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Subclass Distribution of Salivary Secretory Immunoglobulin A Antibodies to Oral Streptococci

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The ability of specific secretory immunoglobulin A (S-IgA) antibodies to inhibit bacterial colonization of mucosal surfaces may be neutralized by the activity of bacterial IgA1 proteases. Because of the resistance of the IgA2 subclass to these enzymes, the biological effect of IgA1 proteases in vivo may depend on the subclass distribution of the bacterium-specific antibodies. We have estimated the subclass distribution of S-IgA antibodies in saliva samples from 13 individuals against IgA1 protease-producing (*Streptococcus sanguis* and *Streptococcus oralis*) and nonproducing (*Streptococcus gordonii* and *Streptococcus mitis* bv. 2) oral streptococci. IgA1 was found to be the predominant subclass of antibodies against these four bacteria in most of the saliva samples, corroborating previous data suggesting a role of IgA1 proteases in plaque formation. However, variation in the subclass distribution of S-IgA antibodies against the same strain was observed. In one individual, IgA2 was the predominant subclass of antibodies against all four streptococci and of total salivary S-IgA, pointing to the possible significance of genetic variations. The study also addresses methodological problems related to the quantitation of salivary antibodies by solid-phase immunoassays.

Specific immunological defense on mucosal surfaces is mediated primarily by antibodies of the immunoglobulin A (IgA) class. In accordance with their restricted ability to use ancillary effector mechanisms such as activation of complement and phagocytosis, IgA antibodies do not mediate the dramatic defense reactions characteristic of other immunoglobulin isotypes. Whereas the role of IgA in serum is incompletely understood, secretory IgA (S-IgA), the form of IgA present in secretions, seems to mediate protection by interfering with microbial adherence to mucosal surfaces and by inhibiting the penetration of potentially harmful antigens into mucosal tissues (for reviews, see references 6 and 21).

IgA occurs in serum and secretions as two subclasses that differ in amino acid sequences and glycosylation of the α heavy chain (30). IgA1 predominates (approximately 90%) in serum, whereas secretions, including saliva, may contain up to about 50% IgA2 (9). The physiological significance of the subclasses has not been explained. However, it is likely that IgA1 and IgA2 antibodies are of different protective values because of the exclusive susceptibility of the IgA1 subclass to bacterial IgA1 proteases. IgA1 proteases are endopeptidases with specificity for one of several postproline peptide bonds located within a sequence of 13 amino acids present in the hinge region of IgA1, but not IgA2, molecules. As a consequence of IgA1 protease activity, S-IgA1 molecules are left as antigen-binding Fab_α fragments devoid of the Fc · SC part, which is particularly responsible for the effector functions of S-IgA antibodies (21).

IgA1 proteases are produced by a number of overt pathogens (23). In relation to dental disease, it is relevant that such enzymes are produced by several members of the oral streptococcal floras. These include *Streptococcus sanguis*, *Streptococcus oralis*, and *Streptococcus mitis* bv. 1 (19, 22). These species are among the first to colonize a clean tooth surface (31), thereby initiating the formation of dental plaque, which may eventually cause caries and periodontal disease. In addition, a number of suspected periodontal pathogens contribute to IgA1 protease activity in the oral cavity (23).

Previous studies in our laboratory have indicated that IgA1 protease activity may promote the adherence of oral streptococci to tooth surfaces (34) and that this effect is accompanied by the occurrence of Fab_α fragments on the bacterial surface (1). In view of the resistance of the IgA2 subclass to protease activity, it is possible that bacterium-specific IgA2 antibodies, if present, limit the potential effect of IgA1 protease. To investigate this possibility, we have estimated the subclass distribution of salivary S-IgA antibodies to plaque-forming oral streptococci including IgA1 protease-producing and nonproducing species. As part of the study, we have addressed methodological problems related to the quantitation of salivary antibodies by performing solid-phase immunoassays.

MATERIALS AND METHODS

Collection of whole saliva and isolation of salivary S-IgA. Whole saliva samples from 13 healthy individuals were collected by expectoration into a tube on ice. The saliva was clarified by centrifugation at 12,000 × *g* for 10 min at 5°C and mixed with an equal volume of 80% glycerol in distilled water containing 0.1 M EDTA (disodium salt) to inhibit streptococcal IgA1 protease activity (19). The saliva samples were frozen in liquid nitrogen and stored at −65°C.

To isolate salivary S-IgA, 2 ml of whole saliva was subjected to size exclusion chromatography on a column (55 by 1.6 cm) of Sepharose CL-2B (Pharmacia, Uppsala, Sweden) equilibrated with 0.01 M phosphate-buffered saline, pH 7.4, containing 0.02% sodium azide (PBS). The column had been calibrated with standards including S-IgA (400 kDa) and dimeric and monomeric myeloma IgA purified as described previously (35). Eluent fractions containing S-IgA were identified by an enzyme-linked immunosorbent assay (ELISA) in which S-IgA was detected in microwells coated with rabbit anti-α-chain antibody and by using peroxidase-conjugated antibody specific for the secretory component as a third layer (24). Eluent fractions were also tested in IgA

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TABLE 1. ELISA procedures used to determine the subclass distribution of salivary S-IgA antibodies to streptococcal cells (assays A and B) and the subclass content of total salivary S-IgA (assay C)

Step	Procedure in assay:		
	A	B	C
1	Glyoxal-treated streptococcal cells (4×10^8/ml, 4 h)	Rabbit anti-human α-chain antibodies (1:3,000, 4 h)	Rabbit anti-mouse immunoglobulin antibodies (1:3,000, 4 h)
2	Salivary S-IgA (serial twofold dilutions, 16 h)	S-IgA subclass standard (serial twofold dilutions, 16 h)	Monoclonal anti-IgA1 or anti-IgA2 antibody (1:3,000, 4 h)
3	Monoclonal anti-IgA1 or anti-IgA2 antibody (1:3,000, 4 h)	Monoclonal anti-IgA1 or anti-IgA2 antibody (1:3,000, 4 h)	Salivary S-IgA or S-IgA subclass standard (serial twofold dilutions, 16 h)
4	Biotinylated rabbit anti-mouse immunoglobulin antibodies (1:5,000, 2 h)	Biotinylated rabbit anti-mouse immunoglobulin antibodies (1:5,000, 2 h)	Peroxidase-conjugated rabbit anti-human α-chain antibodies (1:2,000, 2 h)
5	Peroxidase-conjugated streptavidin (1:5,000, 1 h)	Peroxidase-conjugated streptavidin (1:5,000, 1 h)	

subclass-specific assays (Table 1, assay C). Fractions corresponding to the elution position of the S-IgA marker and representing the peak of reactivity in ELISAs (see Results) were pooled, supplied with glycerol to a concentration of 20% (vol/vol), frozen in liquid nitrogen, and stored at −65°C until used.

Preparation of S-IgA1 and S-IgA2 standards. S-IgA was purified from human colostrum by methods including ammonium sulfate precipitation, size exclusion, and anion-exchange chromatography (28). To separate S-IgA1 from S-IgA2 molecules, colostral S-IgA was subjected to affinity chromatography on a column of agarose-bound jacalin (Vector Laboratories, Burlingame, Calif.) by using the procedure recommended by the manufacturer. Unbound proteins collected in the effluent (S-IgA2 fraction) and protein eluted with 0.8 M galactose (S-IgA1 fraction) were then chromatographed on a calibrated size exclusion column (30 by 0.8 cm) of Superose 6 (Pharmacia) equilibrated in PBS. With each subclass, eluent fractions representing S-IgA of 400 kDa were pooled and the protein concentration was determined spectrophotometrically assuming $E^{1\ cm,\ 1\%}_{280\ nm} = 13.4$ (17). By using the S-IgA2 preparation as the standard in a subclass-specific ELISA (Table 1, assay C), it was found that the S-IgA1 preparation contained less than 5% S-IgA2, whereas, in a similar assay, by using the S-IgA1 preparation as the standard, S-IgA2 was found to contain less than 0.5% S-IgA1.

Bacterial strains. *S. sanguis* ATCC 10556 (American Type Culture Collection, Rockville, Md.), *Streptococcus gordonii* ATCC 10558, *S. oralis* ATCC 10557, and *S. mitis* bv. 2 NCTC 12261 (National Collection of Type Cultures, Colindale, United Kingdom) were propagated in Trypticase Peptone Broth (BBL Microbiology Systems, Cockeysville, Md.) prepared as described by Gibbons and Fitzgerald (14). After 20 h of growth, the cultures were dispensed into 10-ml sterile tubes and stored at ~20°C. Thawed bacterial cells were washed twice in 10 ml of PBS before use.

Subclass distributions of total salivary S-IgA and S-IgA antibodies. Subclass distributions of total salivary S-IgA and S-IgA antibodies to the individual streptococci were estimated by analysis of salivary S-IgA preparations instead of whole saliva. The distribution of total S-IgA was estimated by parallel titration of salivary S-IgA and S-IgA subclass standards in assay C (Table 1). The distributions of specific S-IgA antibodies in the 13 S-IgA preparations were determined in two steps. First, all 13 preparations were titrated in parallel in Maxisorp ELISA wells (Nunc, Roskilde, Denmark) coated with streptococcal cells and developed for the detection of S-IgA1 and S-IgA2 antibodies (Table 1, assay A). One S-IgA preparation (no. 13) was chosen as the reference standard. Second, the relative levels were transformed into absolute concentrations (nanograms per milliliter) based on estimates of the absolute concentrations of antibodies in the reference standard. These estimates were made by using assay A (Table 1), as calibrated by parallel titration of S-IgA subclass standards in wells of the same plate coated with anti-α-chain antibodies (Table 1, assay B). Salivary antibody levels were expressed as the percentage of antibody relative to total S-IgA of the same subclass. All assays were performed with duplicate, serial twofold dilutions of salivary S-IgA and of standards. Dilutions were in ELISA washing solution, i.e., PBS (0.5 M NaCl) containing 0.15% Tween 20 and 0.005 M EDTA.

IgA subclass-specific monoclonal antibodies were purchased from Nordic (Tilbury, The Netherlands). Polyclonal antibodies and streptavidin were from Dakopatts (Glostrup, Denmark). With the exception of peroxidase-labeled antibodies against human α chains, all antibodies were of affinity-isolated grade. Bacteria were immobilized in ELISA wells in 0.1 M sodium bicarbonate, pH 8.3, containing 0.3% methyl glyoxal (Sigma Chemical Co., St. Louis, Mo.) (8). Antibodies used for coating were diluted in 0.1 M borate-buffered saline, pH 8.4. In later steps, antibodies were diluted in ELISA washing solution. Washing solution also served to block residual binding sites in coated wells. ELISA wells were developed with *o*-phenylenediamine substrate and read at 492 nm with a Titertek Multiskan spectrophotometer (Flow Laboratories, Glasgow, United Kingdom). Optical densities were processed by the computer program Titersoft (Flow). By using a four-parameter logistic model, the program constructs a dose-response curve based on optical densities in wells used for the titration of a standard or an unknown.

RESULTS

Background reactions caused by salivary samples in the ELISA. When, in initial experiments, unfractionated saliva was used for the titration of S-IgA1 and S-IgA2 antibodies by ELISA, the majority of the saliva samples gave rise to strong background reactions as detected in blocked control wells not coated with bacteria. Some saliva samples caused considerable reaction, even when added at a dilution of 1:128 (Fig. 1). By systematic variation of the assay conditions, it was found that neither change of the blocking agent from Tween 20 to bovine serum albumin (1%) or gelatin (0.25%)

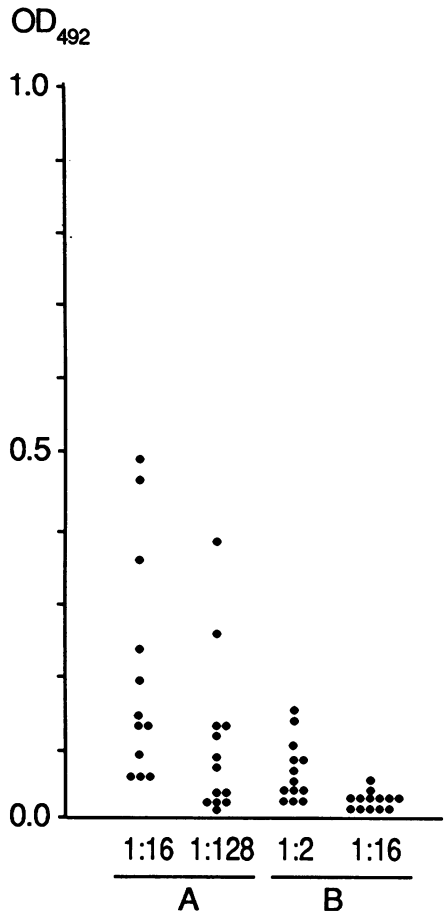

FIG. 1. Background reaction (optical density at 492 nm) in the ELISA for salivary S-IgA1 antibodies against streptococci (Table 1, assay A) as obtained by parallel incubation of 13 individual whole saliva samples (A) and the corresponding purified S-IgA preparations (B), diluted as indicated, in blocked wells not coated with antigen. Note that the strong background reaction obtained with some samples of whole saliva is not displayed by the corresponding S-IgA preparations.

nor the use of more-intensive washing procedures resulted in a reduced reaction in blocked wells (results not shown). No reaction in blocked wells occurred when the incubation with IgA subclass-specific monoclonal antibodies was omitted.

After fractionation of whole saliva on a column of Sepharose CL-2B, a reaction in blocked wells was obtained with fractions corresponding to the void volume (Fig. 2). With later-eluted fractions representing S-IgA of both subclasses, including antibodies to streptococci, such a reaction was insignificant (Fig. 2). Therefore, subclass distributions of S-IgA antibodies and of total S-IgA were estimated by using a pool of the later eluent fractions instead of whole saliva.

Calibration of ELISAs. In assays for total S-IgA1 and S-IgA2, the dose-response curves of sample and the S-IgA subclass standard were parallel (data not shown), and estimates were made at a dilution corresponding to the midsegment of the sigmoid curve.

S-IgA1 and S-IgA2 antibody levels relative to the levels in preparation no. 13 were calculated as the means of three estimates made with consecutive twofold dilution steps of the sample. With all 12 preparations, and irrespective of the

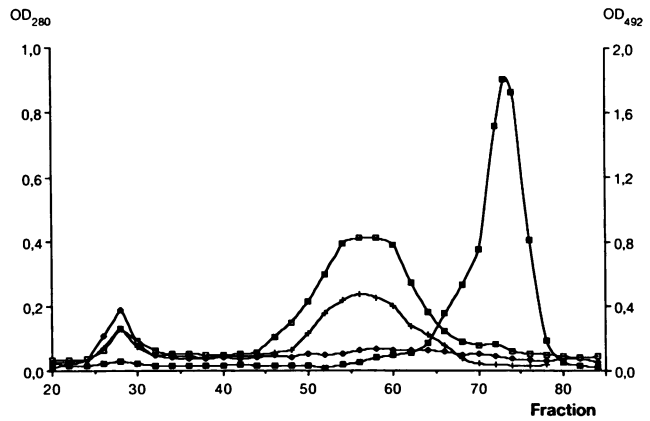

FIG. 2. Analysis of whole saliva fractionated on a column of Sepharose CL-2B. ■, protein profile (optical density at 280 nm [OD_{280}]); □, total S-IgA profile (OD_{492}) determined by ELISA. Similar profiles were obtained with subclass-specific ELISAs (Table 1, assay C). +, IgA antibody activity (OD_{492}) detected in ELISA wells coated with *S. sanguis* ATCC 10556 and developed with enzyme-conjugated anti-α-chain antibodies; ◆, ELISA profile (OD_{492}) resulting from incubation in blocked ELISA wells followed by development with anti-IgA1 antibody and conjugates according to assay A (Table 1). A similar profile was obtained by development with anti-IgA2 antibody.

subclass of S-IgA antibodies measured, the coefficient of variation of the three estimates was less than 10%, testifying to a high degree of parallelism among the dose-response curves of the 13 preparations.

Absolute concentrations of specific S-IgA1 and S-IgA2 antibodies in the reference preparation were estimated by using assay A (Table 1), as calibrated by the parallel titration of S-IgA1 and S-IgA2 standards in wells coated with anti-α-chain antibody. Calibrated in this way, the ELISA system did not meet the requirement of parallel dose-response curves of the sample and standard (Fig. 3). Accordingly, calculated antibody concentrations in the undiluted prepara-

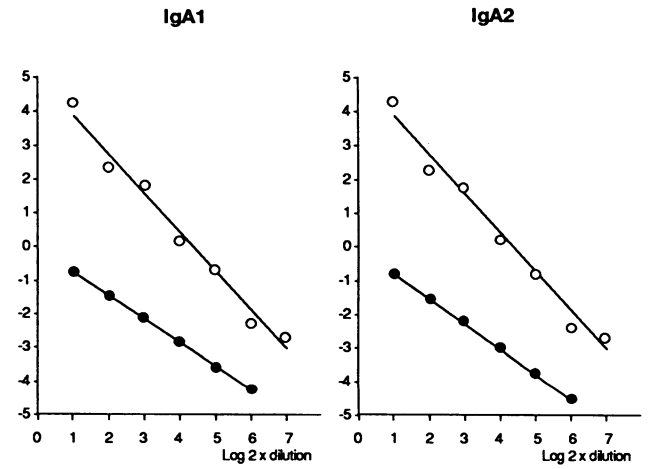

FIG. 3. ELISA dose-response curves of S-IgA1 and S-IgA2 antibodies against *S. sanguis* in saliva sample no. 13 (●) in relation to the dose-response curves of the corresponding S-IgA subclass standards (○). To illustrate the similar geometric relationship between antibody and standard dose-response curves in S-IgA1 and S-IgA2 assays, the logit-log method of interpolation (38) has been used.

TABLE 2. Concentrations and relative amounts of S-IgA1 and S-IgA2 antibodies against four oral streptococci in the S-IgA preparation from saliva no. 13

Dilution	*S. sanguis*			*S. oralis*			*S. gordonii*			*S. mitis* bv. 2		
	S-IgA1 (concn)[a]	S-IgA2 (concn)	S-IgA1 (%)[b]	S-IgA1 (concn)	S-IgA2 (concn)	S-IgA1 (%)	S-IgA1 (concn)	S-IgA2 (concn)	S-IgA1 (%)	S-IgA1 (concn)	S-IgA2 (concn)	S-IgA1 (%)
1:2	51.3	95.5	34.9	54.5	101.7	34.9	37.0	46.3	44.5	31.1	39.7	43.9
1:4	67.0	133.2	33.5	68.5	152.7	31.0	46.1	61.1	43.0	43.8	57.3	43.4
1:8	85.2	182.6	31.8	91.8	219.4	29.5	61.7	75.6	44.9	67.3	82.1	45.1
1:16	112.8	241.5	31.8	125.9	290.2	30.3	ND[c]	ND	ND	103.1	114.6	47.8

[a] Antibody concentration (nanograms per milliliter). Each value is the mean of duplicate determinations.
[b] % S-IgA1 antibody = [S-IgA1/(S-IgA1 + S-IgA2)] × 100.
[c] ND, not done.

tion varied depending on the dilution at which the preparation was tested (Table 2). However, estimates made at different dilutions maintained constant ratios of the two subclasses (Table 2). With each of the four streptococci, the estimates recorded were those made with a twofold dilution of the S-IgA preparation (Table 2). Optical densities obtained at this dilution corresponded to the midsegments of the S-IgA1 and S-IgA2 standard curves.

Subclass distributions and levels of salivary antibodies. Subclass distributions of S-IgA antibodies against the four streptococci in all of the 13 saliva samples were compiled (Fig. 4). It appears that IgA1 was the predominant subclass in the vast majority of cases and that IgA1 often constituted more than 80% of the bacterium-specific antibodies. The predominance of S-IgA1 antibodies is reflected also in the mean levels of S-IgA1 and S-IgA2 antibodies (Table 3). In most saliva samples, IgA1 was the predominant subclass also of total S-IgA (Fig. 4). However, in several saliva samples, the subclass distributions of antibodies to the individual streptococci and of total S-IgA were not related (Fig. 4). Saliva sample no. 13 was exceptional in that IgA2 predominated in antibodies to all four streptococci and in total S-IgA (Fig. 4).

No systematic difference was found in the subclass distribution of antibodies to IgA1 protease-producing (*S. sanguis* and *S. oralis*) compared with nonproducing (*S. gordonii* and *S. mitis* bv. 2) streptococci (Fig. 4).

DISCUSSION

As the present study amply demonstrates, the quantitation of salivary S-IgA antibodies according to subclasses in solid-phase immunoassays is a problematic issue. The quantitation may be complicated by extreme background reactions, as detected by incubation of whole saliva in control wells not containing antigen. The common practice of correcting for such background simply by subtraction may lead to erroneous results, since the contribution of background to the reaction in wells containing antigen is likely to be different from the reaction in the control wells.

In this study, background reaction was not observed when the incubation with subclass-specific monoclonal antibodies (Table 1, assay A) was omitted, indicating that the phenomenon resulted from nonspecific immobilization of IgA and not of conjugate. After Sepharose CL-2B fractionation of saliva, the ability to cause a reaction in blocked wells was found in void volume eluent fractions, which are known to contain primarily the high-molecular-weight mucin (16). When adding to this picture the previously reported evidence of noncovalent association of S-IgA molecules to mucins in saliva (33, 37), it is likely that the background reaction resulted from S-IgA immobilized indirectly as part of macromolecular complexes, with the highly tenacious mucin as the main component. Consequently, subclass distributions were estimated by the analysis of eluent fractions containing nonaggregated S-IgA. Such fractions are also equivalent substitutes for unfractionated saliva in measurements of antibody levels, provided that levels are expressed in terms of the amount of antibody relative to the amount of total S-IgA.

Unlike the secretions of other salivary glands, parotid saliva is devoid of high-molecular-weight mucins (37), and preliminary experiments have confirmed that ELISA quantitation of S-IgA antibodies in parotid saliva involves a minimum of background reaction.

Ideally, calibration standards for use in isotype-specific enzyme immunoassays (or radioimmunoassays) should closely resemble the unknown with respect to antigen specificity, affinity, isotype, and molecular form. A standard that meets these criteria will result in a dose-response curve parallel to that of the unknown, hereby providing for accuracy in the calibration. This situation was approached when antibodies in 12 salivary S-IgA preparations were estimated with preparation no. 13 serving as the reference standard.

Total S-IgA1 and S-IgA2 in salivary S-IgA preparations were estimated with an assay that used colostral S-IgAs of the respective subclasses as standards. Colostral S-IgA purified according to commonly used procedures contains large proportions of higher polymers (28). Accordingly, parallel dose-response curves of the standard and unknown could be obtained only by using S-IgA standards matched to the 400-kDa salivary S-IgA by appropriate gel filtration.

To estimate the subclass distribution of bacterium-specific antibodies, it was necessary to express antibody levels in absolute concentration units. While this might have been achieved by preparing affinity-purified antibody standards of each subclass (5), this was considered impractical, and differences in affinity as well as the impurity or denaturation of the standards might have added to the inaccuracy of the calibration. We therefore adopted a previously described approach (26, 36, 39) based on the use of heterologous standards (i.e., of different antigenic specificities) titrated in wells coated with anti-immunoglobulin antibodies. That this type of calibration introduced bias is evident from the increasing antibody concentrations obtained by assaying at increasing dilutions of the salivary S-IgA preparations (Table 2). However, because the geometric relationships of the sample and standard dose-response curves were similar in assays for S-IgA1 and S-IgA2 antibodies (Fig. 3), estimates of concentrations made at the same dilution could still be expected to reflect the subclass distribution of antibodies.

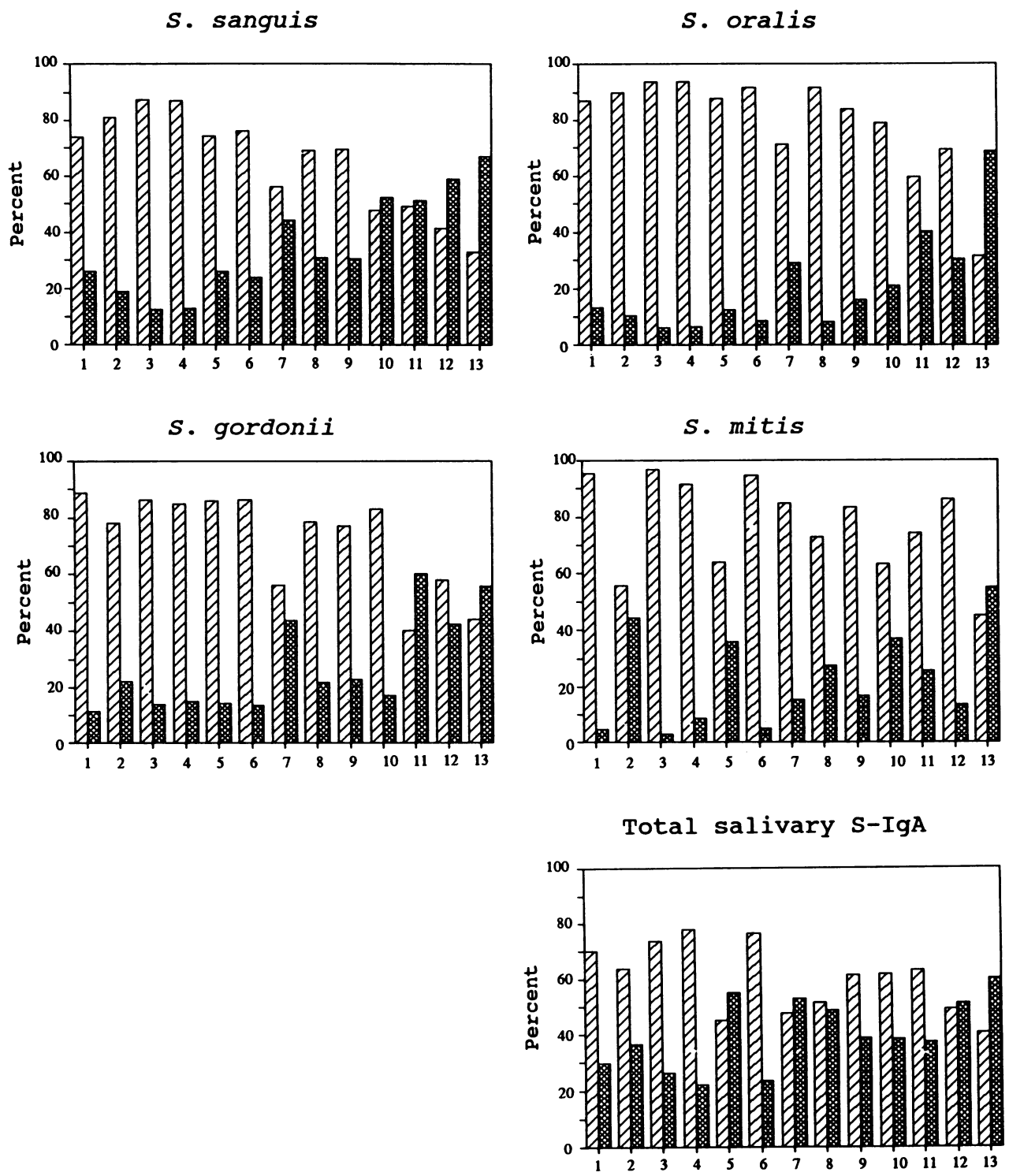

FIG. 4. Subclass distributions of S-IgA antibodies against four oral streptococci and of total S-IgA in saliva samples of 13 individuals. S-IgA1 (hatched bars) and S-IgA2 (cross-hatched bars) percentages were calculated from estimates of concentrations in salivary S-IgA preparations.

Several studies have suggested that the biochemical type of an antigen influences the outcome of the IgA antibody response with respect to subclass (for a review, see reference 29). Thus, in secretions, including saliva, antibodies specific for protein antigens have been found predominantly in the IgA1 subclass, whereas antibodies to bacterial lipopolysaccharide and lipoteichoic acid were predominantly of the IgA2 subclass (3, 29). Such antigen-dependent subclass

TABLE 3. Salivary S-IgA1 and S-IgA2 antibody levels against four oral streptococci

Species	Mean (coefficient of variation) % antibody[a]	
	S-IgA1	S-IgA2
S. sanguis	1.85 (65.7)	1.12 (54.3)
S. oralis	1.35 (55.3)	0.42 (130.9)
S. gordonii	1.21 (74.5)	0.50 (62.6)
S. mitis bv. 2	1.25 (64.0)	0.38 (62.1)

[a] Mean (coefficient of variation) antibody level in 13 saliva samples. % S-IgA1 antibody = [S-IgA1 antibody (ng/ml)/total S-IgA1 (ng/ml)] × 100.

restriction has been observed also in studies of IgG antibodies. As IgG subclasses vary in their effector functions such as complement fixation, opsonization, and mast cell degranulation, it may be speculated also that the subclasses of IgA represent a functional differentiation.

Although subtle functional differences between IgA1 and IgA2 molecules have recently become apparent in various experimental systems (29), the evidence for differential effects of the two IgA subclasses under physiological conditions is still sparse (15, 29). However, the hypothesis that IgA1 and IgA2 antibodies have different efficiencies in vivo because of the effect of bacterial IgA1 proteases has received considerable support (for reviews, see references 21 and 23). In the context of dental plaque formation, it is relevant that IgA1 protease has been found to promote the adherence of oral streptococci to hydroxyapatite beads (simulating dental enamel) in saliva by counteracting the adherence-inhibiting effect of S-IgA antibodies (34). A subsequent study (1) indicated that IgA1 protease activity resulted in the presence of Fab_α on the streptococcal surface. Fab_α was recovered also from bacteria in incipient dental plaque, suggesting that IgA1 protease promotes the bacterial colonization of teeth in vivo. Further support for a role of IgA1 proteases in plaque formation comes from our observation that 88% of the streptococci that colonize a newly cleaned enamel surface produce IgA1 protease, as opposed to a low percentage of the streptococci on the oropharyngeal mucosa and the dorsum of the tongue (25).

S-IgA antibodies seem to inhibit bacterial colonization by blocking epitopes serving as adhesins, by making bacteria more hydrophilic, and by contributing to the formation of large bacterial aggregates (6). The surface antigens of plaque-forming organisms, which may be the targets of inhibiting S-IgA antibodies, are only beginning to be identified (11, 13, 27). Therefore, whole bacteria were chosen as antigens in the present study.

The level of antibodies against streptococci (Table 3) is roughly 15 times higher than the levels reported by Butler et al. (4) in a study of salivary IgA antibodies against two other members of the normal oral floras, *Streptococcus mutans* and *Actinomyces viscosus*. As the ELISA methods used by Butler et al. differ greatly from those used by us, this discrepancy is probably a methodological issue rather than a biological one. In support of this explanation, Russell et al. (36), by using an assay configuration similar to ours, found that in saliva the concentration of IgA1 antibodies to various surface antigens of *S. mutans* was on the order of 0.35 to 1.30 μg/ml. Assuming the total salivary IgA1 concentration to be 100 μg/ml (2, 9), this corresponds to antibody levels of 0.36 to 1.3%, i.e., similar to the levels against oral streptococci reported here.

The observation of similar antibody levels against the four streptococci (Table 3) is in keeping with the constant presence of these species in the oral cavities of most individuals (10). Serological examinations have revealed that, with the possible exception of *S. mitis*, the four streptococcal species included in the study are not totally antigenically distinct (7, 22). Some of the salivary antibodies may therefore be directed against surface antigens shared by two or more strains.

The observation of large, and in most cases predominant, proportions of S-IgA1 antibodies against oral streptococci (Fig. 4) is in accordance with our previous finding of a combination of Fab_α and intact S-IgA on streptococci exposed to saliva and IgA1 protease, with intact S-IgA presumably representing S-IgA2 (1).

Because of the specificity of IgA proteases for the IgA1 subclass, it has been speculated (32) that the IgA2 subclass evolved in response to the occurrence of IgA1 protease-producing microorganisms. Our finding that, in most individuals, S-IgA1 antibodies predominated against IgA1 protease-producing as well as nonproducing streptococci does not corroborate this hypothesis. Direct evidence against the hypothesis comes from a recent study by Kawamura et al. (18), demonstrating that ancestral α-chain genes are closely related to the α2, but not to the α1, gene of humans and hominoid primates.

In spite of the overall predominance of S-IgA1 antibodies, the 13 individuals varied considerably with respect to the subclass distribution of antibodies against the same bacterium (Fig. 4). This outcome is not incompatible with the above-mentioned observations of a correlation between the biochemical type of an antigen and the subclass of the IgA antibodies induced, since whole bacteria represent several epitopes of different types to which the individuals may have responded to different extents. Different levels of antibodies against an epitope may also have arisen from different exposures to cross-reacting microorganisms or food components (12). Interestingly, in one individual, IgA2 predominated in antibodies to all four streptococci and in total salivary S-IgA, pointing to the possibility that the different subclass distributions could also reflect genetic variations in the secretory immune system.

Irrespective of the underlying regulatory mechanisms, the existence of individuals with contrasting subclass distributions of S-IgA antibodies to oral streptococci is of interest in relation to the significance of S-IgA antibodies in the protection of oral tissues. It raises the interesting question of whether such variations may be partly responsible for differences in the rate of plaque formation (20, 31) and in the incidence of dental disease. This question is of relevance to ongoing attempts to stimulate a protective secretory immune response against cariogenic plaque bacteria.

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REFERENCES

1. **Ahl, T., and J. Reinholdt.** 1990. Detection of immunoglobulin A1 protease-induced Fab_α fragments on dental plaque bacteria. Infect. Immun. **59**:563–569.
2. **Brandtzaeg, P., I. Fjellander, and S. T. Gjeruldsen.** 1970. Human secretory immunoglobulins. I. Salivary secretions from

individuals with normal or low levels of serum immunoglobulins. Scand. J. Haematol. Suppl. **12**:1–83.
3. **Brown, T. A., and J. Mestecky.** 1985. Immunoglobulin A subclass distributions of naturally occurring salivary antibodies to microbial antigens. Infect. Immun. **49**:459–462.
4. **Butler, J. E., J. E. Spradling, J. H. Peterman, K. S. Joshi, M. Satam, and S. J. Challacombe.** 1990. Humoral immunity in root caries in an elderly population. I. Establishment of a reliable approach for measuring specific antibodies in serum and saliva, their specific activities, rate of salivary secretion and relationship to other salivary proteins. Oral Microbiol. Immunol. **5**:98–107.
5. **Challacombe, S. J., L. A. Bergmeier, C. Czerkinsky, and A. S. Rees.** 1984. Natural antibodies in man to *Streptococcus mutans*: specificity and quantitation. Immunology **52**:143–150.
6. **Childers, N. K., M. G. Bruce, and J. R. McGhee.** 1989. Molecular mechanisms of immunoglobulin A defense. Annu. Rev. Microbiol. **34**:503–536.
7. **Cole, R. M., G. B. Calandra, E. Huff, and I. K. M. Nugent.** 1976. Attributes of potential utility in differentiating among "group H" streptococci or *Streptococcus sanguis*. J. Dent. Res. **55**(special issue A):A142–A153.
8. **Czerkinsky, C., A. J. Rees, L. A. Bergmeier, and S. J. Challacombe.** 1983. The detection and specificity of class specific antibodies to whole bacterial cells using a solid phase radioimmunoassay. Clin. Exp. Immunol. **53**:192–200.
9. **Delacroix, D. L., C. Dive, J. C. Rambaud, and J. P. Vaerman.** 1982. IgA subclasses in various secretions and in serum. Immunology **47**:383–385.
10. **Frandsen, E. V. G., V. Pedrazzoli, and M. Kilian.** 1991. Ecology of viridans streptococci in the oral cavity and pharynx. Oral Microbiol. Immunol. **6**:129–133.
11. **Ganeshkumar, N., P. M. Hannam, P. E. Kolenbrander, and B. C. McBride.** 1991. Nucleotide sequence of a gene coding for a saliva-binding protein (SsaB) from *Streptococcus sanguis* 12 and possible role of the protein in coaggregation with actinomyces. Infect. Immun. **59**:1093–1099.
12. **Gibbons, R. J., and I. Dankers.** 1981. Lectin-like constituents of foods which react with components of serum, saliva, and *Streptococcus mutans*. Appl. Environ. Microbiol. **41**:880–888.
13. **Gibbons, R. J., I. Etherden, and W. Peros.** 1985. Aspects of the attachment of oral streptococci to experimental pellicles, p. 77–84. *In* S. E. Mergenhagen and B. Rosan (ed.), Molecular basis of oral microbial adhesion. American Society for Microbiology, Washington, D.C.
14. **Gibbons, R. J., and R. J. Fitzgerald.** 1969. Dextran-induced agglutination of *Streptococcus mutans* and its potential role in the formation of microbial dental plaques. J. Bacteriol. **98**:341–346.
15. **Gregory, R. L., J. C. Kinella, L. C. Hobbs, S. J. Filler, and H. J. Malmström.** 1990. Function of anti-*Streptococcus mutans* antibodies: inhibition of virulence factors and enzyme neutralization. Oral Microbiol. Immunol. **5**:181–188.
16. **Hay, D. J., R. J. Gibbons, and D. M. Spinell.** 1971. Characteristics of some high molecular weight constituents with bacterial aggregating activity from whole saliva and dental plaque. Caries Res. **5**:111–123.
17. **Heremans, J. F.** 1974. Immunoglobulin A, p. 365–522. *In* M. Sela (ed.), The antigens, vol. 2. Academic Press, Inc., New York.
18. **Kawamura, S., K. Omoto, and S. Ueda.** 1990. Evolutionary hypervariability in the hinge region of the immunoglobulin alpha gene. J. Mol. Biol. **215**:201–206.
19. **Kilian, M., and K. Holmgren.** 1981. Ecology and nature of immunoglobulin A1 protease-producing streptococci in the human oral cavity and pharynx. Infect. Immun. **31**:868–873.
20. **Kilian, M., M. J. Larsen, O. Fejerskov, and A. Thylstrup.** 1979. Effects of fluoride on the initial colonization of teeth *in vivo*. Caries Res. **13**:319–329.
21. **Kilian, M., J. Mestecky, and M. W. Russell.** 1988. Defense mechanisms involving Fc-dependent functions of immunoglobulin A and their subversion by bacterial immunoglobulin A proteases. Microbiol. Rev. **52**:296–303.
22. **Kilian, M., L. Mikkelsen, and J. Henrichsen.** 1989. Taxonomic study of viridans streptococci: description of *Streptococcus gordonii* sp. nov. and emended descriptions of *Streptococcus sanguis* (White and Niven 1946), *Streptococcus oralis* (Bridge and Sneath 1982), and *Streptococcus mitis* (Andrewes and Horder 1906). Int. J. Syst. Bacteriol. **39**:471–484.
23. **Kilian, M., and J. Reinholdt.** 1986. Interference with IgA defence mechanisms by extracellular bacterial enzymes, p. 173–208. *In* C. S. F. Easmon and J. Jeljaszewicz (ed.), Medical microbiology, vol. 5. Academic Press Ltd., London.
24. **Kilian, M., J. Reinholdt, S. B. Mortensen, and C. H. Sørensen.** 1983. Perturbation of mucosal immune defence mechanisms by bacterial IgA proteases. Bull. Eur. Physiopathol. Resp. **19**:99–104.
25. **Kilian, M., J. Reinholdt, B. Nyvad, E. V. G. Frandsen, and L. Mikkelsen.** 1989. IgA1 proteases of oral streptococci: ecological aspects. Immunol. Invest. **18**:161–170.
26. **Mäkelä, O., and F. Peterfy.** 1983. Standard sera in solid-phase immunoassays. Eur. J. Immunol. **13**:815–819.
27. **McBride, B. C., E. S. Morris, and N. Caneshkumar.** 1985. Relationship of streptococcal cell surface proteins to hydrophobicity and adherence, p. 85–93. *In* S. E. Mergenhagen and B. Rosan (ed.), Molecular basis of oral microbial adhesion. American Society for Microbiology, Washington, D.C.
28. **Mestecky, J., and M. Kilian.** 1985. Immunoglobulin A. Methods Enzymol. **116**:37–75.
29. **Mestecky, J., C. Lue, A. Tarkowski, I. Ladjeva, J. H. Peterman, Z. Moldoveanu, M. W. Russell, T. A. Brown, J. Radl, J. J. Haaijman, H. Kiyono, and J. R. McGhee.** 1990. Comparative studies of the biological properties of human IgA subclasses. Protides Biol. Fluids Proc. Colloq. **36**:173–182.
30. **Mestecky, J., and M. W. Russell.** 1986. IgA subclasses. Monogr. Allergy **19**:277–301.
31. **Nyvad, B., and M. Kilian.** 1987. Microbiology of the early colonization of human enamel and root surfaces in vivo. Scand. J. Dent. Res. **95**:369–380.
32. **Plaut, A. G.** 1983. The IgA1 proteases of pathogenic bacteria. Annu. Rev. Microbiol. **37**:603–622.
33. **Prakobphol, A., M. J. Levine, M. S. Reddy, and L. A. Tabak.** 1983. Purification and characterization of a higher molecular weight salivary mucin, abstr. 511, p. 226. Abstr. Annu. Meet. Am. Assoc. Dent. Res. 1983. American Association of Dental Research, Washington, D.C.
34. **Reinholdt, J., and M. Kilian.** 1987. Interference of IgA protease with the effect of secretory IgA on adherence of oral streptococci to saliva-coated hydroxyapatite. J. Dent. Res. **66**:492–497.
35. **Reinholdt, J., P. Krogh, and P. Holmstrup.** 1987. Degradation of IgA1, IgA2, and S-IgA by Candida and Torulopsis species. Acta Pathol. Microbiol. Immunol. Scand. Sect. C. **95**:265–274.
36. **Russell, M. W., T. A. Brown, J. Radl, J. J. Haaijman, and J. Mestecky.** 1986. Assay of human IgA antibodies in serum and secretions by means of monoclonal antibodies. J. Immunol. Methods **87**:87–93.
37. **Tabak, L. A., M. J. Levine, I. D. Mandel, and S. A. Ellison.** 1982. Role of salivary mucins in the protection of the oral cavity. J. Oral Pathol. **11**:1–17.
38. **Tijssen, P.** 1985. Practice and theory of enzyme immunoassays, p. 385–421. *In* R. H. Burdon and P. H. van Knipperberg (ed.), Laboratory techniques in biochemistry and molecular biology, vol. 15. Elsevier Science Publishers, Amsterdam.
39. **Zollinger, W. D., and J. W. Boslego.** 1981. A general approach to standardization of the solid-phase radioimmunoassay for quantitation of class-specific antibodies. J. Immunol. Methods **46**: 129–140.