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Unexpectedly High Levels of Some Presumably Protective Secretory Immunoglobulin A Antibodies to Dental Plaque Bacteria in Salivas of Both Caries-Resistant and Caries-Susceptible Subjects

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The role of salivary antibodies in protection against cariogenic bacteria is actually a matter of debate. Correlation between caries experience and naturally occurring antibodies was extensively investigated. Comparison of salivary antibodies from 21 caries-resistant and 22 caries-susceptible subjects was carried out by using a new quantitative method. Secretory immunoglobulin A (S-IgA) antibodies to *Streptococcus sobrinus* and *Streptococcus sanguis* cells were detected in all salivas and at similar levels in both groups. When assayed with two major antigens from *S. sobrinus*, i.e., protein antigen I/II and cell wall carbohydrates, only specific activities of antibodies to the protein component were increased ($P < 0.001$), but this occurred unexpectedly in the caries-susceptible group. Western blot (immunoblot) analysis with the culture supernatant and cell wall proteins from *S. sobrinus* showed the same antibody specificity in both groups. No selective increase of the protease-resistant S-IgA2 subclass was found, and avidities of antibodies to both antigen I/II and cell wall carbohydrates were similar. Our results demonstrate that naturally induced S-IgA antibodies against *S. sanguis*, *S. sobrinus*, and the major antigens of the latter are not sufficient to inhibit caries development.

It is well established that caries develop from dental plaque, an accumulation of bacteria associated with the tooth surface. These bacteria include several different species, such as those of the *Streptococcus mutans* group (31) and, at a lower degree, *Streptococcus sanguis* (38). Immune defenses against caries (34) have been said to involve secretory immunoglobulins (S-Ig), which consist essentially of immunoglobulin A (IgA) dimers transported through the epithelial cells of salivary glands by the poly-immunoglobulin receptor (35). It is well known that most adults exhibit a large number of dental caries lesions (caries susceptible [CS]), whereas only few individuals are caries resistant (CR) (41). The correlation between caries experience and levels of salivary IgA antibodies to the *S. mutans* group is actually a matter of great debate (5, 10–12, 19, 43). Previous reports have shown that a lower susceptibility to dental caries was associated with high levels of S-IgA antibodies to whole cells of *S. mutans* (9, 27, 30). One of the major etiologic agents of this group of bacteria is a surface protein of about 185 kDa (23) named antigen I/II (Ag I/II) by Russell et al. (45). We used a strain of *Streptococcus sobrinus* isolated from active human dental caries since this species is known to be highly cariogenic (14). We compared CR individuals with a control group of CS subjects by using a new quantitative method and two major antigens, Ag I/II and cell wall carbohydrates (CHO), purified from *S. sobrinus*. Furthermore, a qualitative analysis of salivary antibodies to *S. sobrinus* is given.

MATERIALS AND METHODS

Subjects. The CR group consisted of 22 subjects, whose ages ranged from 22 to 64 years, selected either for their lack of dental caries or (for the eldest) their having fewer than two decayed, missing, or filled teeth. The CS group consisted of 21 subjects, whose ages ranged from 20 to 63 years, selected for their high number of caries lesions (>10 decayed, missing, or filled teeth). Resting mixed salivas were collected for 10 min before mealtime, and volumes were measured for flow rate determination; no mechanical or chemical stimulations were used. After centrifugation, the samples were immediately stored at −80°C until use (8).

Antigens. Two strains of cariogenic bacteria were used: *S. mutans* serotype g (as determined by B. Guggenheim, Zurich University; now called *S. sobrinus* [13]) and *S. sanguis* biovar 2 (25) (as determined by A. Bouvet, Paris VI University). These strains were isolated on blood agar from human dental caries, lyophilized after five subcultures, and designated BC93087 and BC93088, respectively. The bacteria were grown in a sterile dialysate of 10× Todd-Hewitt broth (Diagnostic Pasteur-Sanofi, Marnes la Coquette, France) against 10 volumes of distilled water. After 24 h of culture in 2-liter flasks, the medium was centrifuged at 8,000 × *g* for 30 min. The sedimented cells were washed twice with phosphate-buffered saline (PBS) in a volume corresponding to 1:50 of the culture medium and resuspended at the same concentration. The culture supernatant was dialyzed for 3 days against distilled water and then concentrated 50-fold through Diaflo hollow fibers (H1 P10-20; Amicon, Danvers, Mass.). CHO from the same *S. sobrinus* strain were purified by nitrous extraction by the method of Swanson et al. (46). Briefly, a washed pellet of bacteria from 5 liters of culture was suspended in PBS and incubated for 10 min at room temperature with 100 μl of 4 M sodium nitrite and 50 μl of glacial acetic acid. After centrifugation, the supernatant was adjusted to pH 7.5 and dialyzed against saline. The absence of protein contaminants was controlled with a protein high-sensitivity assay (Bio-Rad Laboratories, Richmond, Calif.), and the CHO molecular weight was established by sodium

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dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) after silver staining by the method of Fomsgaard et al. (16). Protein antigen (Ag I/II) was also extracted from the same *S. sobrinus* culture supernatant and purified by the method of Russell et al. (45). Briefly, the supernatant was concentrated by 70% ammonium sulfate precipitation, followed by DEAE cellulose chromatography at pH 7.4. Ag I/II was eluted with 80 mM potassium phosphate buffer, and this fraction underwent an additional gel filtration on a Sephacryl S300 (Pharmacia, Uppsala, Sweden) column. The purity of this antigen was verified by SDS-PAGE and showed a major band of about 185 kDa.

Salivary immunoglobulins. IgA concentrations were measured by symmetrical capture enzyme-linked immunosorbent assay (ELISA) on plates from Nunc (Roskilde, Denmark), with anti-human α-chain antibodies of sheep origin prepared (21) and horseradish peroxidase (HRPO) labeled in our laboratory. The plates were coated overnight at 4°C with 3 μg of the anti-α-chain antibodies per ml (100 μl per well) and then blocked with 2% skim milk powder in PBS for 2 h at 37°C and washed three times with PBS containing 0.05% (vol/vol) Tween 20. Three twofold dilutions (ranging from 1:2,000 to 1:8,000) of each sample in duplicate were applied for 1 h at 37°C. After three washes, the anti-α-chain HRPO-labeled antibodies were added for 1 h at 37°C, and HRPO activity was revealed with *o*-phenylenediamine (Sigma Chemical Co., Saint Louis, Mo.) and read at 492 nm with a Titertek Multiskan spectrophotometer (Flow Laboratories, Glasgow, United Kingdom). The ELISA was specific, with a sensitivity of 5 ng/ml for S-IgA. The results were drawn from a standard curve of a pool of human whey containing 460 μg of S-IgA per ml. This value was obtained by single radial immunodiffusion with pure S-IgA (21) from colostrum. The absolute protein content of the standard was determined by measurement of optical density (OD) at 280 nm, taken as 1.4 OD units for a 1-mg/ml concentration.

Salivary IgG and IgM were measured by symmetrical sandwich ELISA with anti-human Fcγ antibodies of anti-human μ-chain antibodies, prepared and peroxidase labeled in our laboratory. A large pool of 250 normal human serum samples was taken as a standard for IgG and IgM. This pool was considered to contain 12 mg of IgG per ml and 1.2 mg of IgM per ml.

Subclasses of salivary IgA. Levels of IgA2 were measured by capture ELISA, after complete absorption of S-IgA1 with a large excess of insoluble jacalin (26) purified from jackfruit seeds (kindly provided by X. L. Truong Thi, Institut Pasteur, Ho Chi Minh City, Vietnam) and coupled to Sepharose CL4B (Pharmacia) with cyanogen bromide (20). The volume of insoluble jacalin used to absorb all S-IgA1 molecules in salivas was established with a known concentration of pure colostral S-IgA1, largely exceeding that found in the most concentrated saliva.

We prepared a standard of S-IgA2 from pure S-IgA (8 mg/ml) after total absorption of S-IgA1 with insoluble jacalin. The purity of this standard was controlled by complete lack of precipitation by double diffusion with soluble jacalin (sensitivity, ~2 μg). Pure S-IgA1 was released from the insoluble jacalin column with 0.5 M D-galactose and verified by SDS-PAGE.

Lactoferrin. The lactoferrin determinations were carried out by capture ELISA, with sheep antibodies prepared in our laboratory, and revealed by antilactoferrin rabbit HRPO antibodies (Cappel-Organon Technica, West Chester, Pa.) which did not react in ELISA with either S-IgA or pure secretory component (SC). Standard lactoferrin was purified from human whey by affinity chromatography with heparin-Ultrogel (IBF, Villeneuve-la-Garenne, France) by the method of Bläckberg and Hernell (4). After purity control (no detection with antibodies to SC, a common contaminant of lactoferrin preparations), the standard was quantified at 465 nm, i.e., 0.54 OD unit corresponding to 1 mg/ml (22).

Salivary antibody levels. ELISA plates were coated overnight at 4°C with 100 μl of either bacteria (10^5 cells per ml), CHO (0.1 μg/ml), or Ag I/II (10 μg/ml) per well. High background reactions, observed with saliva concentrations above 1:20 on uncoated plates (1), were avoided with 5% skim milk, which did not modify antibacterial antibody titers. After being blocked for 2 h at 37°C and washed three times with PBS-Tween 20 (0.05% [vol/vol]), salivas (diluted 1:10, 1:20, and 1:40 in the same medium) were incubated overnight at 4°C; all subsequent ELISA steps followed. The S-IgA antibody standard consisted of a human whey sample selected for its high level of antibodies to *S. sobrinus* and *S. sanguis* and calibrated by our "enzyme radio immuno comparative assay," which we termed ERICA, derived from the method of Butler et al. (7) but with several modifications. Transformation of ELISA OD units into nanograms was carried out with radiolabeled colostral S-IgA and with the same anti-α-chain HRPO-labeled antibodies. Colostral S-IgA was prepared as described previously (21) and controlled by SDS-PAGE analysis. Purified S-IgA1 and S-IgA2 were mixed at a ratio of 70:30 (wt/wt), corresponding to their relative proportions in saliva (1, 6, 36, 37) as well as to our results in this article (see Table 3), and further iodine labeled to serve as a standard. Radiolabeling was carried out with [^{125}I]Na (Amersham, Little Chalfont, United Kingdom) and Iodobeads (Pierce Chemical Co., Rockford, Ill.) by the method of Markwell (32), free iodine being removed by dialysis. Specific activities of labeled S-IgA were determined with a Minigamma LKB counter and ranged from 16×10^6 to 25×10^6 cpm/μg, depending on the experiment.

Immulon 2 Removawells (Dynatech, Zug, Switzerland) were coated overnight at 4°C with 100 μl of a 3-μg/ml concentration of rabbit anti-human $F(ab')_2$ antibodies, prepared in our laboratory. After being blocked, dilutions of the iodinated S-IgA standard were incubated overnight at 4°C, and an ELISA was carried out by using identical detection systems. The relationship between the OD at 492 nm, observed after 10 min of substrate incubation, and counts per minute values in each well showed that 1 OD corresponded to 0.4 ng of the S-IgA standard. This relationship was confirmed with different ^{125}I-S-IgA samples.

The antibody levels in the former whey standard were determined under identical conditions, by an antigen-specific ELISA, by using Removawells and our anti-α-chain HRPO antibodies. For each of the four antigens, the titration curve was parallel to that of the capture ELISA with ^{125}I-S-IgA. The percentage of unbound antistreptococcal antibodies in each well was estimated with a subsequent identical ELISA by the method of Fomsgaard and Dinesen (15). Transformation of absorbance into nanograms was calculated by the 0.4 ng per ELISA unit factor as described in the previous paragraph. For each antigen, the values found with the standard whey served as an internal reference curve to obtain the antibody levels in each saliva sample.

Bacterium-bound antibodies were extracted from whole saliva pellets with 0.2 M glycine-HCl (pH 2.8). This extract was neutralized at pH 7.4 and subjected to the anti-*S. sobrinus* activity ELISA under the same conditions as described above.

Western blot (immunoblot) analysis. The 50-fold-concen-

TABLE 1. Individual data of the CS subjects

Subject	Sex	Age (yr)	Flow rate (ml/min)	S-IgA (μg/ml)	S-IgA2 (μg/ml)	Lactoferrin (μg/ml)	S-IgA antibody (ng/ml)			
							S. sobrinus	*S. sanguis*	*S. sobrinus* antigens	
									Ag I/II	CHO
01	F	24	0.28	219	87.8	4.5	84	70	40	190
02	F	25	0.26	143	26.1	5.4	72	36	88	109
03	F	25	0.64	86	20.9	1.3	76	123	55	154
04	F	27	0.43	74	19.3	3	50	54	20	134
05	F	31	0.20	108	32	2	60	25	30	158
06	F	32	0.46	52	3.0	6.9	48	118	135	75
07	F	35	0.24	94	32.9	5	32	70	73	106
08	F	37	0.33	198	59.4	0.8	400	208	488	312
09	F	44	0.20	59	14	2	760	43	760	442
10	F	56	0.43	105	31.6	1.8	116	86	120	436
11	M	25	0.23	97	28.7	4.9	28	55	27	74
12	M	25	0.55	72	16.7	1.2	130	155	920	320
13	M	26	0.63	71	17.1	11	102	102	102	296
14	M	26	0.26	115	41.5	6	78	33	470	94
15	M	31	0.28	117	57.1	1.2	80	105	20	270
16	M	32	0.58	108	38.3	4	87	180	13	440
17	M	34	0.60	110	25.3	3.5	84	60	22	306
18	M	36	0.35	92	48.3	6	60	85	86	160
19	M	37	0.46	90	18.0	3	34	280	5	110
20	M	49	0.23	166	73.5	4.5	110	60	44	316
21	M	63	0.30	100	26.3	2	46	34	33	144
Mean		34.3	0.38	108	34.2	3.8	121	94	169	221
SD		10.7	0.15	42.4	20.9	2.46	165	65	260	124
Median		32	0.33	100	28.3	3.5	78	70	55	160
Interquartiles		26–37	0.26–0.46	86–115	19–41	2–5	50–102	54–118	27–120	110–312

trated 48-h culture supernatant of *S. sobrinus* was 70% ammonium sulfate precipitated, dialyzed, and subjected to SDS-7.5% PAGE (28). The protein cell wall extract was prepared by the method of McBride et al. (33). Briefly, washed *S. sobrinus* cells were sonicated three times for 1 min and centrifuged at 3,000 × *g*. The pellet, treated with 2% SDS and 1% 2-mercaptoethanol, was placed in a boiling water bath for 15 min and centrifuged. The supernatant was analyzed by SDS-PAGE and then electrotransferred to nylon membranes (Immulon P; Millipore Corp., Bedford, Mass.). The membranes were saturated with 5% skim milk, washed, and incubated for 1 h at 37°C with salivas diluted 1:10. Our anti-α-chain HRPO-labeled antibodies (1:1,000) were incubated for 1 h at 37°C and visualized with 2,3′-diaminobenzidine (Sigma).

Avidity of salivary IgA antibodies. Avidities to Ag I/II and CHO were estimated by the method of Pullen et al. with slight modifications (42). Briefly, ELISA plates were coated with either Ag I/II (10 μg/ml) or CHO (0.1 μg/ml). After the plates were coated with skim milk, they were incubated overnight at 4°C with a single constant dilution of each saliva, chosen to give a measurement of approximately 1 OD unit with the anti-α-chain HRPO-labeled antibodies. After several washings, 100 μl of appropriate molarities of sodium thiocyanate in PBS was added in duplicate for 30 min at room temperature. After further washings, the usual ELISA procedure was carried out. Absorbance values were plotted, and the interpolated point, corresponding to 50% of the control value without thiocyanate, was determined for each saliva sample. The relative avidities (avidity index) were expressed as the concentration (in moles) equivalent to this point.

Statistical analysis. All assays were carried out in duplicate with serial twofold dilutions. The two groups were compared by using the rank order test of Mann and Whitney.

RESULTS

Nonspecific and specific parameters. All individual results (Tables 1 and 2) were found to vary considerably in each group. The median (*m*) of the flow rates was not significantly increased in the CR group (m = 0.48 ml/min) in comparison with that of the CS group (m = 0.33 ml/min). In addition, total S-IgA and S-IgA2 medians, expressed in micrograms per minute, were similar in both groups, and the S-IgA2/S-IgA ratios were also identical (Table 3). Nevertheless, the lactoferrin secretion rate was higher in the CR group but without statistical significance (Table 3). Similarly, no significant difference was found in the flow rates of all antibodies.

In all salivas, IgG and IgM never reached more than 10 and 5%, respectively, of the total IgA content (data not shown).

Specific activities (another accurate way to express antibody responses [17]) of IgA antibodies to *S. sobrinus* and *S. sanguis* cells were also similar in the CR and CS subjects (Fig. 1a). For the purified antigens of *S. sobrinus*, the specific activities of CHO antibodies also did not differ in both groups (Fig. 1b), whereas a statistically significant difference ($P < 0.001$) was observed between specific activities of antibodies to Ag I/II, in favor of the CS group.

The levels of IgA antibody to *S. sobrinus* found in the extracts of the saliva pellets were far below 1% of the total amount in the fluid phase.

Specificity of antibodies to the *S. sobrinus* antigens was studied by Western blot (Fig. 2). The culture supernatant

TABLE 2. Individual data of CR subjects

Subject	Sex	Age (yr)	Flow rate (ml/min)	S-IgA (μg/ml)	S-IgA2 (μg/ml)	Lactoferrin (μg/ml)	S-IgA antibody (ng/ml)			
							S. sobrinus	*S. sanguis*	*S. sobrinus* antigens	
									Ag I/II	CHO
22	F	22	0.96	66	14.9	1.4	21	20	21	92
23	F	26	0.30	78	11.7	2.2	35	18	13	280
24	F	31	0.91	31	9.9	3	46	46	7	142
25	F	31	0.43	73	35.2	4	86	270	34	326
26	F	32	0.64	93	38.4	2	10	26	10	49
27	F	33	0.76	67	5.8	0.9	42	137	41	256
28	F	42	0.26	101	39.6	5.6	31	50	20	100
29	F	48	0.40	121	24.2	2	180	81	23	480
30	F	62	0.26	118	41.5	7.2	23	38	42	223
31	M	28	0.33	69	22.1	1.2	16	67	18	104
32	M	28	0.58	62	23.4	4	44	30	33	76
33	M	29	0.53	73	20.4	9	76	165	117	270
34	M	33	0.65	91	8.8	0.9	32	162	13	196
35	M	35	0.74	79	18.9	6.4	29	27	14	156
36	M	36	0.40	109	33	2	30	65	15	84
37	M	36	0.34	108	25.3	3.1	70	70	170	96
38	M	37	0.33	44	18.8	1.6	21	25	8	57
39	M	40	0.54	43	8.9	0.5	17	23	5	50
40	M	41	0.20	94	52.5	7.9	60	80	48	220
41	M	56	0.93	366	181	13.4	460	290	218	864
42	M	57	0.90	33	8.8	3.1	20	13	5	38
43	M	64	0.26	138	48.1	9	76	52	22	446
Mean		38.5	0.53	93.5	31.4	4.1	65	80	41	209
SD		11.8	0.25	67.3	36.1	3.4	96	78	56	192
Median		35	0.48	78	22.1	3	32	50	20	148
Interquartiles		30–42	0.33–0.65	66–101	12–38.4	1.3–6.4	21–76	26–81	13–34	84–256

(Fig. 2a) shows three main bands, a major band of 60 kDa, another of 94 kDa, and the 185-kDa band of Ag I/II (45). The overall patterns of both groups were very similar. For the cell wall proteins (Fig. 2b), a heavy band of 94 kDa and three major bands of 60, 80, and 185 kDa were observed. Like the pattern of the culture supernatant (Fig. 2a), the overall pattern of the cell wall proteins showed no major difference in the antibody specificity of CR and CS salivas (Fig. 2b).

Antibody subclass. The specific activities of S-IgA1 and S-IgA2 to Ag I/II and CHO were studied with nine salivas selected for their high antibody content (Table 4) and found to be higher for Ag I/II in the CS group but regardless of the subclass. In contrast, no difference was observed for antibodies to CHO. When the ratios of S-IgA2 antibodies/total antibodies in the nine former selected salivas were compared (Table 4), no difference was found between the groups.

Avidity index. No difference was found in a comparison of CHO and Ag I/II antibody avidities of three samples of CS and CR subjects selected for their high antibody titers (Fig. 3). It must be noted that the same samples were not used for both antigens since some salivas exhibited high titers against CHO but low titers against Ag I/II and vice versa.

DISCUSSION

In this study, we reassessed the hypothesis that there is an increased salivary antibody response to cariogenic bacteria in CR individuals and observed no increase or qualitative difference of specific antibodies in CR subjects in comparison with CS subjects.

The sampling method allowed a simple approach for the spontaneous flow rate of the tooth-bathing fluid and has been shown recently to give the highest concentration of salivary S-IgA (3). We found the flow rate in the CR group to be elevated in comparison with that of the CS group, but the increase was not statistically significant and thus cannot be a major nonspecific parameter in protection. Nevertheless, this determination was necessary to express our results as secretion rates under physiological conditions. This mode of expression is more accurate than the sole salivary concen-

TABLE 3. Nonspecific and specific parameters of the salivas

Subjects	S-IgA (μg/min)	S-IgA2 (μg/min)	S-IgA2/S-IgA (%)	Lactoferrin (μg/min)	S-IgA antibodies (ng/min)			
					S. sobrinus	*S. sanguis*	*S. sobrinus* antigens	
							Ag I/II	CHO
CR (n = 22)	36[a] (25–54)[b]	10 (6.8–13.5)	29.4 (22–39.2)	1.45 (0.68–2.5)	20 (10–29)	19.5 (13.3–37)	9.4 (5.9–19.5)	71 (34–128)
CS (n = 21)	37 (29–55)	10 (6.8–16.5)	29.2 (23.6–36)	1.12 (0.44–1.5)	22 (15–50)	29 (12.6–64)	17.5 (8.6–52)	57.6 (34.5–103)

[a] Median.
[b] Interquartiles.

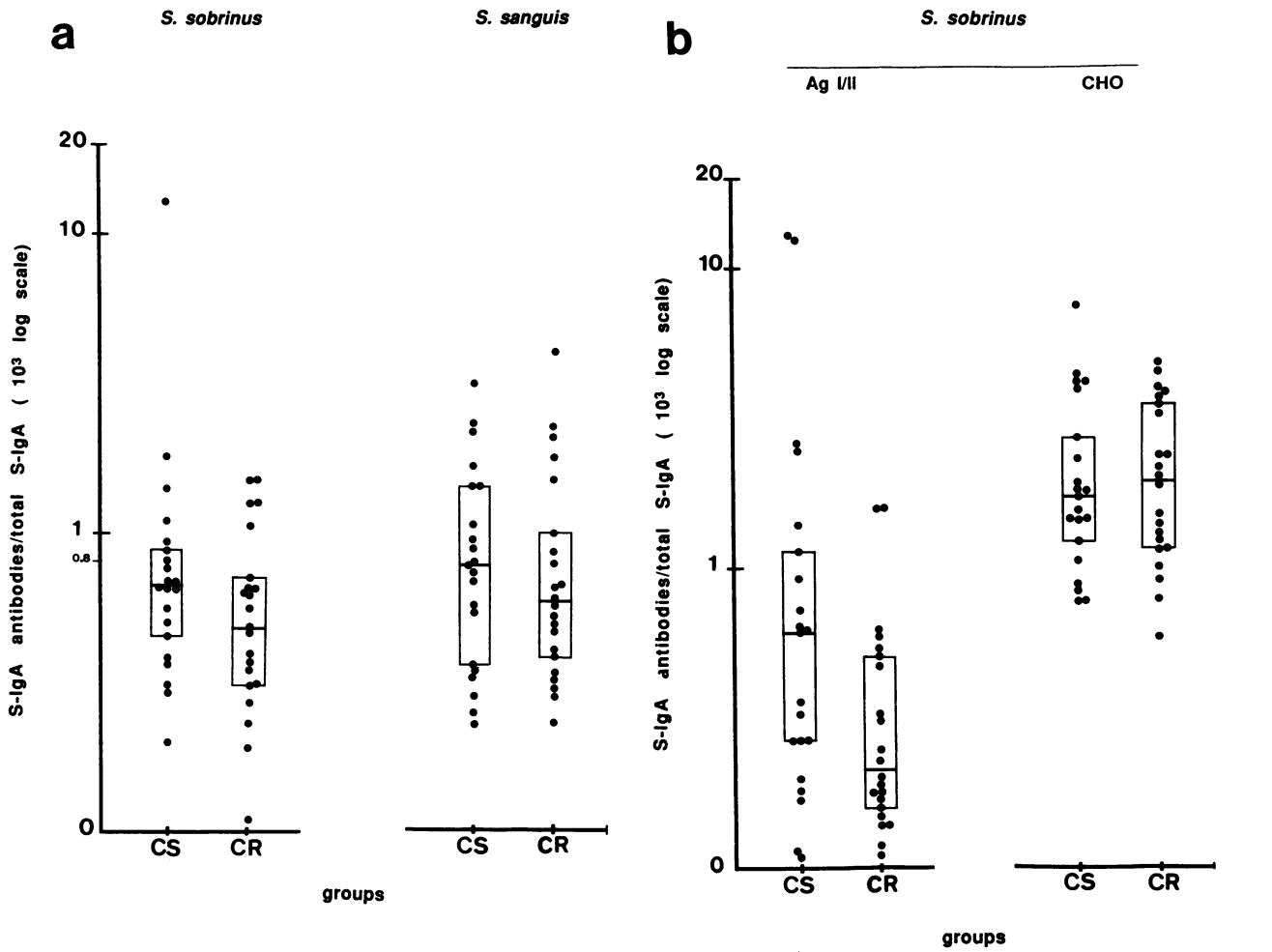

FIG. 1. Specific activities of salivary antibodies to cariogenic bacteria (a) and purified antigens (b) in CS and CR subjects. Plates were coated with either 100 μl of a suspension containing 10^5 cells of *S. sobrinus* or *S. sanguis* (a) or a solution containing either 10 μg of Ag I/II per ml or 0.1 μg of CHO purified from *S. sobrinus* per ml (b). The boxes correspond to interquartiles. Each dot represents an individual, and the bar denotes the median.

tration since it also allows for calculation of the total delivery of the secreted antibodies. Our results for *S. sobrinus* are in agreement with those of Butler et al. (7).

Secretion rates of total S-IgA and its two subclasses were equal in both groups, and the S-IgA2/S-IgA ratio (*m*, ~29%) was in the normal range (6). Similarly, in CR salivas, the rate of secretion of lactoferrin, a well-known antibacterial agent which could interfere with resistance to infection either by itself (2) or through its combination with S-IgA (44), was increased, although not significantly (Table 3). Thus, this parameter cannot explain the difference in caries protection and will not be discussed further. Identical S-IgA secretion rates, as well as subclass ratios in both groups, permitted us to compare their S-IgA specific activities (Table 4). A fresh isolate from a caries lesion was used as a source of antigen with a minimal number of subcultures necessary for sufficient production. This small number allowed us to avoid eventual deleterious mutations of the strains (48). One of the two strains was of the *S. sobrinus* species, which shares large antigenic communities with other representatives of the *S. mutans* group. Moreover, in rats, *S. sobrinus* appeared to be more cariogenic than *S. mutans* (14).

Our ERICA method enabled antibodies to be expressed in nanograms, instead of the unsatisfactory ELISA titer, although, on a theoretical basis, our units cannot be considered as true absolute weights. Both groups showed no significant difference in their salivary antibody contents when tested with bacterial cells. Our results confirm those of Grahn et al. (18) but not the reported tendency of CS subjects to have lower levels of salivary S-IgA antibodies to *S. mutans* (9). When assayed against purified major immunogenic molecules from *S. sobrinus*, the specific activity of anti-CHO antibodies, known to be serotype specific (34), was similar in both groups, whereas that of anti-Ag I/II antibodies was unexpectedly higher in the CS subjects (Fig. 1). This difference for the Ag I/II antibodies is significant ($P < 0.001 \times n$, i.e., $P < 0.015$) even when the number ($n = 15$) of our statistical analyses is taken into account. Nevertheless, this difference needs to be confirmed by another set of investigations since it was not observed when the flow rate of this antibody was taken into account (Table 3). In addition, experimental studies successfully using Ag I/II as a parenteral anticaries vaccine in monkeys (29) have shown the sole serum antibodies to be associated with protection. The apparent efficiency of these serum antibodies seems to be restricted to the IgG isotype (47).

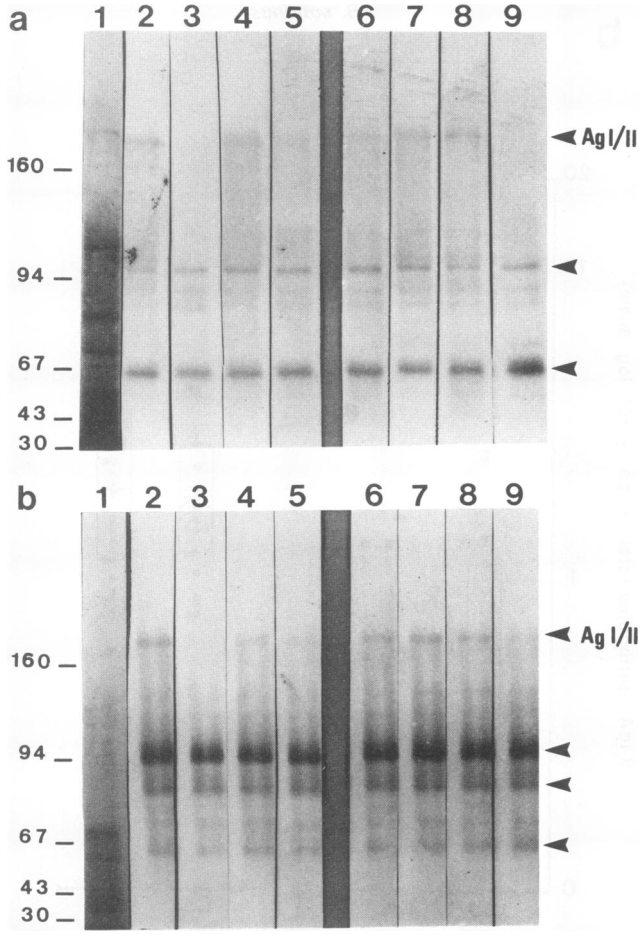

FIG. 2. Specificity of *S. sobrinus* antibodies from CR and CS subjects investigated by Western blot analysis. An SDS-7.5% polyacrylamide gel of culture supernatants and of cell extracts is shown. Culture supernatant (a) and cell wall proteins (b) were absorbed on a nylon membrane, reacted with diluted salivas (1:10), and then incubated with anti-α-chain HRPO-labeled antibodies. Lanes: 1, Coomassie staining of the supernatant (a) and of the cell extract (b); 2 to 5, CS salivas; 6 to 9, CR salivas. Dashes indicate the migration of control molecular mass markers (in kilodaltons), and arrows point to major bands including Ag I/II.

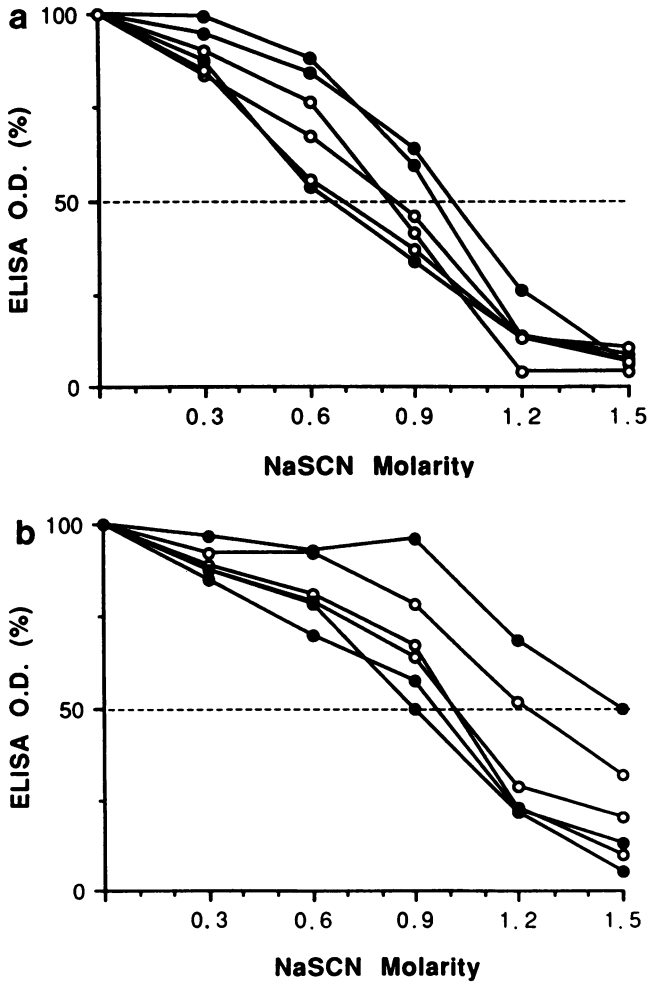

FIG. 3. Avidity indexes of selected S/IgA antibodies to purified surface antigens from *S. sobrinus*, namely, Ag I/II (a) and CHO (b). The horizontal dotted lines correspond to the molarity of NaSCN, inducing 50% dissociation of the complexes. Avidities were similar in the CR (closed circles) and CS salivas (open circles).

For each sample, the sum of antibodies against Ag I/II and CHO exceeded the total amount of antibodies measured with the cells. This excess could be due to hidden epitopes on the bacterial surface which become accessible on the soluble purified antigens. Alternatively, part of the difference could be related to our method of antibody calculation.

In addition to quantitative results, Western blot analysis showed no qualitative difference in the specificity of the antibodies found in both groups, which is not favorable to the hypothesis of an antigen-restricted antibody protection.

It has been postulated that S-IgA2 are the most efficient

TABLE 4. Specific activities of S-IgA1 and S-IgA2 and ratios of S-IgA2 antibodies to total antibodies in selected salivas

Subjects	S-IgA1 antibodies/total S-IgA1[a]		S-IgA2 antibodies/total S-IgA2[a]		S-IgA2 antibodies/total antibodies[b]	
	Ag I/II[c]	CHO[c]	Ag I/II	CHO	Ag I/II	CHO
CR (n = 9)	0.44[d] (0.23–0.56)[e]	2.6 (2.2–3.1)	0.52 (0.38–0.95)	4.7 (2.3–11)	39 (23–54)	47 (36–66)
CS (n = 9)	1.7 (0.9–2.8)	1.55 (1.3–2.9)	1.8 (1.5–8.2)	3.1 (2.7–7.1)	30 (25–52)	50 (22–66)

[a] In per mille.
[b] In percent.
[c] Ag I/II and CHO from *S. sobrinus*.
[d] Median.
[e] Interquartiles.

antibodies in secretions since they can resist IgA1 proteases (24) from a large spectrum of bacteria and, namely, streptococcal species (39, 40). Therefore, we investigated these antibodies in the saliva of subjects with the highest titers and also found no difference, in terms of specific activity, between both subclasses from each of these selected groups.

The possibility of a higher antibody affinity, which could lead to better efficiency in protection, was ruled out by the similar avidities observed against the two antigens. It must be noted that the high avidity index of anti-CHO antibodies might be due to the classical repetitive nature of the carbohydrate epitopes. In conclusion, in both groups, the levels of the CHO antibodies were high and relatively close to the median, indicating that all subjects had been stimulated by this cariogenic serotype.

The presence of S-IgA antibodies to cariogenic bacteria in saliva confirms the involvement of the secretory immune system in responses to oral microorganisms and reflects a consequence of mucosal challenges by the bacteria (19). In conclusion, the similarities in IgA subclass ratio, avidity, and fine antigenic specificity of the salivary antibodies in CS and CR subjects show that even high levels of these molecules are not sufficient to inhibit caries development. This means that caries protection by S-IgA antibodies can be bypassed by pathogens.

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