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Production of Monoclonal Antibody Against a Glucosyltransferase of *Streptococcus mutans* 6715

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A mouse hydrid cell line secreted monoclonal antibody which reacted specifically with *Streptococcus mutans* 6715 (serotype *g*) glucosyltransferase (GTase)-synthesizing water-insoluble glucan and inhibited with enzyme reaction. The antibody was cross-reactive with GTase of serotype *d* but not with GTase of other serotypes of *S. mutans* when an enzyme-linked immunosorbent assay was used.

Streptococcus mutans, one of the prime pathogens of dental caries, produces at least two kinds of glucosyltransferase (GTase) that synthesize water-soluble and water-insoluble glucans. The ability to produce water-insoluble glucan adherent to solid surfaces has been demonstrated to be a virulence factor of *S. mutans* in dental caries development (5). *S. mutans* strains are classified serologically into seven serotypes, *a* to *g*. Immunological studies of *S. mutans* antigens have been performed from various points of view; investigations with antisera against partially or highly purified GTases of *S. mutans* have shown that antisera elaborated against GTase in rodents and rabbits inhibit glucan synthesis to various extents, depending on the serotype of *S. mutans* employed (3, 4, 10, 13, 14).

Current advances in hybridoma technology (9, 11) have made it possible to use monoclonal antibodies as an immunological probe. However, to our knowledge, monoclonal antibodies specific for GTases have not been obtained. We therefore attempted to prepare hybridoma clones which produce anti-GTase monoclonal antibodies.

S. mutans 6715 GTase (GTase-G) was prepared by dissociation of an insoluble glucan-GTase complex with 6 M guanidine-hydrochloride by the method of Smith et al. (15) for use as the immunogen. BALB/c mice (Charles River Japan Co., Kanagawa, Japan) were primed with an intraperitoneal injection of alum-precipitated GTase-G (120 μg of protein per mouse) supplemented with 10^9 heat-killed cells of *Bordetella pertussis*. Two weeks later, the same dose of immunogen in saline was injected without adjuvant. The second booster injection of GTase-G (120 μg of protein) was given intravenously without adjuvant 6 weeks after the first injection, and the spleen of a hyperimmune mouse was removed 3 days later for use in hybridoma production. The protocol for the fusion and selection of antibody-producing hybrid cell lines used in this study have been outlined by Oi and Herzenberg (11). Spleen cells were fused to BALB/c Sp2/O-Ag14 myeloma cells (kindly donated by K. Ozato, National Cancer Institute) at a ratio of 5:1 with 50% polyethylene glycol 4000 (Merck, Darmstadt, Germany). After selection of hybrid cells with HAT medium (11), the presence of the anti-GTase-G antibodies in culture fluids was tested by an enzyme-linked immunosorbent assay (ELISA) (2) with alkaline phosphatase-conjugated rabbit anti-mouse immunoglobulin. Mouse antibody isotypes were determined by ELISA and/or immunodiffusion with commercially available anti-mouse immunoglobulins (Bionetics, Inc., Kensington, Md.).

In screening tests for viable hybrids producing antibodies against GTase-G, 33 of 180 growing hybrid cell cultures produced significant antibodies. The antibody-producing cultures were cloned at least twice by limiting dilution, and 10 stable hybrid cell lines producing antibodies directed to GTase-G were established. Of these clones, IG-3E7 cells secreting immunoglobulin G1 isotype produced the highest titer of the antibody as detected by ELISA. Therefore, the cells were propagated and used in the present study. Culture fluid of IG-3E7 cells was concentrated ×10 by precipitation with 50% saturated ammonium sulfate followed by dialysis against phosphate-buffered saline and characterized as an IG-3E7 monoclonal antibody.

Crude GTase preparation (GTase-C), obtained by precipitating culture supernatant of *S. mutans* 6715 with 50% saturated ammonium sulfate, contained many proteins as revealed by 8 M urea-polyacrylamide slab gel electrophore-

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sis (Fig. 1A). When the slab gel was incubated with phosphate-buffered sucrose solution (pH 6.8), an opaque white band appeared in the band-I zone. The middle of the band was stained only wine color with subsequent periodic acid-Schiff base staining (7). In contrast, the glucans synthesized in the band-S zone were visible only after periodic acid-Schiff base staining as a wide dark-purple band (Fig. 1B). Thus, GTase-C could be separated into two active GTase proteins synthesizing water-insoluble (band I) and water-soluble (band S) glucans by slab gel electrophoresis. The proteins of the slab gel (Fig. 1A) were transferred to a nitrocellulose sheet by using the electrophoretic blotting technique described by Towbin et al. (16). After confirming the quantitative transfer of the proteins by staining of a portion of the sheet with Coomassie blue, another portion was used for determination of the target antigens of IG-3E7 monoclonal antibody. The antibody bound by the immobilized replica proteins on the sheet was detected only in the band-I zone by solid-phase immunoassay with horseradish peroxidase-conjugated rabbit anti-mouse immunoglobulin (16) (Fig. 1C). A similar analysis was done by using more purified GTase preparations that synthesize water-insoluble glucan (GTase-I) and water-soluble glucan (GTase-S) obtained by the chromatofocusing method (8). It was found that GTase-I and GTase-S contained protein band I and band S, respectively, and that IG-3E7 antibody was bound specifically to band I only (data not shown). These results indicate that IG-3E7 monoclonal antibody reacts exclusively with GTase-I.

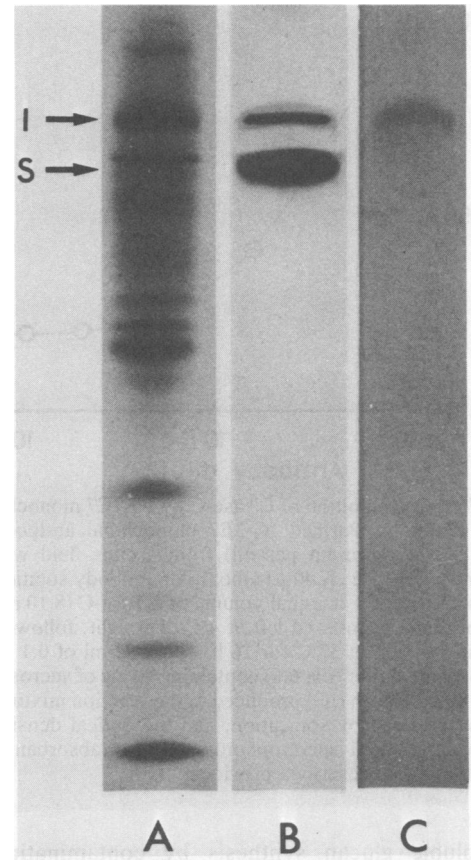

FIG. 1. Determination of GTase antigen reactive with IG-3E7 monoclonal antibody. (A) 8 M urea-3% polyacrylamide slab gel electrophoretic pattern of GTase-C. A portion of the slab gel was stained with Coomassie brilliant blue. (B) Periodic acid-Schiff base staining of the glucans produced on the gel. The gel was incubated with 5% sucrose solution overnight at 37°C. (C) IG-3E7 monoclonal antibody bound to an immobilized antigen on a nitrocellulose sheet transferred by an electrophoretic blotting procedure. The antibody was detected by solid-phase immunoassay with horseradish peroxidase-conjugated rabbit anti-mouse immunoglobulin. I, GTase-I; S, GTase-S.

TABLE 1. Inhibition effect of monoclonal antibody excreted in culture fluid of IG-3E7 cells on glucan synthesis from sucrose by various GTase-preparations

GTase preparation	IG-3E7 antibody	Glucan synthesis[a] (cpm)	Inhibition rate (%)
GTase-C	−	6,200	
	+	3,980	35.8
GTase-G	−	8,500	
	+	4,000	52.9
GTase-S[b]	−	10,100	
	+	8,600	14.9
GTase-I[b]	−	22,600	
	+	3,990	82.3

[a] GTase (5 μl) was mixed with IG-3E7 monoclonal antibody (15 μl), followed by overnight incubation at 4°C. The mixture was then incubated with 10 μl of 40 mM sucrose solution containing 0.008 μCi of [U-^{14}C]sucrose (New England Nuclear Corp., Boston, Mass.) and 10 μl of 0.4 M phosphate buffer (pH 6.8) at 37°C for 60 min. The amount of ^{14}C-glucans produced in the reaction mixture was determined by the method of Chludzinski et al. (1).

[b] Dextran T10 (8 μg) was added in sucrose solution as a primer to quantitate glucan synthesized. For details, see reference 8.

The inhibitory effect of IG-3E7 monoclonal antibody on glucan synthesis from sucrose was then examined by using various GTase preparations (Table 1). Total GTase activity was measured quantitatively with [^{14}C]sucrose by the method of Chludzinski et al. (1). The strongest inhibition (82.3%) was observed against the GTase-I preparation. A slight inhibition (14.9%) was observed in glucan synthesis by GTase-S and was probably due to inhibition against the

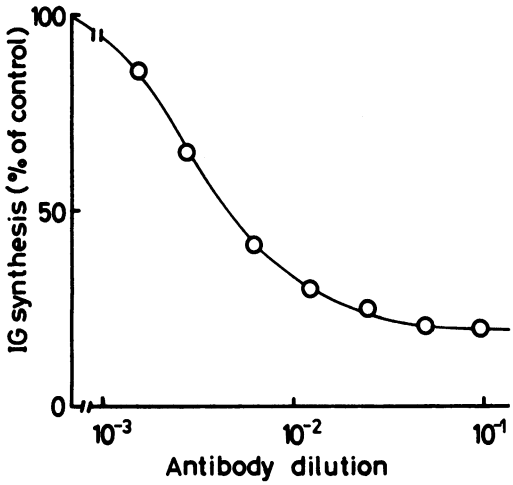

FIG. 2. Inhibition of GTase-C by IG-3E7 monoclonal antibody. Purified IG-3E7 monoclonal antibody (6.12 mg of protein per ml) from ascites fluid was diluted stepwise. A 40-μl amount of antibody solution was mixed with an equal volume of GTase-C (8.10 mg of protein per ml) and left at 4°C overnight, followed by incubation at 37°C for 16 h with 2.92 ml of 0.1 M phosphate buffer (pH 6.8) containing 90 mg of sucrose. Insoluble glucan (IG) produced in the reaction mixture was dispersed by sonication, and the optical density was determined spectrophotometrically (absorbance at 550 nm) as described previously (10).

insoluble glucan synthesis by contaminating GTase-I in the GTase-S preparation, since the presence of a trace amount of the band-I protein in the GTase-S preparation had been confirmed by 8 M urea-polyacrylamide slab gel electrophoresis. It appears that the difference in the extent of inhibition of GTase activity might be due to the ratio of GTase-I to GTase-S in each preparation.

The inhibitory effect of IG-3E7 monoclonal antibody against insoluble glucan synthesis by the GTase-C preparation was tested by using increasing concentrations of the antibody (Fig. 2). IG-3E7 monoclonal antibody was purified from ascites fluid as follows. IG-3E7 cells (10^6 cells per mouse) were injected intraperitoneally into BALB/c mice pretreated with pristane (2, 6, 10, 14-tetramethylpentadecane; Aldrich Chemical Co., Milwaukee, Wis.) (18), and the monoclonal antibody was purified from the resulting ascites fluid by precipitation with 50% saturated ammonium sulfate and by ion-exchange chromatography on a DEAE-Sephacel (Pharmacia, Uppsala, Sweden) column followed by gel filtration on a Sepharose 6B column. IG-3E7 monoclonal antibody was found to inhibit glucan synthesis of GTase-C by 35.8% (Table 1). Figure 2 shows that the antibody inhibited insoluble glucan synthesis by GTase-C at a maximum rate of 80%. This inhibition rate was similar to that obtained with GTase-I by the antibody (Table 1).

It has been found that antiserum against *S. mutans* serotype *g* GTase reacted with GTase of serotype *a*, *d*, and *g* strains, but not with those of serotype *b* and *c* strains (14). In contrast, the antiserum specific for *S. mutans* serotype *e* GTase was only inhibitory against the GTase activities of serotype *c*, *e*, and *f* strains (6). Immunological cross-reactivity of IG-3E7 monoclonal antibody was then tested with GTase-C prepared from culture supernatants of *S. mutans* strains belonging to serotype *a* to *g* (Fig. 3). The antibody strongly reacted with GTase-C of *S. mutans* B13 (serotype *d*) as well as 6715 GTase-C (serotype *g*), but not with those of the other serotypes. This result corresponds to the previous observation that the electrophoretic patterns of GTase or whole cell proteins demonstrated by sodium dodecyl sulfate-gel electrophoresis are

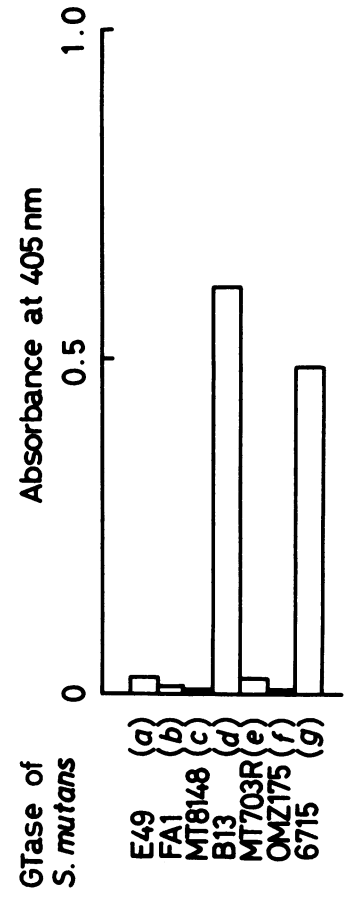

FIG. 3. Binding of IG-3E7 monoclonal antibody with GTase-C from *S. mutans* serotypes *a* to *g*. The reactivity of the antibody was quantitated by ELISA with alkaline phosphatase-conjugated rabbit anti-mouse immunoglobulin.

quite similar between serotype *d* and *g* strains (as among serotype *c*, *e*, and *f* strains), whereas protein patterns of serotype *a* are similar to a certain degree to those of serotype *d* and *g* strains (12, 17). More recently, Fukui et al. (3) found that rabbit antiserum against *S. mutans* 6715 GTase synthesizing insoluble glucan gave a positive precipitin reaction with GTase from serotype *a* as well as those of *S. mutans* serotypes *d* and *g*. However, it was also found that immunological specificity between serotype *a* and serotype *d*/*g* was completely different. Our IG-3E7 monoclonal antibody can be considered more specific than their rabbit polyclonal antiserum in distinguishing serotypes of *S. mutans*. In human populations, *S. mutans* serotype *a* or *b* has rarely been isolated. Furthermore, it should be noted that serotypes *d* and *g* and serotypes *c*, *e*, and *f* constitute two major subgroups in immunological, biological, and genetic terms (5).

We are now characterizing the other monoclonal antibodies produced by other hybrid cell lines obtained in this experiment. The availability of several different monoclonal antibodies directed against a plurality of antigenic determinants of the GTase protein will prove useful in studying the serotype specificity and function of this enzyme and in effective purification of GTase from complicated crude enzymes.

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