Streptococcus sobrinus Antigens That React to Salivary Antibodies Induced by Tonsillar Application of Formalin-Killed S. sobrinus in Rabbits

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We previously found that tonsillar application of antigen induces a strong antibody response to Streptococcus sobrinus in saliva and blood plasma. Rabbits immunized against S. sobrinus by tonsillar application were highly resistant to experimental dental caries triggered by oral inoculation of living S. sobrinus organisms with sucrose. In the present study, we examined the reaction of S. sobrinus antigens to the antibodies induced by the tonsillar application of S. sobrinus AHT-k in rabbits and compared them to those antibodies induced by intramuscular injection. In an enzyme-linked immunosorbent assay using ultrasonic fragments from mutants group streptococci, the saliva and blood plasma selectively reacted to S. sobrinus AHT-k (serotype g) and serologically related streptococci (serotypes a, d, and h) in the sixth week after tonsillar application, whereas the blood plasma in the sixth week after intramuscular injection reacted to the unrelated streptococci (serotypes b, c, e, and f) in addition to the aforementioned streptococci. The antibody reactivity induced after tonsillar application was not lost after treatment of the antigen with heat or proteinase digestion, whereas these treatments resulted in a 70% decrease of the antibody reactivity induced by intramuscular injection. The inhibition by haptenic sugars and the decrease in immunoreactivity by heat treatment and proteinase digestion suggested that 80% of the antibodies induced by tonsillar application reacted to saccharides. These saccharide antigens appeared to be involved in a specific reaction with S. sobrinus-specific streptococci and a selective reaction with serologically related streptococci. These antigens are probably involved in anticaries reactions in experimental dental caries.

Over the past several years, we have been developing tonsillar application, a new immunization route for mucosal immunity, specifically for the oral cavity (3–7, 13). Tonsillar application of sheep erythrocytes (4) and formalin-killed bacterial cells (7, 13) induced production of salivary immunoglobulin A (IgA) and blood plasma IgG more effectively than did intragastric instillation or nasal application. In rabbits immunized by tonsillar application of formalin-killed Streptococcus sobrinus, a cariogenic bacterium, the experimental dental caries were suppressed to one-fifth of the level found in non-immunized rabbits and to one-third of the level in rabbits immunized by the intragastric route (7). The number of S. sobrinus bacteria recovered from the tooth surface in the tonsillar immunized rabbits was also suppressed. The cause of this suppression was suggested to be the elimination of the bacterial cells from the oral cavity, most likely through agglutination by the induced antibodies.

In research into anticaries vaccinations, several antigens which have anticaries potential, such as an antigen I/II (14) and glucosyltransferase (28), have been reported. These antigens are proteins. However, our S. sobrinus antigen appears to be unique in Western blotting analysis (6); therefore, we expected that S. sobrinus antigen recognized by the antibodies induced through tonsillar application would be different from those proteins reported before. The purpose of this study was to examine the reaction of S. sobrinus antigens to the antibodies induced by tonsillar application. Since serum antibodies induced by intramuscular injection have been well studied, we used them as the control.

In this study, we immunized rabbits with whole cells of formalin-killed S. sobrinus by application to the palatine tonsil and by intramuscular injection. The specificity of the antibodies and the antigens of S. sobrinus recognized by the antibodies induced in the saliva and blood plasma were compared using the agglutination test, enzyme-linked immunosorbent assay (ELISA), and Western blotting analysis. The results show that the major S. sobrinus antigens that react to salivary antibodies induced by tonsillar application are saccharides.

MATERIALS AND METHODS

Immunization and fluid collection. S. sobrinus AHT-k (serotype g) (9), isolated from human dental caries (2), was cultured in brain heart infusion broth (Difco Laboratories, Detroit, Mich.). The cells were washed in phosphate-buffered saline (PBS) and then killed in 10% formalin. The dead cells were washed intensively to remove the formalin and placed in PBS to prepare the bacterial cell suspension (1010 cells/ml). The rabbits were placed under general anesthesia with ketamine hydrochloride. Then, 300 μl of the suspension was dropped onto the surface of the palatine tonsil of nine rabbits with a syringe with a hard catheter and intramuscularly injected into both sides of the femoral region of nine other rabbits. In the nine control rabbits, PBS alone was dropped onto the surface of the palatine tonsil. All rabbits received antigen suspension or PBS alone once a week for 6 weeks. Saliva was collected with a pledget under anesthesia with ketamine hydrochloride and ether once a week after initial exposure to the killed S. sobrinus cells. Insoluble substances in the saliva were removed by centrifugation at 3,500 × g for 20 min. Peripheral blood was collected from the posterior auricular vein with a heparinized syringe and spun to obtain blood plasma. The complements in the blood plasma were inactivated by heating at 56°C for 30 min. After these treatments, all saliva and blood plasma samples were snap frozen and stored at −96°C until measurement.

Detection of antibodies. The agglutination titers of the antibodies in the saliva and blood plasma were measured by the indirect agglutination test. At temperatures kept low by ice to avoid the denaturation of the antigens, the S. sobrinus cells were fragmented by an ultrasonic disruptor (200 W; 25 min) with glass beads 0.1 mm in diameter. The ultrasonic fragments were separated from the unbroken

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bacteria and the glass beads by centrifugation (7,000 × g; 30 min). Cell components in the ultrasonic fragments from S. sobrinus were bound to sheep erythrocytes with galactosylaldehyde. The direct agglutination titer with whole cells was also measured. The immunoglobulin concentration in the saliva and blood plasma immunized by tonsillar application was examined by ELISA using plates coated with 100 μl of the ultrasonic fragments of S. sobrinus containing 50 μg of protein per ml. Horseradish peroxidase (HRP)-labeled goat anti-rabbit IgG (1:200 dilution; ICN Pharmaceuticals, Inc., Costa Mesa, Calif.), HRP-labeled sheep anti-rabbit IgM (1:200 dilution; The Binding Site, Ltd., Birmingham, United Kingdom), and HRP-labeled goat anti-rabbit IgA (1:200 dilution; Nordic Immunological Laboratories, Tilburg, The Netherlands) were used. To obtain the standard curve in the ELISA reaction, various concentrations of purified rabbit IgG (ICN Pharmaceuticals Inc.), purified rabbit IgA (Inter-Cell Technologies, Inc., Hopewell, N.J.), or purified rabbit IgM (Rockland, Inc., Gilbertsville, Pa.) were used to directly coat ELISA plates and were reacted with the HRP-labeled antibodies described above. Relative IgG, IgA, and IgM concentrations in blood plasma and saliva were calculated based on the standard curves for ELISA results. The value of the antibody concentrations was calculated based on the results of the competitive assay in an enzyme immunonuclease with purified rabbit IgG. In this system, S. sobrinus cells (50 μl; 10^6 cells/ml) were incubated with equal amounts of diluted blood plasma (1:10 to 1:30 dilutions) from the immunized rabbits for 30 min and were washed intensively to remove nonreactive antibodies. Antibody-bound S. sobrinus cells were incubated with HRP-labeled goat anti-rabbit IgG (1:300 dilution) for 30 min in the presence of the appropriate concentration (0 to 75 μg/ml) of purified rabbit IgG and were washed. S. sobrinus cells were incubated with the reaction mixture, and then absorbance was measured. The IgG concentration in the incubation buffer on S. sobrinus cells was obtained from the titer curve of the purified IgG which caused a 50% inhibition in the assay. In the case of IgM and IgA, immunoglobulin concentrations also were calculated based on the results of the competitive assay in an immunoreaction as above, except for the HRP-labeled sheep anti-rabbit IgM, purified rabbit IgM, HRP-labeled goat anti-rabbit IgA, and purified rabbit IgA were used. The IgA concentration in saliva was represented as that of a monomer. The specificity of the antibodies. The specificity of the antibodies was determined by ELISA. ELISA was performed as above, except that the plates were coated with the ultrasonic fragments from S. sobrinus OMZ176 (serotype d), Streptococcus cricetus HS-1 (serotype a), Streptococcus downei MFC28 (serotype h), Streptococcus mutans IMG (serotype c), S. mutans B14 (serotype e), S. mutans OMZ176 (serotype f), Streptococcus ratti BHT (serotype b), Streptococcus santrizus ATCC 10556, Streptococcus salivarius IFO31936, or Staphylococcus aurous FDA209P, instead of S. sobrinus AHT-k (serotype g). The intensity of the ELISA reaction was expressed as the immunoglobulin concentration. The direct and indirect agglutination titers with whole cells and ultrasonic fragments of the above cells were also measured. The effect of the preincubation of the antibodies with the whole cells of the bacteria was also examined using the ELISA reaction to S. sobrinus AHT-k. To eliminate nonspecific reactions, 10 μl of the saliva or of the blood plasma was absorbed by a sufficient number (10^9 cells/ml) each of whole cells of S. sanguis and S. salivarius in a tube at 37°C for 1 h. After separation from the bacterial cells by centrifugation at 10,000 × g for 20 min, the saliva and blood plasma were absorbed by the whole cells of one of the above bacteria or of streptococci. After centrifugation, these fluids were reacted with ultrasonic fragments from S. sobrinus AHT-k in ELISA.

Antigen analysis. Ultrasonic fragments from S. sobrinus AHT-k, S. sanguis, S. salivarius, and Streptococcus pyogenes ATCC 12844 were heated at 95°C for 3 min with sodium dodecyl sulfate (SDS) and chilled with ice. The SDS-treated ultrasonic fragments from the above bacteria were electrophoresed on a sheet of sodium dodecyl sulfate (SDS)-10% polyacrylamide gel and electroblotted onto a polyvinylidene difluoride membrane. Some of the blotted strips cut from the membrane were reacted with the saliva or blood plasma of the immunized rabbits. The saliva or blood plasma absorbed by whole cells of S. salivarius or S. pyogenes was also reacted with the remaining strips. HRP-labeled goat anti-rabbit IgG (The Binding Site) was used as the secondary antibody. Some of the other strips were directly stained with Coomassie brilliant blue or periodic acid-Schiff stain.

The ultrasonic fragments from S. sobrinus AHT-k cells were heated at 100°C for 10 min or incubated with one of the proteolytic enzymes—trypsin (1.5 μg/ml; pH 8.0), papain (1.5 μg/ml; pH 5.0), proteinase K (1.5 μg/ml; pH 5.0), or natural protease (from Bacillus polymyxa; 1.5 μg/ml; pH 7.0)—at 37°C for 1 h. After hydrolysis by acetic acid (27), low-molecular-weight hydrolysates were removed by dialysis. The saccharide concentration in the retained S. sobrinus fragments was measured by the phenol-sulfuric acid method. To quantify the concentration of saccharide, a mixture of 200 μl of ultrasonic fragments of S. sobrinus diluted in water, 5 μl of 80% phenol, and 500 μl of concentrated sulfuric acid was incubated at room temperature for 10 min. After further incubation of the mixture at 30°C for 30 min, the absorbance at 490 nm was measured. The concentration of the saccharide was expressed as the glucose concentration (45 μg/0.5 A). After these treatments, the ultrasonic fragments were used in the sixth week of immunization as antigens for the ELISA reactions with the saliva and blood plasma. It was confirmed that proteolytic enzymes had no direct effects on the ELISA reaction.

One hundred microliters of saliva or blood plasma was incubated at 37°C for 2 h with 10 μM haptenic sugar (galactose, rhamnose, glucose, lactose, sucrose, maltose, or raffinose) by the modified method described in a previous study (24). Maximum inhibition, under these conditions, was observed with galactose, rhamnose, and glucose. After incubation, the reaction of the fluids with the ultrasonic fragments from S. sobrinus AHT-k was examined by ELISA, and the intensity of the ELISA reaction was expressed as the immunoglobulin concentration.

Statistical analysis. The agglutination titer was expressed as the mean ± the range between minimum and maximum values, and the significance was determined using the Mann-Whitney U test. *P < 0.0005 compared to the initial administration of the respective fluids. Ig concentrations were expressed as means ± SD, and the significance was determined using Student's t test. *P < 0.0005 versus baseline before the initial administration of the respective fluids.

RESULTS

Antibody induction. In rabbits immunized with S. sobrinus for 6 weeks, S. sobrinus antibodies were produced in the saliva and blood plasma (Table 1). The predominant class was IgA in the saliva and IgG in the blood plasma.

Specificity of the antibodies. Before immunization, all of the ELISA reactions with ultrasonic fragments of oral microorganisms in saliva and blood plasma were weak. After tonsillar application, reactions in these fluids to the applied cells and to serologically closely related mutans group streptococci were both intense, whereas the reactions to all other microorganisms, including unrelated streptococci, were as weak as they had been before immunization (*P < 0.01; n = 9) (Fig. 1). The specificity of these microorganisms (the applied cells and serologically closely related mutans group streptococci) in this ELISA reaction for blood plasma after intramuscular injection was not much different from that of the antibodies induced by tonsillar application; the reaction was slightly enhanced with the other microorganisms. The specificity in the ELISA reaction to these microorganisms was similar to that in the direct and indirect agglutination tests (data not shown).

When, after tonsillar application, the saliva was absorbed by whole cells of S. sanguis and S. salivarius, the reactivity of the absorbed saliva for the S. sobrinus fragments decreased slightly. In blood plasma after tonsillar application, the ELISA reaction after absorption decreased to levels similar to those in saliva after tonsillar application as described above (data not shown), whereas the reaction of the blood plasma after intra-

| TABLE 1. S. sobrinus antibodies in saliva and blood plasma after tonsillar application and intramuscular injection of killed S. sobrinus cells* |
| --- | --- | --- |
| Sample | Agglutination titer † | Antibody concn (μg/ml) |
| Before immunization | | |
| Saliva | 2 ± 0 | 2.9 ± 0.7 |
| Blood plasma | 8 ± 0 | 4.2 ± 0.9 |
| Tonsillar application | | |
| Saliva | 128 ± 32* | 30.6 ± 7.3* |
| Blood plasma | 256 ± 64* | 32.5 ± 6.9* |
| Intramuscular injection | | |
| Saliva | 2 ± 0 | 3.9 ± 0.6 |
| Blood plasma | 256 ± 64* | 37.3 ± 7.5* |

* Rabbits were immunized by tonsillar application or by intramuscular injection once a week for 6 weeks. The saliva and blood plasma were collected from the immunized rabbits.
† The indirect agglutination titers were expressed as the medians ± the range between minimum and maximum values, and the significance was determined using the Mann-Whitney U test.
‡ Antibody concn.
In the present study, we conclude that most of the antibodies produced following tonsillar application recognized the antigens containing saccharide, because for the most part the an-
Body reactivity decreased proportionally in response to the decrease in saccharide concentration in *S. sobrinus* fragments. However, much antibody reactivity (80%) remained after the elimination of proteins by heat treatment and proteinase digestion.

**FIG. 2.** Effect of absorption by the whole cells of certain oral bacteria in the ELISA reaction of saliva or blood plasma to ultrasonic fragments from *S. sobrinus*. The saliva before tonsillar application (a) was absorbed by *S. sanguis*, *S. salivarius*, *S. sobrinus* AHT-k, or *S. mutans* Ingbritt. The ELISA reaction between the ultrasonic fragments and the absorbed saliva was examined. The saliva after tonsillar application (b) and blood plasma after intramuscular injection (c) was absorbed by one of the mutans group streptococci, which included *S. sobrinus* AHT-k (serotype g); those serologically closely related to *S. sobrinus* AHT-k, including *S. sobrinus* OMZ176 (serotype d), *S. cricetus* HS-1 (serotype a), and *S. downei* MFe28 (serotype h); and some unrelated to AHT-k, including *S. mutans* Ingbritt (serotype c), *S. mutans* OMZ175 (serotype f), and *S. rattus* BHT (serotype b), following preabsorption with *S. sanguis* and *S. salivarius*. The ELISA reactions showing saliva or plasma absorbed by *S. sobrinus* AHT-k, the serologically closely related mutans group streptococci, and the unrelated types are expressed as solid, dotted, and hatched columns, respectively, and the nonabsorbed ELISA reaction of each fluid is expressed as open columns (non). The results show IgA concentrations in saliva and IgG concentrations in blood plasma. The results are means ± SD (n = 9). The significance was determined using Student’s t test. *, P < 0.05 compared to nonabsorbed saliva or plasma; **, P < 0.01 versus nonabsorbed saliva or plasma; †, P < 0.05 versus saliva after application of the corresponding absorption. S. san., *S. sanguis*; S. sali., *S. salivarius*.

**FIG. 3.** ELISA reaction of saliva or of blood plasma to *S. sobrinus* ultrasonic fragments after heat denaturation or proteolytic enzyme digestion. Ultrasonic fragments from *S. sobrinus* AHT-k were heated at 100°C for 10 min (solid columns) or reacted at 37°C for 1 h with various proteolytic enzymes, including trypsin, pepsin, proteinase K, and natural protease (hatched columns). The nontreated fragments (non) are shown as open columns. Then, the ELISA reactions between the treated ultrasonic fragments and the saliva (a) and blood plasma (b) before immunization, saliva after tonsillar application (c), and blood plasma after intramuscular injection (d) were examined. The results show the means ± SD (n = 9). The significance was determined using Student’s t test. *, P < 0.05 versus nontreatment; **, P < 0.01 versus nontreatment. The antibody reactivity decreased proportionally in response to the decrease in saccharide concentration in *S. sobrinus* fragments. However, much antibody reactivity (80%) remained after the elimination of proteins by heat treatment and proteinase di-
gestion of \( S. \) \( sobrinus \) antigens. Our conclusion is supported by the reactions of the saliva and the blood plasma of \( S. \) \( sobrinus \)-immunized rabbits, which gave a smear identical to that seen in the direct staining for the saccharide in Western blotting analysis. The marked inhibitory effect of antibody reactivity shown by some haptenic sugars also supports our conclusion. In addition, our preliminary results support the hypothesis that the antibodies to \( S. \) \( sobrinus \) antigens produced following tonsillar application are polysaccharides, because the reaction to these antibodies paralleled the saccharide-positive reactions in the fractions obtained by gel filtration of the autoclaved supernatant of \( S. \) \( sobrinus \). In \( S. \) \( sobrinus \) two kinds of saccharide antigens were reported: a rhamnose-glucose polymer (18, 25) and a galactose-glucose polymer, which was found to be a polysaccharide serotype antigen (29).

In this study, we detected \( S. \) \( sobrinus \) AHT-k-specific antibodies in the fluids of immunized rabbits after the absorption by whole cells of related mutans group streptococci. ELISA reactions with this specific antibody in the saliva after tonsillar application (Fig. 6) and intramuscular injection (data not shown) were both inhibited by rhamnose, showing that the antigen reacting to the antibody contains rhamnose. Since the only polysaccharide antigen of \( S. \) \( sobrinus \) containing rhamnose is the rhamnose-glucose polymer, the \( S. \) \( sobrinus \)-specific antibody must react to this polymer. If this is true, it presents some difficulties because less rhamnose-glucose polymer is contained in the cells of \( S. \) \( sobrinus \) AHT-k-related mutans group streptococci (serotypes d, a, and h) than in the cells of \( S. \) \( sobrinus \) AHT-k (serotype g) (23). In the present study, the \( S. \) \( sobrinus \)-specific ELISA reaction proceeded after absorption by the whole bacterial cells but not with cell component antigens (data not shown). The results suggest that a specific reaction depends on the three-dimensional position of the antigen: the rhamnose-glucose polymer is exposed in the cells of \( S. \) \( sobrinus \) AHT-k (serotype g) but covered in the related cells (serotypes a, d, and h).

The other saccharide antigen of \( S. \) \( sobrinus \) AHT-k is a serotype antigen. Previous studies have shown that the serotype antigens of \( S. \) \( sobrinus \) (serotypes d and g) are polysaccharides containing galactose and glucose (10, 12), which have a chemical structure and immunological properties very similar to those of serologically related streptococci (serotypes a and h) (10, 17, 19, 22), while the antigens of the unrelated groups, such as serotypes c, e, f, and b, are immunologically distinct polysaccharides containing rhamnose and glucose (18). In this study, the reactivity for the \( S. \) \( sobrinus \) fragments was decreased by galactose and glucose as well as by rhamnose. The ELISA reactivity for galactose and glucose was lost after the absorption by whole cells of the related streptococci (serotypes d, a, and h) but did not disappear after absorption by the unrelated cells (serotypes c, e, f, and b) (data not shown). Thus, the antibodies reacting to the related streptococci recognize the polysaccharide serotype antigen containing galactose and glucose, especially that part which serotypes g, d, a, and h have in common. This common region must be exposed on the cell surface of \( S. \) \( sobrinus \) AHT-k and of the related serotype groups, because the ELISA reaction was decreased by the absorption by whole cells of streptococci of serotypes g, d, a, and h.

The saliva and blood plasma of rabbits immunized by tonsillar application contained small amounts of antibodies reacting to protein antigens of \( S. \) \( sobrinus \). This corresponds to the amount of preexisting antibodies, most of which react to protein antigen, in these fluids in nonimmunized rabbits. The antibody concentration in the saliva and blood plasma after tonsillar application of bacteria other than \( S. \) \( sobrinus \), according ELISA, also corresponds to that of preexisting antibodies. These antibodies appeared to be broadly reactive types because most were eliminated by preabsorption with \( S. \) \( salivarius \) or \( S. \) \( sanguis \). The blood plasma of rabbits immunized by intramuscular injection contained a large proportion (nearly 70%) of antibodies to protein antigens. In the reaction of the blood plasma after intramuscular injection, the major protein antigens appeared as several bands on Western blotting analysis (Fig. 4). Several bands appeared to have reacted with

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**TABLE 2. Antigen components in \( S. \) \( sobrinus \) ultrasonic fractions after proteinase digestion and hydrolysis by acetic acid**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Treatment time (min)</th>
<th>Protein concn (μg/ml)</th>
<th>Saccharide concn (μg/ml)</th>
<th>Antibody reactivity* (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>0</td>
<td>137.5</td>
<td>57.2</td>
<td>30.7</td>
</tr>
<tr>
<td>Elimination after heat denaturation</td>
<td>10</td>
<td>12.5</td>
<td>52.3</td>
<td>25.5</td>
</tr>
<tr>
<td>Trypsin digestion</td>
<td>60</td>
<td>13.8</td>
<td>55.7</td>
<td>26.1</td>
</tr>
<tr>
<td>Hydrolysis by acetic acid</td>
<td>30</td>
<td>9.7</td>
<td>44.6</td>
<td>20.4*</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>9.3</td>
<td>23.1</td>
<td>10.2*</td>
</tr>
<tr>
<td></td>
<td>90</td>
<td>9.3</td>
<td>3.2</td>
<td>1.7*</td>
</tr>
</tbody>
</table>

* Antibody concentrations were expressed as means ± SD, and the significance was determined using Student’s t test. *P < 0.01 versus nontreatment.
bacteria but not by because these bands disappeared after absorption by certain blotting are involved in cross-reactions with certain bacteria, not shown). Some of the major bands detected by Western

S. sobrinus

cles (8).

not contain antibodies cross-reacting to human cardiac mus-

saliva and blood plasma induced by tonsillar application did

cardiac muscles. In our previous studies, the antibodies in

production in saliva and blood plasma of antibodies directed

52-kDa bands (6, 11, 26) and the bands not clearly visible at

absorption of antibodies by

S. salivarius

and

S. sanguis

ultrasonic fragments and saliva. One hundred microliters of saliva after tonsillar

application (a) or of blood plasma after intramuscular injection (b) was incu-

bated at 37°C for 2 h with 10 μM haptenic sugar: galactose, rhamnose, glucose

(monosaccharides are shown as solid columns), lactose, sucrose, maltose, or raffinose (oligosaccharides are shown as hatched columns). The ELISA reaction of the sugar-treated saliva and blood plasma to the S. sobrinus ultrasonic frag-

ments was examined. The reaction of each nontreated fluid (non) is expressed as

an open column. The reaction of the sugar-treated saliva and blood plasma to the

ultrasonic fragments from S. sobrinus AHT-k was examined and expressed as the

IgA and IgG concentrations, respectively. The results are the means ± SD (n = 9).

The significance was determined using Student’s t test. *, P < 0.01 versus nontreatment; **, P < 0.05 versus nontreatment.

broadly reactive antibodies, because they disappeared after the absorption of antibodies by S. salivarius and S. sanguis (data not shown). Some of the major bands detected by Western blotting are involved in cross-reactions with certain bacteria, because these bands disappeared after absorption by certain bacteria but not by S. salivarius and S. sanguis. The 62- and 52-kDa bands (6, 11, 26) and the bands not clearly visible at 170 kDa (15) were also involved in the cross-reaction to human cardiac muscles. In our previous studies, the antibodies in saliva and blood plasma induced by tonsillar application did not contain antibodies cross-reacting to human cardiac muscles (8).

The present findings show that tonsillar application with whole cells of S. sobrinus AHT-k preferentially induced the production in saliva and blood plasma of antibodies directed against cell surface antigens, including the S. sobrinus-specific

saccharides, probably rhamnose-glucose polymers, and serotype-selective (with a common related group) saccharides, such as galactose-glucose polymers. On the other hand, the intramuscular injection-induced antibodies in the blood plasma reacted to a wide variety of cell surfaces and covered antigens, including the proteins that cross-react with certain bacteria, as well as S. sobrinus-specific and the serotype-selective saccharides.

The present results suggest the possibility that selectivity in the induced antibodies by tonsillar application takes place in any kind of bacterial cell. There have been several reports that differences in the immunization route cause a difference in the specificity of the induced antibodies. However, there are no reports showing similar selective antibody induction by immunization. Even in intragastric instillation, including oral adminis-

tration, the immunization of whole cells of S. mutans induced the salivary antibodies to react not only to the serotype carbohydrate but also to the lipoteichoic acid (21).

The antibodies induced by intramuscular injection reacted to a wide variety of bacterial antigens. With respect to the physiological role of the antibody, this specificity of the anti-

bodies is suitable for eliminating bacteria in blood plasma, where only one kind of bacterium occasionally exists. However, in the oral cavity, where a wide variety and great number of microorganisms exist all the time, antibodies specific and/or selective for surface antigens seem essential for the selective microbial elimination of mutants group streptococci.

There are some differences between the antibodies induced by tonsillar application and by intramuscular injection. We do not know why tonsillar application and intramuscular injection of the same S. sobrinus organisms resulted in differences in recognizing antigens. By both routes, IgG was the predominant antibody in blood plasma, and many specific IgG-producing cells, probably the source of the blood plasma IgG, were detected in the spleen (13). There must be differences in the steps from antigen incorporation to antibody production in the spleen. These differences could be caused by many factors, from selective B-cell stimulation by an unknown mechanism

FIG. 5. Effect of haptenic sugar on the ELISA reaction between S. sobrinus ultrasonic fragments and saliva. One hundred microliters of saliva after tonsillar application (a) or of blood plasma after intramuscular injection (b) was incubated at 37°C for 2 h with 10 μM haptenic sugar: galactose, rhamnose, glucose (monosaccharides are shown as solid columns), lactose, sucrose, maltose, or raffinose (oligosaccharides are shown as hatched columns). The ELISA reaction of the sugar-treated saliva and blood plasma to the S. sobrinus ultrasonic fragments was examined. The reaction of each nontreated fluid (non) is expressed as an open column. The reaction of the sugar-treated saliva and blood plasma to the ultrasonic fragments from S. sobrinus AHT-k was examined and expressed as the IgA and IgG concentrations, respectively. The results are the means ± SD (n = 9). The significance was determined using Student’s t test. *, P < 0.01 versus nontreatment; **, P < 0.05 versus nontreatment.

FIG. 6. Effect of haptenic sugar on the ELISA reaction between the S. sobrinus ultrasonic fragments and saliva absorbed by the mutans group streptoco-

celli related to S. sobrinus. Following absorption by S. cricetus and S. downei after tonsillar application, 100 μl of saliva was incubated at 37°C for 2 h with 10 μM haptenic sugar, including galactose, rhamnose, glucose (monosaccharides are shown as solid columns), and lactose (shown as a hatched column). Saliva absorbed by S. cricetus and S. downei and saliva not treated with sugars (non) are shown as an open column. The ELISA reaction of the sugar-treated saliva to the ultrasonic fragments from S. sobrinus AHT-k was examined and expressed as the IgA concentration. The results are the means ± SD (n = 27). The significance was determined using Student’s t test. *, P < 0.01 versus nontreatment; **, P < 0.05 versus nontreatment.
during antigen processing to presentation or selective B-cell suppression by tolerance. The possibility of selective antigenic repertory in T and B cells is unlikely because immunological induction took place in the spleen.

The animals infected with S. sobrinus showed more severe lesions than those infected with the other mutans group streptococci, such as S. mutans (2, 20, 30). In epidemiological studies of humans, the frequency of severe caries seems to correlate with the presence of S. sobrinus (16). Our results showing that a serotype-selective polysaccharide antigen must be involved in the elimination of S. sobrinus and in the decrease in S. sobrinus-induced caries provide for the development of effective vaccinations against dental caries caused by S. sobrinus.

We know that the intramuscular immunization of S. mutans induces antibodies to the serotype-selective polysaccharide antigen involved in the reaction with serologically related S. mutans (9); therefore, if a similar reaction was to occur after tonsillic application, the combination of our antigen and the S. mutans (9); therefore, if a similar reaction was to occur after tonsillic application, the combination of our antigen and the S. mutans

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