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Inhibition of *Streptococcus mutans* Adherence to Saliva-Coated Hydroxyapatite by Human Secretory Immunoglobulin A (S-IgA) Antibodies to Cell Surface Protein Antigen I/II: Reversal by IgA1 Protease Cleavage

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The effect of human secretory immunoglobulin A (S-IgA) and serum antibodies to surface protein antigen (Ag) I/II on the adherence of Ag I/II-bearing *Streptococcus mutans* and of free Ag I/II to saliva-coated hydroxyapatite (SHA) was investigated. The inhibition by S-IgA of binding of both *S. mutans* and free Ag I/II to SHA was dependent on antibody to Ag I/II. Essentially no difference was found between S-IgA1 and S-IgA2 with respect to antibody-dependent inhibition of Ag I/II binding to SHA, but S-IgA1 inhibited *S. mutans* adherence more effectively than did either serum immunoglobulin A1 (IgA1) or IgG antibodies. The antiadherence effect of S-IgA was abrogated after cleavage by IgA1 protease. Purified Fabα fragments containing Ag I/II-binding activity enhanced the binding of free Ag I/II to SHA and showed greater binding to SHA than did intact S-IgA1. Despite its relative inability to interact with precoated SHA, S-IgA1 containing antibody to Ag I/II was readily incorporated into the salivary pellicle during coating, but this did not promote Ag I/II binding. These data suggest that S-IgA antibodies can inhibit the initial adherence of *S. mutans* to salivary pellicle-coated tooth surfaces in an adhesin-specific fashion, but the presence in the oral cavity of bacterial IgA1 proteases would potentially interfere with this antiadherence mechanism.

The colonization of smooth tooth surfaces by *Streptococcus mutans* involves adherent interactions between this cariogenic microorganism and the salivary pellicle that coats the dental enamel. Although weak and reversible, this initial binding appears to be an indispensable preliminary step to the eventually irreversible adhesion and accumulation of *S. mutans* cells mediated by extracellular polysaccharides (12). Both *S. mutans* (13) and its cell surface protein antigen (Ag) I/II (37) bind selectively to saliva-coated hydroxyapatite (SHA), which simulates pellicle-coated enamel, but isogenic Ag I/II-deficient mutants of *S. mutans* lack the protein fuzzy coat on the cell surface and bind poorly to SHA compared with the parent strains (19, 23). These findings suggest that Ag I/II can function as a major adhesin in mediating the initial adherence of *S. mutans* to salivary pellicle-coated tooth surfaces, although this may not be the only mechanism (3, 39).

Secretory immunoglobulin A (S-IgA) antibodies act as the host's first line of mucosal defense by interfering with microbial adherence and colonization (15). For example, human salivary S-IgA antibodies inhibit the adherence of oral streptococci to isolated epithelial cells from the buccal mucosa (44), and murine intestinal S-IgA antibodies to *Vibrio cholerae* reduce the adsorption of vibrios to the intestinal mucosa (10). In earlier studies on the effect of naturally occurring human S-IgA antibodies on the binding of *S. mutans* to SHA, no conclusive results were obtained: either adherence was unaffected (11), or the observed inhibition could not be ascribed necessarily to a specific antibody (17). S-IgA-mediated inhibition of *S. mutans* adherence was clearly demonstrated in a more recent report (32), although the antigen specificity of the antibodies involved was not addressed. In the same study, bacterial immunoglobulin A1 (IgA1) proteases, which specifically cleave IgA1 in the hinge region, yielding intact Fabα and Fcα fragments, abrogated the adherence-inhibiting effect of S-IgA. Inhibition of the binding of *S. mutans* to experimental pellicles in an antigen-specific fashion was shown by using rabbit IgG polyclonal antibodies to antigen B (Ag I/II) (9) or mouse monoclonal IgG antibodies to antigen P1 (Ag I/II) (4).

The experiments in this study were designed (i) to determine whether human S-IgA antibodies to Ag I/II inhibit the binding of free Ag I/II or Ag I/II-bearing *S. mutans* to SHA, (ii) to examine the effect of cleavage of S-IgA antibodies by IgA1 protease on the inhibition of adherence, and (iii) to compare the antiadherence activities of S-IgA and serum IgA and IgG antibodies.

MATERIALS AND METHODS

Streptococcal strains and growth conditions. The *S. mutans* strains used in this study were MT 8148 (provided by S. M. Michalek, University of Alabama at Birmingham); IB 162 (provided by K. W. Knox, Institute of Dental Research, Sydney, Australia); and NG8 and 834 (both donated by A. S. Bleiweis, University of Florida, Gainesville). Strains MT 8148 and NG8 express and retain cell surface Ag I/II, strain IB 162 does not retain Ag I/II on its cell surface, and strain 834 is an isogenic Ag I/II-deficient mutant of the NG8 strain. Inocula from stock cultures were grown anaerobically in Todd-Hewitt broth (Difco Laboratories, Detroit, Mich.) at 37°C. Early-stationary-phase cells were used in all experiments.

For adherence studies, the microorganisms were metabol-

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ically radiolabeled by including in the culture medium 10 μCi of [^{3}H]thymidine (Amersham Corp., Arlington Heights, Ill.) per ml. The bacteria were washed three times with buffered KCl (50 mM KCl, 1 mM potassium phosphate, 1 mM $CaCl_2$, 0.1 mM $MgCl_2$ [pH 6.0]) and suspended in buffered KCl containing 0.5% bovine serum albumin (BSA; Sigma Chemical Co., St. Louis, Mo.) at 2×10^8 cells per ml (7). The streptococcal suspensions were dechained by passing them through a 27-gauge syringe needle 10 to 15 times. Microscopic examination verified that this treatment resulted in single or paired cells, whereas chains with three or more cells were sparse. Bacterial numbers were estimated turbidimetrically at 660 nm by comparison to direct bacterial cell counts. The specific activities of the washed *S. mutans* cells were 0.8×10^{-3} to 1.2×10^{-3} cpm per cell.

Preparation of *S. mutans* Ag I/II. Ag I/II was purified from culture supernatants of *S. mutans* IB 162 by ammonium sulfate precipitation and chromatography on DEAE-cellulose and Sephacryl S-300 (34). Purity was assessed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) with Coomassie blue staining, which revealed one band with an M_r corresponding to that of Ag I/II (approximately 185,000). For adherence studies, Ag I/II was radiolabeled with ^{125}I (0.25 mCi/10 μg of protein) by the chloramine T method and separated from unbound ^{125}I on a Sephadex G-25 column (Pharmacia LKB Biotechnology, Piscataway, N.J.) in phosphate-buffered saline containing 1% BSA (37). The specific activity was 1.2×10^7 cpm/μg of Ag I/II.

Enzyme-linked immunosorbent assay (ELISA). All immunological reagents were obtained from Dakopatts (Glostrup, Denmark), unless otherwise specified. To quantify total immunoglobulin concentrations, the unknown samples were titrated in five duplicate twofold dilutions in parallel with human serum standards (The Binding Site Ltd., Birmingham, England) on microtiter plates coated with anti-human IgA, IgG, or IgM (1:1,000). The plates were developed with the corresponding peroxidase-conjugated anti-human immunoglobulin isotype. The color developed with a substrate of *o*-phenylenediamine and H_2O_2 was measured at 490 nm after 10 to 15 min. Standard curves were generated, and the unknowns were interpolated by means of a computer program based on four-parameter logistic algorithms (35). Antibody to Ag I/II was quantified as described above, except that the unknown samples were titrated on plates coated with Ag I/II (5 μg/ml). Fabα fragments were assessed for anti-Ag I/II binding activity by using intact S-IgA1 antibodies as standards, and the plates were developed with peroxidase-conjugated anti-κ- and anti-λ-chain antibodies.

To assess the separation of S-IgA1 and S-IgA2 preparations, samples were incubated in wells coated with anti-human IgA, and plates were developed with mouse monoclonal antibodies to human IgA1 or IgA2 (6) and peroxidase-conjugated rabbit anti-mouse immunoglobulins. To quantify the subclasses, purified polymeric myeloma IgA1 and IgA2 were used as standards (provided by J. Mestecky, University of Alabama at Birmingham).

Cleavage of S-IgA by IgA1 protease was detected by a modification of a previously described assay (31). Briefly, digested and control samples were incubated on plates coated with rabbit anti-mouse immunoglobulin and mouse monoclonal antibody to human Fcα (Dako clone 6E2C1) and developed with peroxidase-conjugated anti-κ- and anti-λ-chain antibodies. To detect cleavage of anti-Ag I/II S-IgA1 antibodies, plates were coated with Ag I/II and developed with mouse monoclonal antibody to Fcα or IgA1 (or IgA2 as a control) and peroxidase-conjugated anti-mouse immunoglobulin. To verify the identity of Fabα fragments purified by size exclusion chromatography, fractions were tested for their reactivity with anti-human IgA and peroxidase-conjugated anti-κ and anti-λ antibodies.

The effectiveness of adsorption of anti-*S. mutans* antibodies on *S. mutans* was evaluated in plates coated with 4×10^8 cells per ml. The bacteria were fixed in the wells with 0.3% methyl glyoxal in 0.1 M sodium bicarbonate (pH 8.3) (1). Development was done with appropriate peroxidase-conjugated anti-human immunoglobulin isotypes.

Purification of immunoglobulins. S-IgA was isolated as described previously (27) from human colostrum, which is a much richer S-IgA source than saliva and contains antibodies to oral bacteria (2). Cells and lipids were removed by centrifugation, and clarified colostrum was depleted of casein by acidification (to pH 4.2 with 2% acetic acid) and centrifugation. Crude S-IgA was precipitated with 50% saturated ammonium sulfate, and lactoferrin was removed by affinity chromatography on heparin-Sepharose 4B (Pharmacia). The preparation was then subjected to anion-exchange chromatography on a MonoQ column (Pharmacia), and fractions were tested for the presence of IgA by the ELISA. The purity of the preparation was tested by gradient SDS-PAGE with unreduced and reduced samples (22), and by the ELISA for IgA, IgG, and IgM. Under reducing conditions, three bands with M_rs corresponding to those of secretory component (SC), α chains, and light chains were detected; under nonreducing conditions, one band that migrated more slowly than the M_r-200,000 marker was detected. In addition to IgA, the ELISA detected trace amounts of IgM and IgG.

S-IgA1 and S-IgA2 were separated from S-IgA, purified as described above, by affinity chromatography on a column of agarose-bound jacalin (Vector Laboratories, Burlingame, Calif.) equilibrated in 0.175 M Tris buffer (pH 7.5) (20). Unbound S-IgA2 was collected in the effluent, and bound S-IgA1 was eluted with 0.1 M melibiose in 0.175 M Tris buffer (pH 7.5). The S-IgA1 preparation contained 97% subclass 1, and the S-IgA2 preparation contained more than 90% subclass 2, as shown by the ELISA.

Serum IgA1 and IgG containing antibody to Ag I/II from a patient with subacute bacterial endocarditis (SBE) due to *S. mutans* (38) and normal IgA1 and IgG from a healthy donor were purified essentially as described previously (36) by anion-exchange chromatography (MonoQ column). IgA1 was further purified by affinity chromatography on jacalin-agarose as described above and on protein G-Sepharose 4 Fast Flow (Pharmacia) to remove residual IgG. The purity of the preparations was verified by SDS-PAGE, and the ELISA showed that the IgA1 preparation contained more than 99% subclass 1.

Preparation of IgA1 protease and cleavage of S-IgA. IgA1 protease was prepared by ammonium sulfate precipitation (60% saturation, pH 7.2) of *Streptococcus sanguis* ATCC 10556 (donated by M. Kilian, University of Aarhus, Denmark) culture supernatants and size-exclusion chromatography on a Superose 12 HR 16/50 column (Pharmacia) (33). After overnight incubation at 37°C of S-IgA with IgA1 protease, cleavage was detected with the ELISA as explained above. Fabα fragments were purified by fractionating the digest by size exclusion chromatography on Superose 12 equilibrated in phosphate-buffered saline and calibrated with M_r markers ranging from 1,350 to 670,000. The fractions corresponding to M_rs of approximately 45,000 were collected and verified to contain Fabα fragments that retained their antigen-binding capacity in the ELISA.

Adsorption of antibodies. To remove specific antibodies, immunoglobulin preparations (0.5 ml containing approximately 2 mg of immunoglobulin per ml) were adsorbed on *S. mutans* strains (approximately 5×10^{10} cells) at 37°C for 1 h and then at 4°C overnight. The cells were removed by centrifugation, and the supernatant was evaluated by the ELISA. If undesired antibodies remained, the adsorption was repeated.

Adherence assays. The binding of Ag I/II, S-IgA1, and Fabα to SHA was assayed by a modification (37) of the method of Clark et al. (7). Briefly, SHA was prepared by treating 5 mg of hydroxyapatite powder (HTP; Bio-Rad, Richmond, Calif.) with 100-μl samples of clarified whole human saliva. In certain experiments, hydroxyapatite was coated with saliva plus exogenous S-IgA. Saliva was collected freshly for each experiment from a healthy donor with a low level of IgA anti-Ag I/II antibody (<1 μg/ml). After three washes in buffered KCl containing 0.5% BSA to block any uncoated regions, SHA was incubated with approximately 5×10^4 cpm of ^{125}I-Ag I/II, ^{125}I-S-IgA1, or ^{125}I-Fabα in 100 μl of buffered KCl containing 1% BSA. S-IgA1 and Fabα fragments were labeled as described above for Ag I/II and showed similar specific activities (1.1×10^7 to 1.3×10^7 cpm/μg of protein). The samples were incubated at room temperature with end-over-end mixing for 1 h; then unbound molecules were removed with three washes in buffered KCl. Bound radioactivity was measured with a Gamma 4000 counter (Beckman Instruments, Irvine, Calif.). Background binding was assessed on BSA-coated hydroxyapatite.

Binding of [^{3}H]thymidine-labeled *S. mutans* cells (2.5×10^7 bacteria) to SHA was assayed as described above, except that hydroxyapatite beads were used (Macrosorb C or BDH; Gallard-Schlesinger, Carle Place, N.Y.) instead of hydroxyapatite powder, the assay volume was 125 μl, and bound radioactivity was measured with a liquid scintillation spectrometer (Beckman Instruments) (18).

To determine the effect of antibodies on the binding of purified Ag I/II or *S. mutans*, immunoglobulin preparations were added to SHA and antigen or bacteria in the reaction tubes.

RESULTS

Inhibition of the binding of Ag I/II to SHA. Human S-IgA containing anti-Ag I/II antibody inhibited the binding of Ag I/II to SHA in a dose-dependent fashion (Fig. 1). When S-IgA was depleted of antibody to Ag I/II by adsorption on the cell surface Ag I/II-retaining *S. mutans* MT 8148 (Table 1), the antiadherence activity was abolished (Fig. 1), suggesting that the inhibition was antibody specific. In contrast, control adsorption of S-IgA on *S. mutans* IB 162, which sheds its surface Ag I/II, did not alter its ability to inhibit the binding of Ag I/II to SHA (Table 1, Fig. 1). When S-IgA was cleaved by overnight pretreatment with IgA1 protease (Table 2), the antiadherence effect was counteracted (Fig. 1). Residual inhibitory activity could be due to uncleaved S-IgA1 or to S-IgA2 antibodies present in the S-IgA preparation (Table 2). To examine whether both subclasses were able to inhibit the binding of Ag I/II to SHA, S-IgA1 and S-IgA2 purified from four human colostral samples and adjusted to contain equal concentrations of antibody were tested in the Ag I/II adherence assay. Generally there was little difference in inhibitory activity between the subclasses or among samples obtained from different colostrums (Fig. 2).

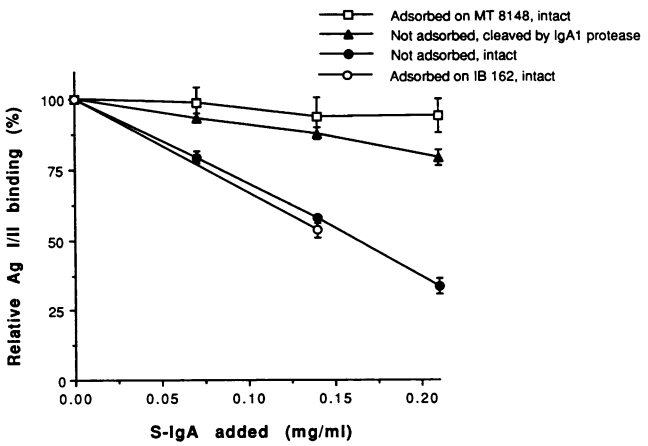

FIG. 1. Inhibition of the Ag I/II binding to SHA by specific and intact S-IgA antibody. Intact S-IgA contained 4.28 μg of anti-Ag I/II antibody per mg of total IgA; at the S-IgA concentrations tested, the anti-Ag I/II antibody concentrations were 0.3, 0.6, and 0.9 μg/ml. Each point represents the mean ± standard deviation (SD) of triplicate determinations.

Effect of Fabα fragments on the binding of Ag I/II to SHA. To determine whether Fabα fragments retain some ability to inhibit Ag I/II adherence, purified Fabα fragments containing Ag I/II-binding activity (Table 2) were tested in the Ag I/II adherence assay. The results (Fig. 3) suggested that Fabα antibody fragments enhanced the binding of Ag I/II to SHA and therefore that Fabα fragments might bind more readily than S-IgA1 to SHA. In support of this concept, radiolabeled Fabα fragments were found to bind to SHA more than S-IgA1 did (Fig. 4A). The binding of radiolabeled Fabα or S-IgA1 was inhibited by unlabeled Fabα or S-IgA1, respectively, but not by unrelated proteins (data not shown). In contrast to its relative inability to interact with preformed pellicle, S-IgA1 was readily incorporated into the pellicle during its formation (Fig. 4B). Although this might be expected to promote the adherence of Ag I/II, binding was either unaffected or even inhibited when pellicles were prepared in the presence of threefold-higher concentrations of S-IgA (Fig. 4C).

Inhibition of *S. mutans* adherence to SHA by human anti-Ag I/II S-IgA. To determine whether S-IgA antibody to Ag I/II can inhibit the binding of *S. mutans* to SHA, we investigated

TABLE 1. Specific antibodies determined by ELISA in colostral S-IgA preparations before and after adsorption on *S. mutans* strains

S. mutans strain used for adsorption	Mean optical density at 490 nm (n = 2)			
	Total IgA (1:2,000)[a]	IgA antibody to:		
		MT 8148 (1:400)	NG8 (1:400)	Ag I/II (1:200)
None	1.317	0.767	ND[b]	0.682
MT 8148	1.233	0.028	ND	0.003
IB 162	1.182	0.125	ND	0.696
None	1.057	ND	0.540	0.369
NG8	1.075	ND	0.027	0.007
834	1.025	ND	0.054	0.314

[a] Numbers within parentheses indicate dilutions.
[b] ND, not determined.

TABLE 2. Assay of S-IgA preparations before and after cleavage by IgA1 protease

Assay for:	ELISA method		Sample	Concn of sample in assay (μg/ml)	Mean optical density at 490 nm (n = 2)	
	Coating	Development			Intact IgA	Protease-treated IgA
Intact total IgA	Anti-Fcα	Anti-κ and -λ	S-IgA	0.24	0.444	0.109
Intact anti-Ag I/II IgA	Ag I/II	Anti-Fcα	S-IgA	19.2	0.178	0.055
Intact anti-Ag I/II IgA1	Ag I/II	Anti-IgA1	S-IgA	19.2	0.469	0.045
Intact anti-Ag I/II IgA2	Ag I/II	Anti-IgA2	S-IgA	19.2	0.124	0.116
Anti-Ag I/II Fab	Ag I/II	Anti-κ and -λ	S-IgA1	3.2	0.207	
Anti-Ag I/II Fab	Ag I/II	Anti-κ and -λ	Fabα	3.2		0.464

the binding to SHA of two cell surface Ag I/II-expressing *S. mutans* strains, NG8 and MT 8148, in the presence of various S-IgA preparations. S-IgA was depleted of anti-*S. mutans* antibody other than anti-Ag I/II by adsorption on *S. mutans* 834, an Ag I/II-deficient isogenic mutant of NG8, or *S. mutans* IB 162, which does not retain Ag I/II on its surface. S-IgA was also depleted of all anti-*S. mutans* antibodies by adsorption on the NG8 and MT 8148 strains (Table 1). The adherence of *S. mutans* NG8 and MT 8148 was inhibited by intact and unadsorbed S-IgA (Fig. 5). Removal from S-IgA of anti-*S. mutans* antibody except for antibody to Ag I/II did not affect its antiadherence function, but removal of all anti-*S. mutans* antibody resulted in diminished antiadherence activity. The inhibitory effect on *S. mutans* adherence of S-IgA containing antibody to Ag I/II was counteracted by pretreatment with IgA1 protease (Fig. 5).

Adherence inhibition by S-IgA1 compared to serum IgA1 and IgG. Serum IgA1 and IgG with antibody activities against Ag I/II were isolated from the serum of a patient with SBE due to *S. mutans*. Anti-*S. mutans* antibody other than anti-Ag I/II was removed by adsorption on *S. mutans* 834 (Table 3). S-IgA1 was adsorbed on either strain 834 or strain NG8 to obtain preparations that contained or lacked antibody to Ag I/II (Table 3). IgA1 and IgG isolated from a healthy donor were depleted of all anti-*S. mutans* antibody by adsorption on *S. mutans* NG8 (Table 3). The specific activity of each antibody preparation was adjusted to 4.5 μg of antibody per mg of immunoglobulin by adding an appropriate amount of corresponding antibody-free (adsorbed) immunoglobulin. All immunoglobulin preparations containing antibody to Ag I/II inhibited the binding of *S. mutans* NG8 to SHA in a dose-dependent manner (Fig. 6), but S-IgA1 exhibited more adherence inhibition than did either serum IgA1 or IgG, which demonstrated comparable activities. Control immunoglobulin preparations lacking anti-Ag I/II antibody were without effect (Fig. 6).

DISCUSSION

In this study we demonstrated that Ag I/II was a prominent target of the anti-*S. mutans* adherence activity of human S-IgA antibody. Inhibition by S-IgA of the binding of both free Ag I/II and *S. mutans* cells was dependent upon antibody to Ag I/II. Removal of anti-*S. mutans* antibody other than anti-Ag I/II did not significantly affect the adherence-inhibiting effect of S-IgA. These findings suggest either that Ag I/II is a major *S. mutans* adhesin for binding to SHA or that other surface structures involved in adherence are sterically blocked by antibody bound to cell-surface Ag I/II. However, the finding that Ag I/II-deficient mutants bind poorly to SHA, in contrast to their parent *S. mutans* strains (3, 19, 23), favors the first interpretation. The pellicle receptor for Ag I/II has not been identified, but our earlier report that the binding of Ag I/II to SHA is inhibited by amino sugars and such substances as Tris (37) has received recent

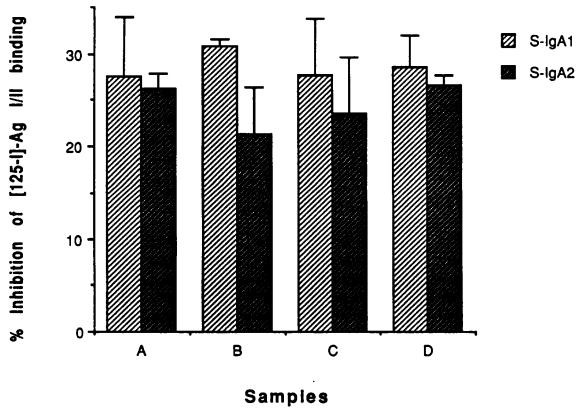

FIG. 2. Inhibition of the binding of Ag I/II to SHA by S-IgA1 and S-IgA2. S-IgA subclass samples from four different colostrum samples were adjusted to equal concentrations of anti-Ag I/II antibody activity (0.4 μg/ml). Data are presented as the means ± SDs of triplicate determinations.

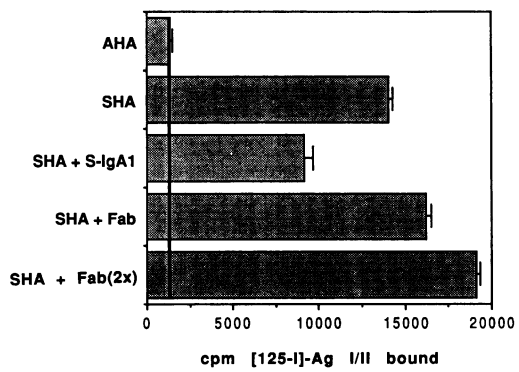

FIG. 3. Effect of Fabα fragments on the binding of Ag I/II to SHA. S-IgA1 was used at 0.6 μg/ml of anti-Ag I/II antibody (equivalent to approximately 0.3 μg/ml of anti-Ag I/II Fabα), whereas Fabα was used at 0.175 μg/ml and 0.35 μg/ml (2×) anti-Ag I/II Fabα. AHA, BSA-coated hydroxyapatite. Results are expressed as the means ± SDs of triplicate assays.

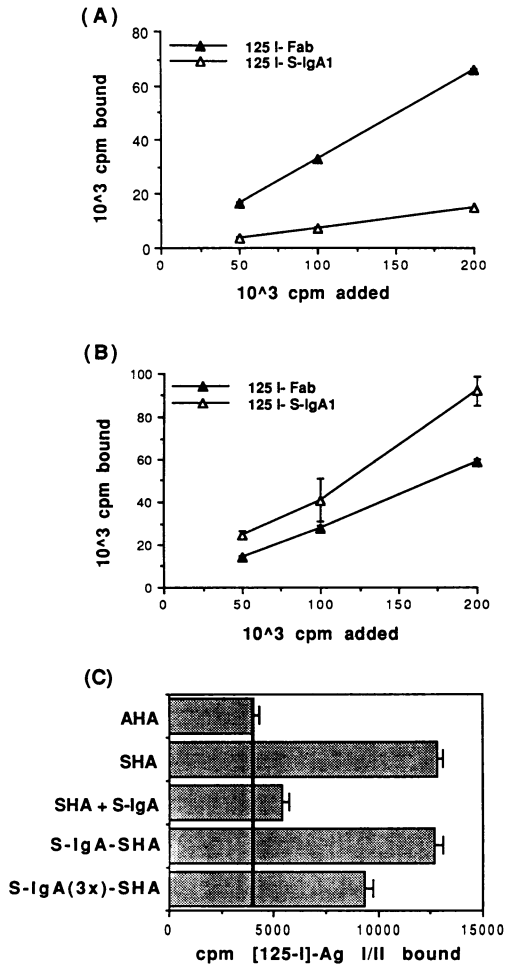

FIG. 4. (A) Binding of Fabα and intact S-IgA1 to preformed experimental salivary pellicle. (B) Incorporation of S-IgA1 into the pellicle during its formation. In this experiment, ^{125}I-S-IgA1 or ^{125}I-Fabα was present together with saliva during the formation of the pellicle. (C) Pellicle-bound S-IgA does not promote the binding of Ag I/II. Hydroxyapatite was coated with saliva plus S-IgA (S-IgA-SHA) containing antibody to Ag I/II (0.8 μg/ml or 2.4 μg/ml [3×]). AHA, BSA-coated hydroxyapatite. Results are expressed as the means ± SDs of triplicate assays.

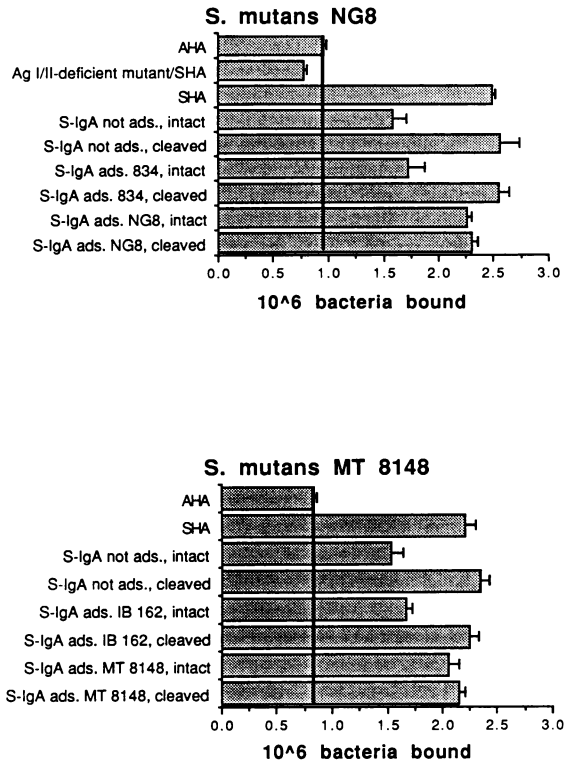

FIG. 5. Differential modulation of the adherence of *S. mutans* NG8 and MT 8148 by intact or IgA1 protease-cleaved human anti-Ag I/II S-IgA antibodies. S-IgA was used at 0.2 mg/ml either as prepared or after adsorption (ads.) on various strains, either intact or after cleavage by IgA1 protease. Background binding was estimated on albumin-coated hydroxyapatite (AHA), and binding of *S. mutans* lacking Ag I/II was evaluated by using the 834 mutant strain. Results are presented as the means ± SDs of triplicate determinations.

support from the finding that amino compounds inhibit the adherence of *S. mutans* to salivary proteins coated on hydroxyapatite (18). Additional mechanisms by which anti-Ag I/II S-IgA antibodies might impair the adherence of *S. mutans* cells could involve a reduction of their hydrophobicity or the formation of large bacterial aggregates that would succumb to the flow of saliva and result in their clearance (15).

Except for susceptibility to IgA1 proteases, only subtle biological differences among the IgA subclasses are known (28); accordingly, we found essentially no difference between S-IgA1 and S-IgA2 with respect to antibody-dependent inhibition of Ag I/II binding to SHA. However, differences in inhibition of *S. mutans* adherence between S-IgA1 antibodies and serum antibodies of the IgA1 and IgG isotypes were found. S-IgA shows better agglutinating properties than does monomeric IgA (14), and S-IgA also reduces the hydrophobicity of bacteria (25), a property that may not be shared to the same extent by serum IgA, which lacks SC (15), or by serum IgG. Furthermore, the special molecular configuration of S-IgA antibodies may impose a steric hindrance on bacterial adhesins that is greater than that imposed by monomeric antibodies. It must be noted, however, that the different isotypes were not the only variable in this experiment: since polyclonal antibodies were used, some differences concerning the epitopes of Ag I/II to which the antibodies were directed, or their affinities, could have contributed to the findings. In this respect, different monoclonal IgG antibodies to antigen P1 (Ag I/II) show different anti-*S. mutans* adherence activities (4). Nevertheless, the facts that both serum IgA1 and IgG from a patient with *S. mutans* systemic infection showed similar inhibitory activity and that colostral S-IgA1 and S-IgA2 from four individuals were also comparable suggest that the differences found were mainly due to the different molecular structures of the antibodies. In this context, it is of interest to note that S-IgA inhibits the attachment of influenza virus to host epithelial cells, in contrast to monomeric IgA and IgG (41). Furthermore, S-IgA inhibits the adherence of *Escherichia coli* to human urinary tract epithelial cells in a manner similar to that of IgG, despite having 100-fold-lower antibody activity (40).

Since naturally occurring antibodies to Ag I/II in saliva and colostrum are predominantly in the S-IgA1 subclass (5, 21), we examined whether cleavage of anti-Ag I/II S-IgA

TABLE 3. Specific antibodies determined by ELISA in colostral S-IgA1 and serum immunoglobulin preparations before and after adsorption on *S. mutans* strains

Immunoglobulin	Strain used for adsorption	Mean optical density at 490 nm (n = 2)			
		Total immunoglobulin (1:2,000)[a]	Antibody to:		
			NG8 (1:400)	834 (1:400)	Ag I/II (1:200)
S-IgA1	None	0.945	0.504	0.441	0.312
	NG8	0.958	0.007	ND[b]	0.010
	834	0.934	ND	0	0.287
SBE IgA1	None	0.892	ND	0.313	0.238
	834	0.879	ND	0.018	0.227
Normal IgA1	None	0.795	0.096	ND	0.045
	NG8	0.781	0.004	ND	0.006
SBE IgG	None	1.456	ND	0.843	0.687
	834	1.493	ND	0.022	0.669
Normal IgG	None	1.672	0.199	ND	0.097
	NG8	1.647	0.016	ND	0.003

[a] Numbers within parentheses indicate dilutions.
[b] ND, not determined.

antibodies by IgA1 protease could reverse their adherence-inhibiting effect. Binding of free Ag I/II or *S. mutans* cells to SHA was increased when inhibitory S-IgA antibodies were cleaved to Fabα and $Fc\alpha_2$.SC fragments. In contrast, the adherence of *S. mutans* in the presence of intact nonspecific S-IgA was similar to that in the presence of cleaved nonspecific S-IgA. This suggests that S-IgA lacking specific antibody activity, either intact or cleaved to Fabα and $Fc\alpha_2$.SC, essentially has no effect on adherence. Although Fabα fragments retain their antigen-binding capacity (26), they are unable to aggregate or to reduce the hydrophobicity of adhesins or whole bacteria because of their monovalency and relative hydrophobicity (32), respectively. Furthermore, comparison of the anti-Ag I/II adherence activities of separated Fabα fragments with that of S-IgA1 showed that the fragments not only were unable to block but also enhanced the binding of Ag I/II to SHA. This finding supports a previous speculation that cell surface-bound Fabα fragments may mediate bacterial adherence to SHA (32). In a test of this hypothesis, we found that Fabα fragments bind more readily than S-IgA1 does to SHA. As hydrophobic interactions appear to be involved in bacterial adherence to SHA (12, 19, 23, 43), this may explain why Fabα, which is dominated by hydrophobic amino acid residues (32) and lacks the heavily glycosylated $Fc\alpha_2$.SC part (42), interacts with SHA better than the intact molecule does. The binding of anti-Ag I/II Fabα-Ag I/II complexes to SHA via the Fabα component may therefore provide an additional mechanism for the attachment of *S. mutans* to pellicle-coated tooth surfaces. Although *S. mutans* itself does not synthesize IgA1 protease, the production of this enzyme is associated with the ability of other oral streptococci to be primary colonizers of tooth surfaces (16). Ag I/II-like molecules have been described in several such organisms (8, 24). Thus, secreted IgA1 protease may not only promote their own ability to adhere to tooth surfaces but also facilitate colonization by *S. mutans*.

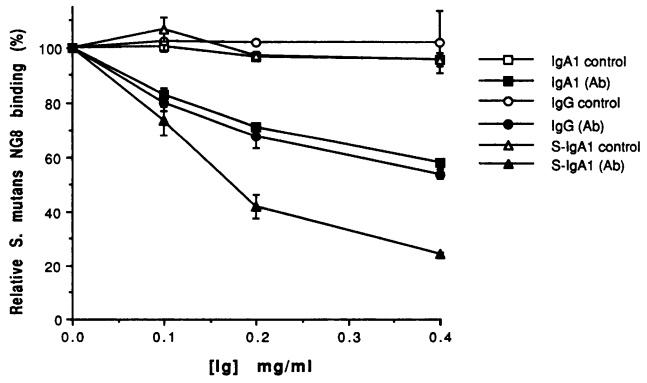

FIG. 6. Anti-*S. mutans* adherence activity of S-IgA1 antibody in comparison with serum IgA1 and IgG antibody. The concentration of the antibody was 4.5 μg/mg of total immunoglobulin. Results are presented as the means ± SDs of triplicate determinations.

In contrast to its relative inability to interact with preformed pellicle, S-IgA1 was readily incorporated in the pellicle (when added together with saliva) during its formation. This is in accordance with previous findings that IgA is a constituent of salivary pellicles (29) and that S-IgA binds to uncoated hydroxyapatite (17). It might be expected that pellicle-bound S-IgA containing antibody to Ag I/II would promote the binding of Ag I/II. However, this was not the case, perhaps because of conformational changes of the antigen-binding sites in the adsorbed state or the orientation of the S-IgA molecules in the pellicle. The finding that higher concentrations of bound S-IgA showed an antiadherence effect might be due to the escape of bound S-IgA to the fluid phase. In this regard, it has been demonstrated that the antibody activity of immunoglobulin incorporated in salivary pellicle is retained in a biological assay based on diffusion of functionally unaltered antibodies from the pellicle (30). These findings further imply that S-IgA antibodies naturally present in salivary pellicle would not contribute to the binding of *S. mutans*.

In conclusion, the data of this study suggest that S-IgA antibodies can inhibit the initial adherence of *S. mutans* to pellicle-coated dental surfaces in an adhesin-specific fashion. This effect of S-IgA seems to be superior to that of serum antibodies, which may reach the tooth surfaces via the gingival crevicular fluid. Therefore, induction of anti-Ag I/II S-IgA antibodies by oral immunization may provide protection against dental caries development through inhibition of initial adherence of *S. mutans* to the salivary pellicle. However, IgA1 proteases produced by oral bacteria may facilitate the Ag I/II-mediated initial adherence of *S. mutans* by abrogating the antiadherence activity of S-IgA antibodies to Ag I/II and generating adherence-enhancing Fabα antibody fragments. Therefore, further studies on the role of bacterial IgA1 proteases in oral microbial ecology are warranted.

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