lipofectamine (GIBCO-BRL) according to the supplier's instructions, and the recombinant adenovirus AdV-VRGB was harvested after 15 days from wells in which bacteriolysis complement was observed (Fig. 1C and D). The titer of the harvested adenoviral suspension was measured using an Adeno-X rapid titer kit (Takara).

Detection of expression of the AdV-VRGB-mediated gene and protein in 293 cells. Total RNA was extracted using TRIzol reagent (Invitrogen) from the 293 cells 3 days after they were infected with AdV-VRGB, and reverse transcription (RT) was performed using SuperScript III (Invitrogen) and oligo(dT)20 primer (Invitrogen). To detect the mRNA of AdV-VRGB-mediated genes, we used primers to amplify the VR (Table 1) and β-actin primers (Invitrogen) as an internal control. In addition, total protein was extracted from the 293 cells 3 days after AdV-VRGB infection and was assessed by Western blotting and enhanced chemiluminescence.

Immunization with DNA vaccine for VR and generation of anti-VR antiserum. The DNA vaccine for VR was used to immunize 6-week-old female mice (BALB/c AnNcrIcj; Charles River Laboratory Japan, Inc.) 4 times every night using retrograde intracorneal ductal injections (43). The mice were injected with AdV-VRGB suspension (1 x 107 PFU in 200 μl of lactated Ringer's solution) the first time according to methods described in a previous report (43) and with 50 μg of pSecTag2B-VRGB plasmid in lactated Ringer's solution the other 3 times. The antiserum titer was measured by ELISA using 10 ng of the purified GtfB as an antigen, and serum was harvested from ascites fluid 2 weeks after the last immunization.

Inhibition assay of GtfB activity by anti-VR antiserum. The effect of anti-VR antiserum on GtfB was analyzed according to a previous method (11).
FIG. 1. Construction of DNA vaccine plasmid and adenovirus. (A) The gene encoding the variable region of gtfB was cloned into pSecTag2B, and pSecTag2B-VRGB was constructed as a DNA vaccine plasmid for VRGB. (B) The expression cassette of VRGB elicited from pSecTag2B-VRGB and pALC3 cosmid vector was ligated, packaged in λ phage, and introduced into Escherichia coli. The cosmid obtained was pALC3-VRGB. (C) pALC3-VRGB and pOG44, a FLP recombinase expression plasmid, were cotransfected into 293 cells, and the infectious recombinant adenovirus AdV-VRGB was harvested. (D) Noncotransfected 293 cells (NC) and cotransfected 293 cells 8 and 15 days after pALC3-VRGB and pOG44 cotransfection are shown.
solution of purified GtfB (1.5 μg/ml) was incubated with an equal volume of anti-VR antiserum, anti-CA-Gtf antiserum, or sham serum undiluted or diluted 1:3 in PBS. The reaction mixtures of GtfB and serum were then incubated with [14C]glucose-sucrose at 37°C for 1 h, and the amount of [14C]glucan was measured as described above. Triplicate samples were analyzed in this experiment. Significant differences were determined using Student’s t test (P < 0.05).

RESULTS

Purification of GtfB. The cell-associated GtfB and GtfC enzymes were extracted from S. mutans MT8148 cells by using 8 M urea and were purified by DEAE–anion-exchange chromatography and the following hydroxyapatite column chromatography (Fig. 2A and B). In DEAE–anion-exchange chromatography, Gtf activity was observed in fractions 6 to 17, which were recovered as active fractions. During hydroxyapatite column chromatography, GtfB and GtfC were eluted in fractions 29 and 24, respectively. SDS-PAGE of GtfB and GtfC gave single protein bands with molecular masses of approximately 165 and 163 kDa, respectively (Fig. 2C). Anti-CA-Gtf antiserum reacted with both GtfB and GtfC (Fig. 2D), and anti-Gtf-I and anti-Gtf-SI antisera reacted with GtfB and GtfC, respectively (Fig. 2E and F). The anti-Gtf-I antiserum cross-reacted slightly with GtfC. The purified GtfB was used in further experiments.

Predicting antigenicity using Kolaskar’s method and the practical antigenicity of CAT and GBD derived from GtfB. As shown in Fig. 3, primary catalytic and glucan-binding sites were included in the group of highly antigenic candidates. Based on this prediction, we designed PCR primers for CAT such that the primer could amplify the region, including primary and secondary catalytic sites. Likewise, the PCR primers for GBD that could amplify the region, including the 6 repeating units of GBD, were designed. The CAT and GBD region fragments amplified by these PCR primers were ligated into pGEX-6P-1 to form pGEX-6P-1-CAT and pGEX-6P-1-GBD, respectively. The recombinant CAT and GBD were expressed as GST fusion proteins, and the GST regions were removed from them by using PreScission protease (Invitrogen). The recombinant CAT and GBD proteins were then used to immunize rabbits, from which anti-CAT and anti-GBD antisera were obtained. The lysates from E. coli recombined with pGEX-6P-1, GEX-6P-1-CAT, and pGEX-6P-1-GBD were electrophoresed by SDS-PAGE and observed as 26, 38, and 72 kDa of GST fusion proteins, respectively (Fig. 4A). In the Western blot analyses used to evaluate the antigenicity of CAT and GBD, it was observed that anti-CA-Gtf antibody did not react with the CAT or GBD proteins (Fig. 4B), while anti-CAT and anti-GBD antisera reacted with the corresponding recombinant proteins, and the native GtfB served as a positive control (Fig. 4C and D). In the inhibition assays of these antibodies (Fig. 5), anti-CAT and anti-GBD antibodies were less able to inhibit GtfB activity than was anti-CA-Gtf antibody, although there was a significant difference between the glucan synthesis inhibited by the anti-GBD antibody and the sham serum. Thus, it was suggested that the CAT and GBD regions are not immunodominant compared with the other antigenic regions that react with anti-CA-Gtf antibodies, although they are antigenic in rabbits.

Reevaluation of the antigenic region in GtfB by using ANTHEPROT software. As it was suggested that CAT and GBD were nonimmunodominant, we reevaluated the antigenic
region of GtfB by the other in silico predictions identified by the Parker and Welling methods by using the ANTHEPROT software (Fig. 6).

According to these analyses, the antigenicity of the primary catalytic site was predicted to be low by both methods, as this region was highly hydrophobic, lowly hydrophilic, and had solvent accessibility. On the other hand, the GBD region was also predicted to have relatively low antigenicity by both methods, although the 6 periodic antigenic peaks corresponding to the 6 repeating structures were identified as antigenic epitopes in Parker's prediction.

When GtfB was roughly divided into 3 parts, VR, CAT, and GBD, Parker's prediction indicated that the N-terminal VR had more antigenic peaks and was more solvent accessible than the other 2 parts. No report has evaluated the antigenicity of VR from GtfB. Thus, this region was adopted for use in the following experiments.

Expression of AdV-VRGB-mediated gene and protein in 293 cells. In the RT-PCR analysis used to confirm AdV-VRGB-mediated mRNA expression, an approximately 1.1-kbp fragment was amplified in the sample from the Adv-VRGB-infected 293 cells (Fig. 7A). Western blot analysis was then used
to confirm that the protein derived from AdV-VRGB was produced; an appropriate protein band with a molecular mass of approximately 46 kDa, including a myc epitope and histidine tag derived from pSecTag2B, was observed in the AdV-VRGB-infected 293 cells (Fig. 7C).

Immunization of mice with AdV-VRGB and pSecTag2B-VRGB. To induce the development of antibodies against VR, AdV-VRGB and pSecTag2B-VRGB were injected at the back of the hind legs of mice once and thrice, respectively. The reason why AdV-VRGB was not used for all 4 immunizations was to prevent the mice from dying due to the injection of adenovirus. To measure the titer of anti-VR IgG, ELISA using the purified GtfB as the antigen and goat anti-mouse IgG antibody as the secondary antibody was performed. It was observed that the titer of anti-VR IgG increased in 2 of 5 mice (Fig. 8). Thus, it was revealed that immunization with AdV-VRGB and pSecTag2B-VRGB could induce the development of antibody against VR. The antiserum against VR recovered from these 2 mice was pooled and used in further experiments.

Evaluation of the antiserum induced by DNA vaccine against VR. Reactivity of the antiserum induced by DNA vaccines against VR was evaluated by Western blot analysis using crude CA-Gtf extracted from S. mutans cells, using urea as an antigen (Fig. 9). The results showed that the antiserum induced by DNA vaccines against VR reacted with natural GtfB. Thus, it was suggested that the VR of GtfB is the antigenic region and is more reactive than CAT and GBD.

Inhibition of GtfB activity by anti-VR antiserum. We carried out an inhibition assay to prove that the anti-VR antiserum from mice contains antibodies that react with a native protein such as anti-CA-Gtf and to evaluate the effect of anti-VR antiserum on the glucan synthesis of GtfB (Fig. 10). In this experiment, the protein concentration of each nondiluted anti-VR antiserum, anti-CA-Gtf antiserum, and sham serum was 60 \(\mu\text{g} / \text{mL}\). When the ELISA using equal volumes of GtfB (concentration, 1.5 \(\mu\text{g} / \text{mL}\) was performed, the absorbances at 405 nm of the nondiluted anti-VR antiserum, anti-CA-Gtf antiserum, and sham serum were 1.2, 1.6, and 0, respectively. These results revealed that the anti-VR antiserum significantly inhibited GtfB activity compared with the sham serum. The anti-VR antiserum inhibited GtfB activity almost to the same degree, as did the anti-CA-Gtf antiserum when 60 \(\mu\text{g}\) protein of the antiserum was used. Thus, these results suggested that the anti-VR antiserum induced by AdV-VRGB and pSecTag2B-VRGB effectively inhibited GtfB activity.

DISCUSSION

In the present study, we reevaluated the effectiveness of the CAT and GBD as epitopes in vaccine production and also
found that the ca. 360-amino-acid VR, which exists at the N terminus of GtfB, is a more reactive vaccine target. The large Gtf molecules hamper complete functional domain mapping. However, studies comparing the amino acid sequences of Gtf from various oral streptococci (Fig. 6) revealed that Gtf consists of 4 regions: a ca. 40-amino-acid conserved signal sequence; a ca. 360-amino-acid VR with unknown function that is species specific and not conserved among the other Gtfs; a ca. 500-amino-acid catalytic domain that contains conserved amino acids that are necessary for sucrose hydrolysis (14, 25, 27); a series of 6 direct repeats that function in glucan binding (1, 9, 28). The latter 2 functional domains were employed as potential vaccine target regions in previous attempts to develop a component vaccine against GtfB, owing to their association with enzyme function and the high degree of sequence conservation among streptococci. However, the antigenicity of those 2 regions was confirmed only by Western blot analyses using antibodies induced by synthetic peptides corresponding to the CAT or GBD regions derived from GtfI of *Streptococcus downei* (40, 41). Thereafter, to make up for antigenicity, the diepitopic antigen consisting of both the CAT and GBD (45), the chimera of CAT and the B subunit of cholera toxin (22), and the chimera of GBD and the saliva-binding region of Ag I/II (50) were applied. On the basis of these successful reports, we also tried to develop a DNA vaccine against CAT and GBD, but we did not obtain the expected results. One result was that the recombinant CAT and GBD did not react with the antiserum induced by native Gtf from *S. mutans* (Fig. 4), although these recombinant proteins did induce antibodies that reacted with native GtfB and the corresponding recombinant proteins. In addition, the DNA vaccine plasmid for CAT and GBD using pcDNA3 did not mediate sufficient amounts of the recombinant proteins to induce specific antibodies, although expression of the objective mRNA was confirmed by RT-PCR in an *in vitro* experiment (data not shown). Thus, we began to doubt the antigenicities of these regions as a way to express antigenic protein and decided to focus on alternative domains.

Secondary structure predictions suggest that the Gtfs are members of the α-amylase superfamily and contain a circularly permuted \((\alpha/\beta)_8\)-barrel motif (8, 19, 26). Our results in Fig. 6 revealed that the primary CAT site (FDSIRVDA...
VDN) of GtfB is highly hydrophobic, and its solvent accessibility was very low. Since these observations suggested that the primary CAT site exists inside the \((\alpha/\beta)_{8}\)-barrel structure, it was thought that this region may be difficult to recognize as an antigen for the immune system, although low-molecular-weight sucrose is able to pass through this structure as an enzyme substrate. Thus, it was suggested that the antigenicity of CAT would be low, as shown by Parker’s prediction.

In our previous study of glucosyltransferase from Streptococcus oralis (GtfR; 11), the incomplete recombinant GtfR without GBD reacted with the antiserum induced by native GtfR from S. oralis. An antiserum preliminarily prepared by immunization with the recombinant GtfR without GBD not only reacted with native GtfR in Western blot analysis but also inhibited glucan synthesis by GtfR (data not shown). On the other hand, GbtA and GBD are homologous, and it was reported that immunization with the entire GbtA protein did not elicit a protective immune response (2, 36). Taken together, these observations indirectly suggest that GBD antigenicity is lower than that of the other Gtf regions. Practically, our results suggested that GBD would not be immunodominant, because few antigenic peaks were seen compared with the other GtfB regions according to the analysis using Parker’s method (Fig. 6).

In this study, we reevaluated the antigenic GtfB region and tried to find a more potent antigenic component. Results of the in silico analysis using Parker’s method suggested that the VR derived from GtfB was immunodominant, as evidenced by its many antigenic peaks compared with those of CAT and GBD (Fig. 6). As it was reported that the VR is specific to the Gtf of individual Streptococcus species (17, 18), our DNA vaccines would induce GtfB-specific antibodies that would have an effect only on S. mutans glucan synthesis and colonization and would not interfere with Gtf-expressing members of the oral commensal microbiota.

It was recently reported that nonimmunodominant regions
were effective as building blocks in a streptococcal fusion protein vaccine (42). Although CAT and GBD were suggested to be nonimmunodominant in this study, they nevertheless may be attractive vaccine targets. Thus, fusion proteins of VR and CAT or GBD may induce more effective antibodies to inhibit glucan synthesis of GtfB.

We adopted the adenovirus vector, which possesses an intensive promoter and a high infectious ability, as a way to deliver recombinant antigen protein sufficient for mucosal immunization as well. Since it was demonstrated that AdVR-RGB expressed sufficient recombinant antigen protein to induce the development of antibodies that could inhibit GtfB activity in murine in vivo experiments, use of AdVR-RGB may also be effective with mucosal route immunization. As a natural consequence, AdVR-RGB ought to be applied in oral experiments in animals to test whether dental caries can be prevented. We realize that there is a safety problem when it comes to application of this DNA vaccine virus. Even if it were safe, its use would not be permitted in the human oral cavity for dental caries prevention. Because of safety issues, we hesitate to use only DNA vaccine virus in all immunization steps; instead, we used DNA vaccine plasmid in the last 3 immunizations to reduce stress in the mice. We plan to use AdVR-RGB to make a hybridoma that produces anti-VR antibody to clone the gene encoding anti-VR antibody by phage display (35) and to transform this gene into a rice plant by using Agrobacterium-mediated transformation (31, 32). Rice harvested in this planned study will be evaluated for passive immunization to prevent dental caries.

FIG. 10. Effect of anti-VR antiserum on GtfB activity. Purified GtfB (1.5 μg protein) that was allowed to react with 0, 20, or 60 μg protein of anti-VR antiserum, anti-CA-Gtf antiserum, or sham serum at 37°C for 1 h was incubated with [14C]glucosyl-sucrose at 37°C for 1 h, and the amount of [14C]glucan was measured. The GtfB activity in the nonserum was defined as 100%. Triplicate samples were analyzed in this experiment, and the asterisks indicate statistical significance based on Student’s t test (*, P < 0.01; **, P < 0.05).

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