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Antibody Responses of Monkeys to Oral and Local Immunization with *Streptococcus mutans*

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Monkeys were immunized with *Streptococcus mutans* by a number of routes in an attempt to elicit exclusively a secretory immunoglobulin A (IgA) response. Antibody responses were detected by a sensitive radioimmunoassay. Monkeys primed subcutaneously and boosted submucosally with formolized cells of *S. mutans* had high serum IgG, IgA, and IgM radioimmunoassay titers and only slight salivary IgG titers. Instillation of killed cells of *S. mutans* into the right parotid salivary duct elicited good IgG, IgA, and IgM responses in both the right parotid saliva and serum, but only a weak IgM response was detected in the left parotid saliva. Administration of killed cells of *S. mutans* in enterically coated capsules did not elicit a detectable antibody response or have a discernible effect on the antibody response to subsequent immunization by instillation. No increase in antibody titer was detected in the serum or whole saliva from monkeys orally immunized with enterically coated capsules containing viable *S. mutans* or in the serum, whole saliva, or intestinal contents from monkeys immunized with uncoated capsules containing killed cells of the same organism. These results do not support the concept that oral immunization with *S. mutans* is effective in stimulating a generalized secretory IgA response in primates.

Immunization with vaccines of *Streptococcus mutans* has been effective in preventing the development of dental caries in laboratory animals fed a high-sucrose diet. Protection of rats from dental caries has been achieved by local injection with killed whole cells of *S. mutans* (16, 23) which, in addition to eliciting humoral antibody, elicited a salivary immunoglobulin A (IgA) response. Oral immunization of rats by including killed cells of *S. mutans* in their drinking water has also afforded protection from caries (18). *S. mutans*-specific IgA antibodies were found in the saliva and milk of the orally immunized rats, but serum antibody could not be detected. Immunization experiments with these two routes have protected rats from caries and are the basis for the proposal that the protection is mediated by IgA antibody in saliva (16, 23).

In monkeys, immunization with *S. mutans* by subcutaneous (2, 3) or oral submucosal (6) injection has afforded protection against caries. High titers of circulating antibody result from both of these routes of immunization, with only low levels of antibody detectable in saliva (3). Protection of monkeys has also been reported to have been obtained by the passive transfer of IgG derived from immune serum (15). These results suggest that caries protection in monkeys may be mediated by humoral antibody.

Emmings et al. (10) have demonstrated an oral secretory IgA (s-IgA) response in monkeys by instilling antigens of *S. mutans* into the parotid salivary ducts; however, the s-IgA response was accompanied by high titers of serum antibody. Mestecky et al. (17) reported that the oral immunization of human volunteers with capsules containing killed cells of *S. mutans* elicited an exclusively s-IgA response in saliva and tears without any detectable increase in humoral antibody. The distribution of the secretory antibody response found in orally immunized humans is similar to that found in orally immunized and protected rats. It is not known whether s-IgA antibody alone is protective against dental caries in either humans or monkeys. To establish experimentally whether s-IgA is protective, methods of immunization must be found which elicit only an s-IgA response.

The aim of the experiments described here was to determine whether a number of different routes of immunization were able to elicit exclusively an s-IgA response in monkeys.

MATERIALS AND METHODS

Animals. *Macaca fascicularis* monkeys were used in these studies. Animals in groups 1 to 5 were born in the wild, were fed a maintenance diet with a low sucrose content, and had been kept in the colony for several years. *S. mutans* could be cultured from both the plaque and feces of these adult monkeys. Monkeys in experiment 14 were born in captivity and were changed to a high sucrose diet during the experiment,

as shown in Fig. 1. *S. mutans* could be consistently isolated from the dental plaque of young monkeys only after the change to the high sucrose diet.

Preparation of antigens. *S. mutans* strain Ingbritt was grown in batch culture with the pH maintained at 6.8 in a glucose-tryptone-yeast extract medium as described previously (28). Cells, harvested by centrifugation, were either (i) killed with 0.2% formaldehyde, washed, frozen, and lyophilized or (ii) suspended in 10% (wt/vol) polyvinylpyrolidone–5% (wt/vol) sodium glutamate (pH 7.0); between 20 to 30 ml of this medium was added for each 6 g (wet weight) of cells, and the slurry was then frozen and lyophilized. The dried formolized cells of *S. mutans* served as both oral vaccine and as solid-phase antigen in the radioimmunoassay (RIA) used to monitor the antibody response. The dried live *S. mutans* cells were used as an oral vaccine after estimating the number of viable organisms per milligram on horse blood agar.

Soluble protein preparations were also used as antigens in the RIA. An extract was obtained from the cell-free supernatant of a culture of *S. mutans* strain Ingbritt, grown in the defined medium of Terleckyj et al. (25), by precipitation with 80% $(NH_4)_2SO_4$ as previously described (28). Pure cell wall protein antigens A and B were kindly provided by R. R. B. Russell. They were purified from culture supernatant as previously reported (19).

Monkey immunization. (i) Subcutaneous and submucosal immunization (experiment 14). Four young home-bred monkeys were immunized with whole formolized cells of *S. mutans*. The cells, grown in Todd-Hewitt broth with 1% glucose (wt/vol) added, were prepared by Wellcome Research Laboratories, Beckenham, Kent, England. The first injection (4 mg of cells) was subcutaneous, the next two injections (8 mg of cells) were given submucosally in the mouth, and the next oral submucosal injection (5 mg of cells) was given together with aluminum hydroxide adjuvant. See Fig. 1 for the immunization schedule. At the beginning of the experiment (day 0), the monkeys were 14.5 ± 4 (mean ± standard deviation) months old.

(ii) Oral and local immunization (groups 1 to 5). Each experimental group comprised two adult female monkeys. All animals were fasted overnight before immunization. Figure 1 shows the immunization schedules, with day 0 denoting either the first day of immunization, collection of body fluids, or both. Group 1 monkeys were given capsules enterically coated as described by Couch et al. (8), each capsule containing 50 mg of formolized *S. mutans*, and were subsequently instilled three times with 10 mg of formolized *S. mutans* cells in 1 ml of phosphate-buffered saline (Oxoid Ltd., Basingstoke, England) into the right parotid salivary duct. Animals in group 2 were instilled three times with 1 mg of formolized *S. mutans* cells in 1 ml of phosphate-buffered saline. The first six enterically coated capsules given to group 3 monkeys contained 10^6 colony-forming units of *S. mutans* per capsule (approximately 47 mg [dry weight] of cells per capsule), and the final 18 capsules contained 10^8 colony-forming units per capsule (approximately 60 mg [dry weight] of cells per capsule). Group 4 monkeys

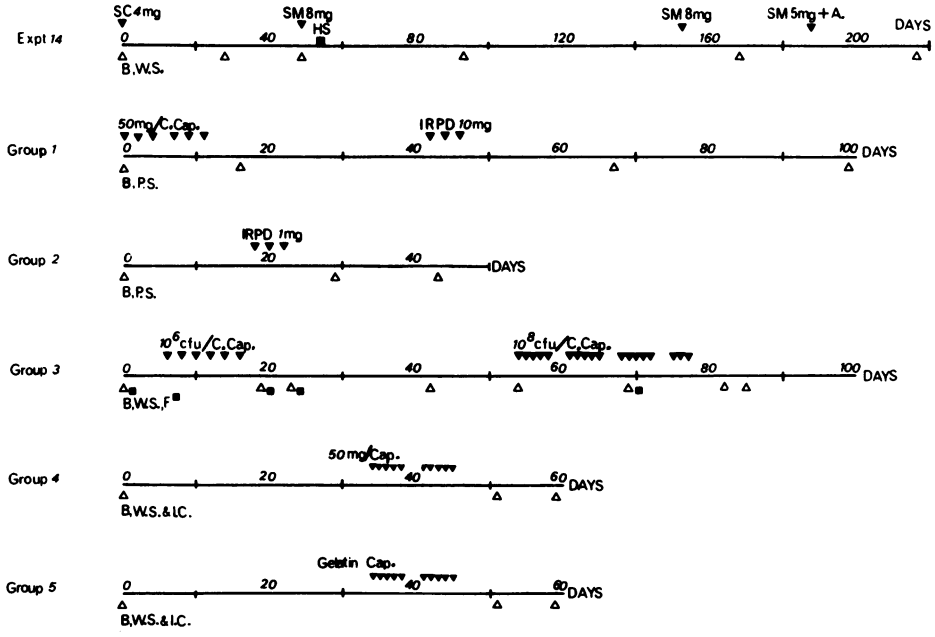

FIG. 1. *Protocols for immunization (solid arrows above line) and sampling of body fluids (open arrows below line). Monkeys were immunized by either subcutaneous (SC), submucosal (SM), or submucosal plus adjuvant (SM + A) injection, instillation of the right parotid duct (IRPD), enterically coated capsules (C.Cap.), or uncoated capsules (Cap.). Samples taken were blood (B), whole saliva (WS), parotid saliva (PS), intestinal contents (IC), and feces (F). HS denotes the change to a high-sucrose diet in experiment 14.*

were given 10 uncoated capsules containing 50 mg of formolized *S. mutans* cells, and group 5 monkeys received 10 similar capsules containing powdered gelatin.

Enterically coated capsules did not dissolve below pH 6 and therefore protected the contents from stomach acid. Encapsulated viable cells of *S. mutans* were administered with the aim of creating a transitory increase in the level of *S. mutans* in the gut and thereby providing a greater antigenic stimulus.

The change to uncoated gelatin capsules given to animals in groups 4 and 5 was based on the reported successful oral immunization of humans with *S. mutans* cells (17) by using uncoated capsules.

Sampling of saliva, serum, and intestinal contents. Samples of saliva, serum, and intestinal contents were collected at intervals during the experiments as shown in Fig. 1. Whole saliva was collected after stimulation by subcutaneous injection of 0.5 mg of pilocarpine nitrate (British Drug Houses, Poole, Dorset) per kg. Left and right parotid saliva was collected separately by cannulating each parotid duct as described previously (28) and then stimulating salivation with 1 mg of pilocarpine nitrate per kg. Atropine sulfate (300 μg per monkey) was given after collection to reduce nausea and salivation. All samples of saliva were frozen immediately after collection and then thawed and clarified by centrifuging at 10,000 × *g* for 10 min. Venous blood was allowed to clot, and the serum was separated with the aid of a Serasieve (Hughes and Hughes, Romford, Essex). Intestinal contents were collected from monkeys in groups 4 and 5 under general anaesthesia induced and maintained by nitrous oxide and halothane. A lateral incision was made through the abdominal wall, and a nylon suture was tied loosely around the small intestine 30 cm from the ileocaecal valve. A similar suture was tied 15 cm proximal to the first, and the contents of the length of intestine between the two loops of suture were sampled. The loops of suture ensured that subsequent samplings were from the same section of intestine. Flat clamps were gently applied at each suture, and the contents were milked to one end of the segment and aspirated by passing a 19-gauge needle attached to a 5-ml syringe through the intestinal wall. Care was taken to avoid major blood vessels. Usually 1 to 2 ml of contents was aspirated, but when little or no material could be obtained in the designated section, additional samples were taken from the 15-cm sections either side of it. Intestinal contents were frozen after collection, but before being assayed for specific antibody they were thawed and mixed in a glass/glass homogenizer with an equal volume of physiological saline. After heat inactivation at 56°C for 30 min, the samples were clarified by centrifugation (12,000 × *g*, 10 min), and the supernatant was precipitated with 50% $(NH_4)_2SO_4$. The precipitate which formed at 4°C after 1 h was dissolved and dialyzed against phosphate-buffered saline containing 0.05% (wt/vol) NaN_3, and the volume was adjusted to half that of the original intestinal contents sample.

All serum, saliva, and intestinal contents samples were stored at −20°C until assayed.

RIA. Samples of serum, saliva, and intestinal contents were assayed for IgG, IgA, and IgM antibodies to a number of antigens of *S. mutans* by RIA by the method described previously (28). In brief, solid-phase antigens of *S. mutans* (formolized whole cells or soluble antigens adsorbed to polystyrene wells) were incubated with single dilutions of either serum (1/100 or 1/200), saliva (1/4 to 1/16) or intestinal contents (1/5 or 1/10). The dilution assayed depended on the sensitivity of the particular labeled antibody preparation (28), which was determined by assaying a reference immune serum. Assays were performed in duplicate for the polystyrene-adsorbed antigens and in triplicate for the whole cell antigens. After incubation at room temperature for 3 h, the solid-phase antigens were washed and incubated with ^{125}I-labeled anti-human IgG, IgA, or IgM antibodies (30,000 cpm per assay) which had been affinity purified on columns of insolubilized monkey immunoglobulin before labeling. The class-specific antibodies, purified from commercially available antisera (anti-human IgG and IgM from Wellcome Reagents, Beckenham, Kent, and anti-human IgA from Meloy Laboratories, Springfield, Va.) were heavy chain specific. The affinity purification ensured that only those anti-human immunoglobulin antibodies which cross-reacted with the corresponding monkey immunoglobulin were labeled. After overnight incubation at 4°C the solid-phase antigens were washed and counted in an ICN Gamma Set 500.

The total counts per minute associated with functional antibody for each labeled antibody preparation was estimated by incubation of the labeled antibody with glutaraldehyde-polymerized monkey serum. The test counts per minute were corrected for counts per minute binding to antigen alone, and the remainder were expressed as a percentage of the counts per minute binding to polymerized monkey serum, i.e., as a percentage of the labeled antibody.

The sensitivity of the RIA to the different classes of antibody can not be presumed to be the same, so that similar percent labeled antibody titers do not necessarily indicate similar levels of different classes of antibody. The sensitivity of the radiolabeled antibody preparations declines with age (28), and to enable comparison between titers of sequential samples, all samples from each experimental group were assayed at the same time.

Enumeration of *S. mutans* in feces. Feces (0.1 to 0.2 g) obtained per rectum with sterile wooden swab sticks were placed into sterile preweighed containers. Half-strength brain heart infusion broth (Oxoid) was added to give a 10-mg/ml suspension, together with sterile glass beads (3.5 to 4.5 mm; Hopkins and Williams Ltd., Essex, England), and the samples of feces were suspended with the aid of a Vortex mixer. The suspensions were serially diluted, and 0.1-ml samples of appropriate dilutions were spread onto mitis salivarius agar plates (Oxoid) supplemented with 0.2 U of bacitracin per ml as described by Gold et al. (12). Colonies resembling those of *S. mutans* were counted, subcultured, and characterized by using the biochemical tests of Colman (7) and serotyped by double immunodiffusion in agarose gel.

Serum absorption. Pre-immunization samples of serum from monkeys in groups 4 and 5 were incubated at ambient temperature for 6 h with an approximately equal volume of packed, heat-killed cells (56°C for 30

min) of either *S. mutans* strain Ingbritt or *Streptococcus azgazardah* (National Collection of Type Cultures no. NCTC 4540). It has been demonstrated (5) that *S. azgazardah* possesses a surface component which reacts non-specifically with the Fc region of human IgG. Cells were sedimented by centrifugation (10,000 × *g*, 10 min), and the absorbed and unabsorbed sera were diluted 1:200 and assayed by RIA for IgG, IgA, and IgM to whole cells of *S. mutans* strain Ingbritt.

RESULTS

Responses to subcutaneous and submucosal immunization. After subcutaneous and submucosal immunization of monkeys in experiment 14 with killed cells of *S. mutans*, strong serum IgG and IgM responses to whole cell antigens were detected by RIA (Fig. 2). A lower serum IgA response was detected. Small increases of salivary IgG antibody titers concomitant with large increases of humoral IgG titers were also apparent, but the IgA and IgM titers showed only slight increases.

The failure of subcutaneous immunization combined with local immunization of the oral mucosa to elicit a significant IgA antibody response in saliva prompted further attempts to stimulate an s-IgA response in monkey saliva by using alternative routes of immunization.

Responses to oral immunization and salivary gland instillation. No rise of specific antibody to *S. mutans* in any of the classes assayed was apparent in group 1 animals after oral immunization with enterically coated capsules. Subsequent instillation (three times with 10 mg of whole cells) into the right parotid salivary duct was effective in stimulating both a serum response and a right parotid salivary response in all classes of antibody assayed (Fig. 3). In the left parotid saliva a slight but significant rise in the IgM titer was detected, with no significant increases being detected for either IgA or IgG. The flow of saliva from the right parotid

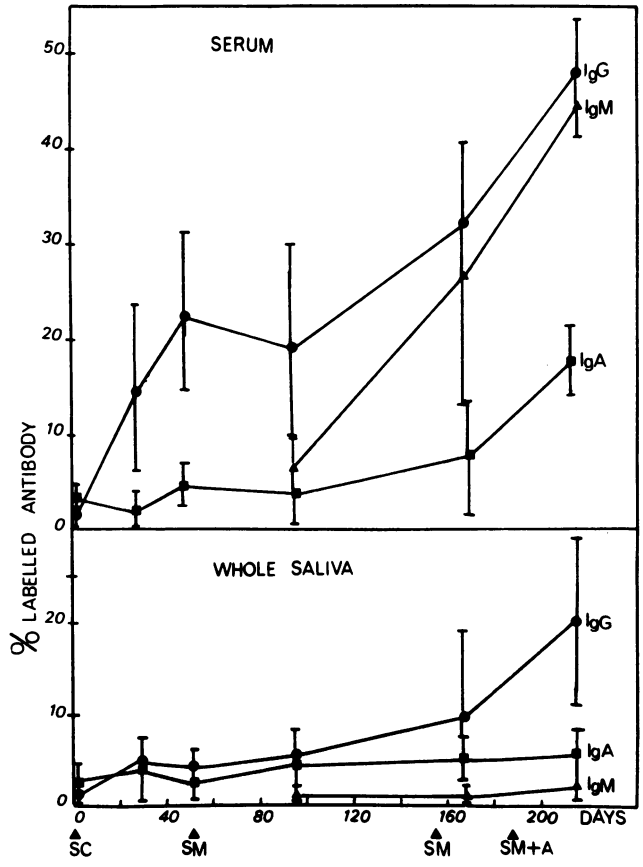

FIG. 2. *Antibody responses of monkeys in experiment 14 as determined by RIA. Each point is the mean of samples from four monkeys ± standard deviation. Samples of serum (1:100) and saliva (1:4) were assayed in triplicate by using formolized cells of S. mutans as antigen. Monkeys were immunized by subcutaneous (SC) and submucosal (SM) injection of killed cells of S. mutans.*

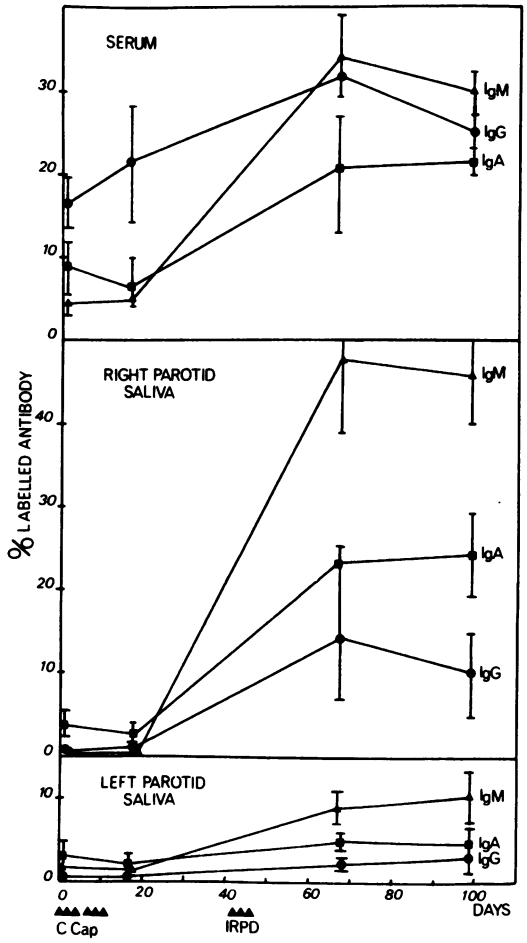

FIG. 3. *Antibody responses of monkeys in group 1 as determined by RIA. Each point is the mean of samples from two monkeys ± standard deviation. Samples of serum (1:100), and saliva (1:4) were assayed in triplicate by using formolized cells of S. mutans as antigen. Monkeys were immunized with killed cells of S. mutans via enterically coated capsules (C.Cap) and instillation of the right parotid duct (IRPD).*

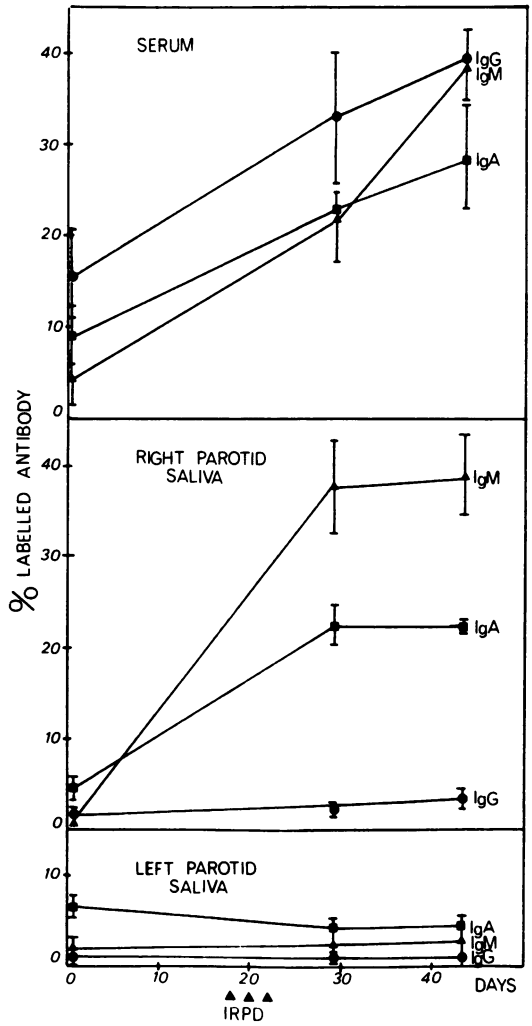

FIG. 4. *Antibody responses of monkeys in group 2. Samples of serum (1:100) and saliva (1:4) were assayed in triplicate by using formolized cells of S. mutans as antigen. Monkeys were immunized by instillation of the right parotid duct (IRPD) with killed cells of S. mutans.*

was greatly reduced by the large degree of local inflammation and swelling after the instillation. This pattern of antibody response to instillation of immunogen into the parotid salivary glands confirmed the results of Emmings et al. (10).

Group 2 monkeys also received immunogens by instillation (three times with 1 mg of whole cells) into the right parotid duct, which induced good IgA and IgM responses detectable in both serum and right parotid saliva (Fig. 4), but no increase of antibody of any class was found in the left parotid saliva. A good serum IgG response, however, was accompanied by a poor IgG response in the right parotid saliva. With the reduced dose of immunogen given to group 2 monkeys, the flow rate of saliva from the right parotid gland was similar to that from the left. In both groups 1 and 2, a high and persistent IgM titer was detected in the right parotid saliva.

Oral immunization with viable *S. mutans* cells. No significant rise in specific antibody to whole cell antigens of *S. mutans* was detected by RIA in the serum and saliva of group 3 monkeys (Table 1). The total dry weight of cells contained in the first six capsules was 280 mg, and the total dry weight of cells in the final 18 capsules was 1,080 mg, each monkey receiving approximately 2×10^9 colony-forming units of

viable *S. mutans*. The number of viable *S. mutans* in the feces fell during the period of oral immunization (Fig. 5); however, the reduction was small when compared with the total number present. The slight reduction indicated that there may have been an intestinal antibody response after oral immunization.

TABLE 1. *RIA titers of group 3 monkeys*[a]

Day	Serum[b]		Whole saliva[c]	
	IgG	IgA	IgG	IgA
0	7 ± 4[d]	10 ± 2	1 ± 0.5	8 ± 3
19	5 ± 3	8 ± 3	1 ± 0.6	7 ± 2
23	4 ± 3	8 ± 2	1 ± 0.4	7 ± 0.5
42	6 ± 4	7 ± 1	1 ± 0.2	5 ± 0.5
54	5 ± 2	6 ± 1	1 ± 0.4	6 ± 2
69	2 ± 1	7 ± 1	1 ± 0.3	6 ± 1
82	6 ± 3	7 ± 2	1 ± 0.2	5 ± 1
85	5 ± 2	6 ± 0.5	1 ± 0.6	5 ± 1

[a] See Fig. 1 for the immunization schedule.
[b] Serum samples were assayed in triplicate at 1:100.
[c] Whole saliva samples were assayed in triplicate at 1:4.
[d] RIA titers are expressed as percentage of labeled antibody ± standard deviation. They were determined by using formolized whole cells of *S. mutans* as antigen and are the mean of samples from two monkeys.

Oral immunization and intestinal antibody. Based on the above results and to investigate further whether oral immunization stimulates intestinal antibody, group 4 and 5 monkeys were orally immunized. Uncoated gelatin capsules were used, since these had previously been reported to be an effective vehicle for oral immunization of humans with *S. mutans* (17). Pre- and post-immunization samples of intestinal contents, serum, and saliva were assayed by RIA using whole cell antigens and supernatant extract adsorbed to polystyrene. No post-immunization increase of either IgA, IgG, or IgM in serum, saliva, or intestinal contents was detected (Table 2); however, pre-immunization samples of serum and saliva of both group 4 and 5 monkeys did have antibody to the above antigens. Absorption of the pre-immunization sera with cells of *S. mutans* greatly reduced the IgG, IgA, and IgM titers to *S. mutans* (Table 3), whereas absorption with *S. azgazardah* showed only a slight or moderate reduction. The slight reduction in titer by *S. azgazardah* could be the result of either nonspecific interaction with IgG reducing the total level of IgG and therefore specific antibody or interaction with specific antibody to antigens common to both *S. mutans*

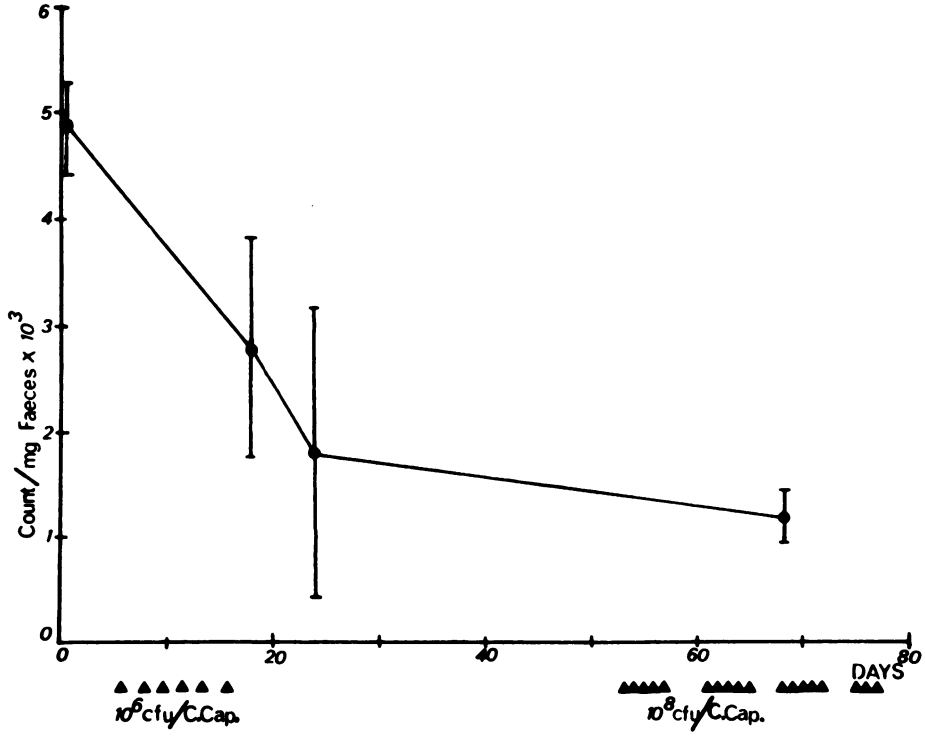

FIG. 5. *Counts of S. mutans in the feces of group 3 monkeys. Each point is the mean of fecal samples from two monkeys ± standard deviation.*

and *S. azgazardah*, for example, lipoteichoic acid. The greater reduction in titer by absorption with *S. mutans* indicated that the preimmune sera did have specific antibodies to *S. mutans*, and since these preexisting antibodies to whole cell antigens may have masked a small specific antibody response after oral immunization, titers to purified cell wall antigens A and B were also determined. These antigens are known to be immunogenic since postinstillation sera and right parotid saliva samples from group 1 monkeys had IgG and IgA titers to both antigens A and B which were 2 to 6 times higher than pre-immunization levels.

Very low levels of antibody to purified antigens A and B were detected in the pre-immunization samples from group 4 and 5 monkeys (Table 2), and no significant increase above these levels occurred after immunization, strongly suggesting that the oral immunization regimen of group 4 was ineffective in stimulating humoral antibody or secretory antibody in either the mouth or the gut.

DISCUSSION

The oral submucosal immunization of monkeys in experiment 14 elicited high titers of circulating antibody, but failed to stimulate a

TABLE 2. *RIA titers of group 4 and 5 monkeys*[a]

Antigen used	Group	Day	Serum[b]			Whole saliva[c]			Intestinal contents[d]		
			IgG	IgA	IgM	IgG	IgA	IgM	IgG	IgA	IgM
Whole cells	4	0	12.0 ± 4.0[e]	7.4 ± 3.0	22.0 ± 2.0	8.0 ± 1.0	6.0 ± 2.0	6.0 ± 2.0	0.4 ± 0.5	1.5 ± 2.0	1.3 ± 1.8
		51	9.0 ± 1.0	8.0 ± 3.0	22.0 ± 2.0	6.0 ± 1.0	5.0 ± 2.0	5.0 ± 2.0	1.0 ± 0.4	3.0 ± 0.7	3.0 ± 1.3
		59	10.0 ± 2.0	8.0 ± 0.1	24.0 ± 2.0	6.0 ± 2.0	3.0 ± 1.0	4.0 ± 2.0	0.9 ± 0.6	3.0 ± 1.5	3.0 ± 3.0
	5	0	13.0 ± 1.0	10.0 ± 6.0	21.0 ± 3.0	8.0 ± 2.0	4.0 ± 0.3	4.0 ± 1.0	0.4 ± 0.2	5.0 ± 6.0	6.0 ± 4.0
		51	13.0 ± 1.0	10.0 ± 4.0	20.0 ± 5.0	6.0 ± 1.0	4.0 ± 2.0	3.0 ± 0.5	1.6 ± 0.2	5.0 ± 4.0	7.0 ± 3.0
		59	11.0 ± 4.0	9.0 ± 4.0	19.0 ± 5.0	5.0 ± 4.0	4.0 ± 1.0	2.0 ± 1.0	1.4 ± 0.8	6.0 ± 5.0	6.0 ± 4.0
Supernatant extract	4	0	7.0 ± 0.4	4.5 ± 0.0	17.0 ± 5.0	0.6 ± 0.3	0.6 ± 0.07	1.0 ± 0.6	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
		51	9.0 ± 2.0	4.4 ± 0.0	14.0 ± 3.0	1.0 ± 0.6	0.25 ± 0.2	1.2 ± 0.2	0.16 ± 0.2	0.1 ± 0.2	0.1 ± 0.2
		59	10.0 ± 0.4	4.4 ± 3.0	18.0 ± 6.0	0.5 ± 0.5	0.2 ± 0.0	1.4 ± 1.6	0.0 ± 0.0	0.0 ± 0.0	0.3 ± 0.06
	5	0	15.0 ± 3.0	11.0 ± 8.0	16.0 ± 7.0	1.1 ± 0.9	1.2 ± 1.4	0.9 ± 0.7	0.1 ± 0.2	0.5 ± 0.5	0.2 ± 0.4
		51	14.0 ± 4.0	11.0 ± 4.0	14.0 ± 9.0	0.5 ± 0.4	0.5 ± 0.1	0.5 ± 0.1	0.2 ± 0.4	0.1 ± 0.05	0.6 ± 0.4
		59	13.0 ± 4.0	10.0 ± 8.0	13.0 ± 7.0	0.25 ± 0.3	0.3 ± 0.1	0.3 ± 0.1	0.05 ± 0.2	0.1 ± 0.0	0.2 ± 0.3
Antigen A	4	0	1.4 ± 0.5	0.4 ± 0.1	3.9 ± 0.2	0.3 ± 0.2	0.3 ± 0.1	0.3 ± 0.2	0.03 ± 0.04	0.05 ± 0.07	0.2 ± 0.2
		51	1.3 ± 0.4	0.3 ± 0.1	4.6 ± 0.4	0.1 ± 0.02	0.1 ± 0.0	0.2 ± 0.1	0.06 ± 0.05	0.0 ± 0.08	0.1 ± 0.1
		59	1.3 ± 0.6	0.6 ± 0.1	4.6 ± 0.4	0.2 ± 0.04	0.2 ± 0.1	0.2 ± 0.1	0.04 ± 0.08	0.06 ± 0.06	0.1 ± 0.05
Antigen B	4	0	0.3 ± 0.0	0.5 ± 0.3	2.4 ± 0.3	0.0 ± 0.0	0.5 ± 0.1	0.3 ± 0.3	0.0 ± 0.0	0.2 ± 0.3	0.3 ± 0.1
		51	0.4 ± 0.1	0.8 ± 0.5	1.9 ± 0.1	0.0 ± 0.0	0.3 ± 0.0	0.2 ± 0.1	0.0 ± 0.0	0.2 ± 0.1	0.2 ± 0.1
		59	0.7 ± 0.3	1.2 ± 0.3	2.8 ± 0.1	0.0 ± 0.0	0.2 ± 0.1	0.2 ± 0.1	0.0 ± 0.0	0.3 ± 0.1	0.3 ± 0.1

[a] See Fig. 1 for the immunization schedules.
[b] Serum samples were assayed in duplicate at 1:200.
[c] Whole saliva samples were assayed in duplicate at 1:16.
[d] Intestinal contents samples were assayed in duplicate at 1:10.
[e] RIA titers are expressed as percentage of labeled antibody ± standard deviation and are the mean of samples from two monkeys.

TABLE 3. *Effect of absorption on the pre-immunization RIA titer of samples of serum from group 4 and 5 monkeys*

Group	Serum treatment	IgG	IgA	IgM
4 (Immunized)	None	13.5 ± 7.8[a]	9.2 ± 7.7	41.0 ± 3.5
	Absorbed *S. mutans*[b]	2.0 ± 2.8 (85)[c]	3.6 ± 1.3 (61)	14.5 ± 10.6 (65)
	Absorbed *S. azgazardah*	9.5 ± 2.1 (30)	7.8 ± 2.1 (15)	31.5 ± 4.9 (23)
5 (control)	None	28.0 ± 16.9	24.9[d]	26.0 ± 15.5
	Absorbed *S. mutans*	4.0 ± 5.6 (85)	13.9 (44)	13.0 ± 9.8 (50)
	Absorbed *S. azgazardah*	20.0 ± 0.7 (28)	22.5 (9.6)	21.5 ± 9.2 (17)

[a] RIA titers are expressed as percentage of labeled antibody ± standard deviation and are the mean of samples from two monkeys.
[b] Sera were absorbed with an equal volume of packed, heat-killed cells of either *S. mutans* or *S. azgazardah* and assayed at 1:200 by using formolized cells of *S. mutans* as antigen.
[c] Figures within parentheses are the percentage of reduction in titer.
[d] Control group IgA titers were obtained using serum from one monkey only.

salivary s-IgA response, which confirms the findings of Emmings et al. (10). In contrast to these findings, good salivary s-IgA responses have been obtained with submucosal immunization of gnotobiotic rats with particulate whole cell vaccines (16) and of hamsters with soluble glucosyltransferase preparations (24).

Challacombe et al. reported that in monkeys immunoglobulins may pass from the serum into the saliva (4), with IgG and IgA being transferred faster than IgM. The detection of IgG, but not IgM, in the salivas of experiment 14 monkeys (Fig. 2) may be the result of the different transfer rates of these immunoglobulins. The low IgA titers detected in saliva were probably a reflection of the relatively low serum IgA titers.

Similar titers reached in the serum and right parotid saliva of group 1 and 2 monkeys after instillation of the right parotid duct indicated that the prior oral immunization of group 1 animals had no discernible effect on the subsequent response to local immunization. The dose of formolized whole cells instilled into group 2 monkeys was only one-tenth that administered to group 1 monkeys, so that the slightly lower antibody response in group 2 monkeys was not unexpected. The high parotid saliva IgG titer of group 1 monkeys may be due to local inflammation, since only a low salivary IgG titer was found in group 2 monkeys when a high serum IgG titer was detected. The induction of tolerance to parenteral antigenic challenge by prior oral immunization of a variety of animals has been widely reported (1, 22, 26), but such unresponsiveness did not appear to have been induced with the orally administered antigen regimen used on group 1 monkeys.

Goldblum et al. (13) reported the induction in colostrum of lactating women of specific s-IgA to an antigenically distinguishable strain of *Escherichia coli* which, when implanted, was able to colonize the intestinal tract. Oral immunization of group 3 monkeys was based on the hypothesis that by introducing viable *S. mutans* into the intestinal tract of the monkeys in enterically coated capsules, the resulting transitory increase in the level of *S. mutans* may have provided a greater stimulation of the gut-associated lymphoid tissue than if a similar dose of killed organisms were given. It is apparent (Table 1) that no increase in specific antibody occurred in any of the classes assayed in either serum or saliva; however, a slight fall in the number of viable *S. mutans* in feces was observed (Fig. 5). The interpretation of this decrease is complicated by subsequent data (not shown) which demonstrated similar levels and fluctuations of *S. mutans* in the feces of nonimmunized control monkeys. Therefore, it is not possible to attribute this decrease conclusively to intestinal antibody produced in response to oral immunization.

The possibility of an intestinal antibody response in group 3, implied by the data in Fig. 5, prompted investigation of the intestinal antibody responses in addition to the humoral and salivary antibody responses to oral immunization. Neither intestinal, humoral, nor salivary antibody responses were detectable after oral immunization of group 4 monkeys (Table 2), even when purified cell wall antigens (proteins A and B) were used as antigens in the RIA. Antigen B has been found to be associated with other streptococci, but antigen A has only been described in cultures of *S. mutans* (20).

It has been suggested that the major function of s-IgA is to limit the adsorption of antigens to, and transport across, mucosal surfaces. Both in vivo (29) and in vitro (27) experiments have shown that antigen-specific antibody can reduce or inhibit antigen absorption and uptake. The serum absorption experiments described in this study (Table 3) demonstrate that adult monkeys have circulating antibodies to *S. mutans*. The absence of either a secretory or humoral immune response to oral immunization may be the result of circulating antibody blocking absorption and therefore effectively exclude antigen and prevent stimulation of gut-associated lymphoid tissue. Alternatively, perhaps too little antigenic challenge occurred because either the level or frequency of antigen administration (or both) was too low. A comparison of the results reported here with those of Mestecky and co-workers (17), who reported obtaining an s-IgA response by orally immunizing human volunteers with *S. mutans*, shows that similar antigenic doses were given. A 50-mg capsule given 10 times to a 4- to 6-kg monkey is comparable to a 100-mg capsule given 14 times to an adult human. A possible difference between the two studies is that the human volunteers received a serotype of *S. mutans* to which they had no antibody, as predetermined by whole cell agglutinations. The pre-immunization antibody in the monkeys may have acted to exclude the orally administered antigen. Further comparative analyses of the human experiments reported previously with the monkey studies reported here do not reveal any differences that could account for the conflicting results.

It has been reported that the ability of intestinal organisms to elicit an immune response is related to their adhesiveness to the mucosa (14), and oral immunization experiments with intestinal pathogens which adhere well to mucosal

surfaces demonstrated that high doses of viable organisms were required to achieve effective oral immunization (9). *S. mutans* does not adhere well to mucosal surfaces (11), as reflected by its distribution in the mouth. Thus, a large antigenic load may be required to elicit an antibody response to oral immunization with *S. mutans*.

The reports of consistently detecting a salivary IgA response in rodents to both the submucosal (16, 23) and oral (18, 21) routes of immunization are in marked contrast to the results presented here. The experiments described in this paper have not demonstrated an s-IgA response in either the saliva (groups 1 to 5) or the intestinal contents (group 4) of monkeys orally immunized with *S. mutans*. Implantation of *E. coli* into the gut flora of lactating women was reported as being unsuccessful in eliciting a salivary s-IgA response (13) even when specific s-IgA could be detected in colostrum by an enzyme-linked immunosorbent assay. These conflicting results may indicate differences between the rodent and primate or human secretory immune systems.

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