Differential Inhibition of *Streptococcus mutans* In Vitro Adherence by Anti-Glucosyltransferase Antibodies

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Antibodies prepared against an insoluble-soluble glucan-synthesizing fraction significantly inhibited in vitro adherence of *Streptococcus mutans*, whereas antibodies directed against a soluble glucan-synthesizing fraction were much less inhibitory.

A significant role for *Streptococcus mutans* in the development of smooth surface plaque and subsequent dental caries has been strongly implied (13). Gibbons and Nygaard (7) have suggested that the ability of this organism to adhere to smooth surfaces is dependent on its ability to synthesize unique water-insoluble glucan polymers. The glucosyltransferases (GTF) (EC 2.4.1.5) of this organism are capable of synthesizing both water-insoluble and soluble glucans (3). Previously, it has been demonstrated that antibodies prepared against either whole cells of *S. mutans* (11, 12) or crude GTF preparations (12) could inhibit the adherence of the organism to smooth surfaces in vitro. However, since the existence of multiple GTF activities in *S. mutans* has been suggested (6, 8–10) and the insoluble glucan product implicated in adherence contains both \( \alpha-1,3 \)- and \( \alpha-1,6 \)-linked glucose units (4), the nature of the anti-GTF antibodies responsible for preventing adherence is not clear. Recently, it has been possible to resolve the GTF activities from strain GS5, serotype c (1) of *S. mutans*, into fractions GTF-A and GTF-B synthesizing insoluble-soluble and soluble (primarily \( \alpha-1,6 \)-linked) glucan polymers, respectively (9). This report is concerned with the effects of antibodies prepared against each fraction on the adherence of strain GS5 in vitro.

*S. mutans* GS5 GTF-A, synthesizing both soluble and insoluble glucans, and GTF-B, synthesizing soluble glucan exclusively, were isolated after chromatography on BioGel A-15 columns (9). Each preparation (2 mg) was mixed with an equal volume of Freund complete adjuvant (Difco) and injected subcutaneously into the backs of female New Zealand rabbits. After 1 month, the animals were injected intravenously with 100 \( \mu \)g of enzyme. Bleedings were commenced 1 week after the second injection. Gamma globulin fractions were obtained after treatment of the pooled sera with ammonium sulfate (2). Soluble and insoluble glucan-synthesizing activities were determined as previously described (9). When antibody effects on enzyme activity were measured, the enzyme fractions were preincubated with antibody preparations for 30 min at 37°C prior to assay. In vitro adherence to glass surfaces utilizing live cells was carried out as previously described (11). The components were added in the following order: cells, antibodies where indicated, saline-sodium azide (0.02%), and sucrose (2%).

Insoluble glucan synthesis by GTF-A was markedly inhibited by anti-GTF-A but not by anti-GTF-B gamma globulins (Fig. 1). The apparent activation of GTF activity by serum components (control gamma globulins) has been previously reported (5) and is currently under investigation. In contrast, total glucan synthetic activity (soluble plus insoluble products) was inhibited by both anti-GTF preparations. The inhibition of total but not insoluble glucan synthesis by anti-GTF-B indicates that this antibody inhibits soluble glucan synthesis by GTF-A. This is further suggested by the observation that anti-GTF-A as well as anti-GTF-B inhibits soluble glucan formation by GTF-B (Fig. 2). These results support the recent suggestion (9) that the GTF-A fraction also contains the soluble glucan-synthesizing activity of GTF-B.

As predicted by the results shown in Fig. 1, it was observed that anti-GTF-A had a marked inhibitory effect on in vitro cellular adherence of *S. mutans*, whereas anti-GTF-B produced a much smaller inhibitory effect (Fig. 3). This latter observation does not appear to result from a contamination of GTF-B with the insoluble glucan-synthesizing activity present in
GTF-A, since GTF-B synthesizes no detectable insoluble glucan and cannot catalyze the adherence of heat-inactivated cells of S. mutans to smooth surfaces (9). Since insoluble glucans synthesized by S. mutans contain both α-1,3- and α-1,6-linked glucose units, it is possible that the inhibition of α-1,6-glucose addition by anti-GTF-B might prevent the formation of the proper branched glucan necessary for maximum cellular adherence.

These observations suggest that at least two antigenically distinct species of GTF exist in S. mutans GS5. One of these, present in GTF-A, is primarily responsible for insoluble glucan synthesis, whereas the other, constituting GTF-B and also present in GTF-A, synthesizes soluble glucan in the absence of other enzymes. Furthermore, these results indicate that any potential anticaries vaccine producing anti-GTF antibodies must be directed against the relevant GTF activities. Since the GTF preparations utilized as immunogens in the present study were not homogeneous, it is not yet possible to rule out completely the possible roles of non-GTF molecules in the adherence process. In this regard, experiments are currently in progress to prepare homogeneous GTF preparations and examine the effects of the corresponding antibody preparations on cellular adherence to smooth surfaces.

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LITERATURE CITED


