

## SYSTEMIC TOLERANCE AND SECRETORY IMMUNITY AFTER ORAL IMMUNIZATION\*

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The phenomenon of immune tolerance after ingestion of antigen was reported as early as 1911 when Wells demonstrated that guinea pigs fed ovalbumin lost the ability to develop systemic anaphylaxis (1). These observations were expanded in the classic experiments of Chase in 1946 (2), demonstrating inhibition of delayed hypersensitivity to dinitrochlorobenzene by prior feeding. Loss of systemic reactivity after oral feeding has now been demonstrated in several different models (3–10). Mice or rats fed sheep erythrocytes show a diminished ability to form plaque-forming cells in the spleen after intraperitoneal challenge, which was ascribed to serum factors in some studies (5, 6) and to suppressor cells in others (7). In addition to suppression of delayed hypersensitivity (8), antigen-induced DNA synthesis of murine lymph node cells after subcutaneous challenge (9) can also be suppressed by prior intragastric administration of soluble protein antigens.

Thus, in various animal models and with different antigens and assays, a general phenomenon has been observed, that deposition of antigen in the gut may, under certain circumstances, lead to inhibition of an immune response to a subsequent parenteral injection of the specific antigen. There is, however, disagreement as to the mechanisms involved and it is possible that different mechanisms are operative in different systems. It also appears that gut-associated lymphoid tissue (GALT),<sup>1</sup> particularly Peyer's patches (PP) and mesenteric lymph nodes (MLN), may play a central role in the induction of systemic suppression by the oral route (7, 10).

PP cells also appear to have a major role in the distribution of antigen-sensitive cells to the secretory immune system (11–13). Deposition of antigen in the gut has been shown to lead to the production of IgA antibodies in secretions at sites distant from the gut such as colostrum, lacrimal, and salivary secretions in man (14), and salivary secretions in the rhesus monkey (15) and rats (16). It appears, therefore, that the secretory immune system can be stimulated centrally, and that precursors of IgA-producing cells migrate from the GALT to several secretory sites in addition to the lamina propria of the gut.

In view of these observations, it seemed possible that intragastric administration of soluble or particulate antigen might lead to concurrent induction of secretory anti-

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<sup>1</sup> Abbreviations used in this paper: B cells, B-enriched preparation(s); Con-A, concanavalin A; FCS, fetal calf serum; GALT, gut-associated lymphoid tissue; IG, intragastric(ally); KLH, keyhole limpet hemocyanin; LNC, lymph node cell(s); LPS, lipopolysaccharide(s); MLN, mesenteric lymph node(s); OVA, ovalbumin; PBS, phosphate-buffered saline; SRBC, sheep erythrocytes; PEC, peritoneal exudate cells; PP, Peyer's patches; PPD, purified protein derivative; SI, stimulation index.

bodies and systemic suppression. The objectives of this investigation were to examine this possibility using *Streptococcus mutans* bacterial cells as an example of a particulate antigen, and to compare the results with those obtained using the soluble antigens ovalbumin and keyhole limpet hemocyanin. The results indicate that secretory immunity and systemic tolerance may appear concomitantly after oral immunization with these antigens, and are associated with the development of antigen-specific suppressor cells.

### Materials and Methods

*Animals.* 8- to 12-wk-old CBA/J mice were obtained from The Jackson Laboratory, Bar Harbor, Maine.

*Antigens.* *S. mutans* Ingbritt (serotype c) was kindly supplied by Dr. C. Schachtele, University of Minneapolis (Minneapolis, Minn.), and was grown for 48 h in Todd-Hewitt broth at 37°C, harvested by centrifugation at 6,000 g, and formalized in phosphate-buffered formalin for 24 h. Cell walls were prepared by ultrasonication on a Branson W200P ultrasonicator (Heat Systems-Ultrasonics, Inc., Plainview, N. Y.) The cells from 1 liter of broth were placed in 10 ml of saline and sonicated for 20 min. Whole cells were removed by centrifugation at 4,000 rpm for 15 min, or longer if any Gram-positive material remained in the supernate. Cell walls were recovered by centrifugation at 10,000 rpm for 30 min and washed three times in saline. The preparation was lyophilized and resuspended in saline at 5 mg/ml.

Ovalbumin (OVA) was obtained from Sigma Chemical Company, St. Louis, Mo., and keyhole limpet hemocyanin (KLH) was obtained from Pacific Biomarine Supply Co., Venice, Calif.

*Preparation of Cell Suspensions.* Cell suspensions from lymph nodes, spleen, or PP were prepared as described (17). For cell transfer experiments, cells were suspended at  $2 \times 10^8$ /ml in RPMI-1640 medium (Grand Island Biological Co., Grand Island, N. Y.), and supplemented with 100 µg/ml streptomycin and 100 U/ml penicillin. Resident peritoneal exudate cells were obtained by flushing the peritoneal cavity with 5-6 ml of cold Earle's balanced salt solution.

*Proliferative Assay.* Animals were injected subcutaneously at the base of the tail with 100 µl of antigen emulsified in H37 Ra adjuvant (Difco Laboratories, Detroit, Mich.) as previously described (18). 8 d later a suspension of cells from the inguinal and paraaortic lymph nodes was prepared in RPMI 1640 that contained 100 µg/ml streptomycin, 100 U/ml penicillin, 25 mM HEPES buffer, 2 mM glutamine (Gibco Laboratories), and supplemented with 2.5% vol:vol pooled AB positive heat-inactivated human serum.  $4 \times 10^5$  cells in 200 µl media were incubated for 4 d with various concentrations of *S. mutans* cells or cell walls, purified protein derivative (PPD) (Connaught Laboratories, Toronto, Canada) 50 µg/ml, OVA 500 µg/ml, mitogens lipopolysaccharide (LPS) (Difco Laboratories) 40 µg/ml, and concanavalin A (Con A) (ICN Nutritional Biochemicals, Cleveland, Ohio) 4 µg/ml final concentration. 1 µCi of tritiated thymidine (New England Nuclear, Boston, Mass.) was added per well for the final 18 h of incubation. In comparative experiments, results were expressed as stimulation indices. All cultures were performed in triplicate and the mean counts per minute  $\pm$  SE recorded.

*Samples.* Serum was collected from clotted blood obtained from the tail. Saliva was obtained by injecting 1 mg of pilocarpine intraperitoneally and allowing the animals to salivate into small glass test tubes. Saliva was centrifuged at 4,000 rpm to remove debris. Samples were heat-inactivated at 56°C for 30 min and absorbed with 0.25 vol packed sheep erythrocytes (SRBC) for 1 h at room temperature. Saliva samples were made isotonic before absorption by the addition of 2 mg/ml 0.14 M NaCl.

*Passive Hemagglutination Test.* Cell walls of *S. mutans* (2.5 mg/ml) or OVA (2 µg/ml) were coated onto SRBC by the chromic chloride method. Briefly, 0.5 ml of antigen was mixed with 0.5 ml of a 50% solution of SRBC in saline, and 0.5 ml of a fresh solution of chromic chloride (1 mg/ml) added dropwise with continuous stirring. After 10 min at room temperature, the sensitized cells were resuspended at 1% in phosphate-buffered saline (PBS), pH 7.2.

Duplicate doubling dilutions (25 µl) of murine saliva or serum samples were made, and 25 µl of sensitized cells added to one series, and unsensitized cells to the other as a control. Cells were allowed to settle for 18 h at 4°C before reading.

To examine the class specificity of salivary antibodies, samples were absorbed with an equal volume of a 1:5 dilution of anti-murine IgG, IgA, or IgM previously incubated with whole cells of *S. mutans* or erythrocytes coated with OVA. After incubation for 1 h at 37°C and 18 h at 4°C, the samples were centrifuged at 15,000 rpm for 30 min, and the antibody activity compared with samples that had been treated with saline.

**Intragastric Immunization.** Animals were anesthetized with ether and a 21-gauge needle inserted into the stomach. A hollow steel ball of 2 mm diameter had been soldered onto the blunted end. In most experiments mice were given  $10^9$  whole cells of *S. mutans*, or to 20 mg of OVA or KLH in 0.5 ml of PBS. Control animals were given 0.5 ml of PBS alone.

**Nylon Wool Treatment.** Enrichment of T cell population was performed by passage of whole cells through nylon wool according to a modification of the method of Julius et al. (19). Briefly, 1.2 g of type 200 nylon wool (Fenwal Laboratories, Inc., Morton Grove, Ill.) was loosely packed into a 10-ml syringe and autoclaved. Columns were presoaked with warm (37°C) RPMI-1640 that contained 5% fetal calf serum (FCS) for 1 h, and then  $10^8$  cells applied and allowed to flow slowly into the matrix at 37°C. Every 15 min the cells were washed further into the matrix with 2.5 ml warm medium. This was repeated a total of four times. At the end of the incubation, the nonadherent cells were eluted with 18–20 ml of medium. The adherent cells were obtained by washing of the nylon wool in a petri dish with cold medium. Recovery of viable nonadherent cells was ~30% for spleen, 40% for MLN cells, and 25% for PP. When nylon wool nonadherent cells were assayed by the fluorescent antibody test for light chain-bearing cells, those from MLN contained <4% anti-kappa-staining cells, and those from PP and spleens ~6%.

**Transfer Experiments.** Single cell suspensions of spleen, PP, or MLN were prepared as described from animals immunized intragastrically with *S. mutans*, OVA, or PBS.  $40 \times 10^6$  cells were given intravenously into syngeneic recipients; 1 h later these animals were challenged subcutaneously in the base of the tail with 100  $\mu$ g of OVA, or  $5 \times 10^7$  *S. mutans* cells in H37 Ra adjuvant.

## Results

**Proliferative Assay with *S. Mutans*.** An in vitro dose-response curve was obtained with whole cells or cell wall preparations from *S. mutans* in H37 Ra adjuvant in CBA/J mice immunized 8 d previously. The optimal immunizing dose was found to be  $5 \times 10^7$  cells, and the optimal in vitro concentrations were  $2 \times 10^8$  for whole cells and 2.5 mg/ml for cell walls (Fig. 1). Cell walls consistently elicited a greater response than

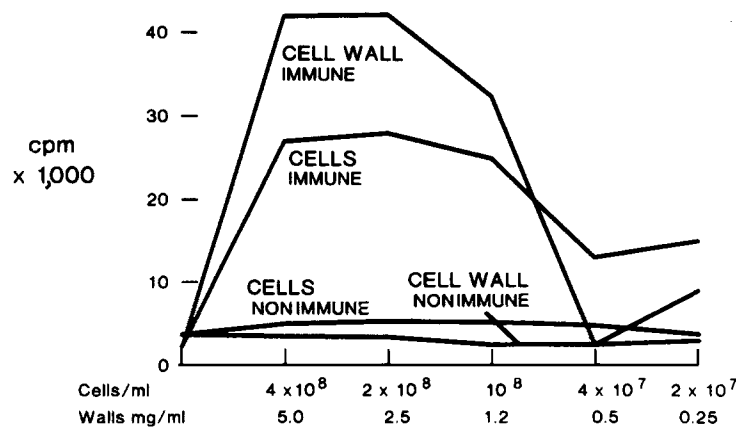


FIG. 1. Proliferative assay with *S. mutans* cells and walls. In vitro dose-response curve. Mean counts per minute of triplicate cultures in 4-d assays of LNC from immunized or control (nonimmunized) mice. Animals immunized 8 d previously with  $5 \times 10^8$  *S. mutans* in H37 Ra complete adjuvant. Walls = *S. mutans* cell walls prepared by ultrasonication. Cells = intact formalinized cells of *S. mutans*.

whole cells. The preparations did not appear to have significant mitogenic activity because the counts obtained with control cells from unimmunized animals were not significantly greater than background.

Inguinal and paraaortic lymph node cells (LNC) were removed from animals immunized subcutaneously with *S. mutans*, and T and B cell preparations obtained on nylon columns as described in Materials and Methods. The T-enriched preparation (hereafter called T cells) contained 4% anti-kappa-staining cells and 95% anti-Thy-1.2 positive cells, whereas the B-enriched preparation (B cells) contained 2% anti-Thy-1.2 positive cells and ~65% positive anti-kappa cells. T cells showed a significant response to both whole cells and cell walls (Table I). The responses were increased to the levels found in unfractionated cells by the addition of 5% resident peritoneal exudate cells (PEC) that had been irradiated (3,300 rad). Treatment of sensitized LNC with anti-Thy-1.2 + complement eliminated proliferation. B cells did not show a response to whole *S. mutans* when supplemented with PEC, and responded to cell wall preparations only if 10% or greater PEC were added, and then the response was <20% of that given by whole LNC. Thus, it appears that the assay is T dependent, and the proliferating cell is primarily a T cell.

*Effect of Intra-gastric (IG) Immunization with OVA.* A single intra-gastric dose of OVA was given to 8-wk-old CBA/J mice, followed 7 d later by subcutaneous challenge. As little as 1 mg of OVA given intra-gastrically led to >80% suppression of the proliferative response in draining lymph nodes, compared with animals given PBS IG (Fig. 2). Saliva taken from mice 14 d after OVA IG (which had not been challenged subcutaneously) showed antibody titers of up to 1:32. The mean titer in animals given 10 mg of OVA IG was  $3.5 \pm 0.5$ , whereas antibodies were not consistently detected in animals given 1 mg or less of OVA IG. Results similar to those shown in Fig. 2 were obtained in two additional experiments, each employing five experimental and five control mice. No serum antibodies were detected in any group after intra-gastric immunization alone, nor when intra-gastric immunization was followed by subcutaneous immunization. These observations indicated that intra-gastric immunization,

TABLE I  
*Proliferative Response in LNC to S. mutans*

LNC	Medium	Cell wall	Whole cells
Whole LNC	2,245 ± 1,019	14,901 ± 1,225	5,609 ± 271
T cells	377 ± 38	6,159 ± 617	2,866 ± 47
+ 1% PEC	400 ± 95	7,558 ± 393	3,877 ± 707
+ 2% PEC	215 ± 24	13,172 ± 791	3,803 ± 760
+ 5% PEC	272 ± 62	13,023 ± 2,160	6,840 ± 76
+ 10% PEC	340 ± 28	13,450 ± 560	6,738 ± 601
B cells	623 ± 63	523 ± 11	485 ± 62
+ 5% PEC	561 ± 224	412 ± 194	ND
+ 10% PEC	1,215 ± 112	2,426 ± 32	886 ± 121
+ 20% PEC	1,132 ± 59	2,590 ± 509	628 ± 93
PEC 20%	118 ± 12	286 ± 57	202 ± 10

T cells = 4% anti-kappa were >95% anti-Thy-1.2 positive. B cells = >65% anti-kappa were 2% anti-Thy-1.2 positive.

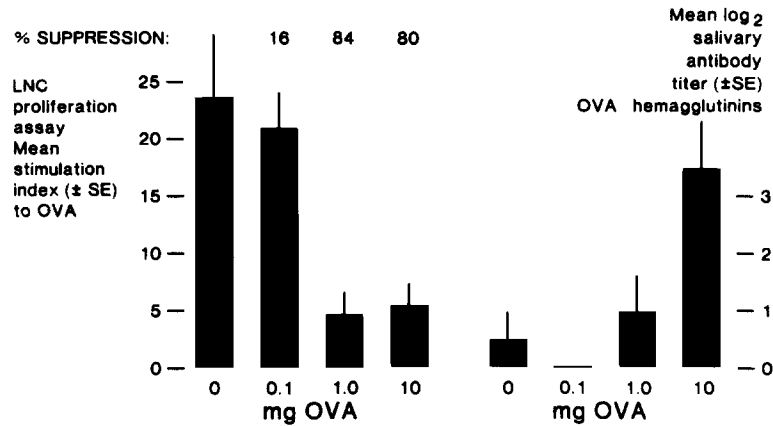


FIG. 2. Salivary antibodies and systemic suppression after intragastric administration of OVA in CBA/J mice. Results show mean  $\pm$  SE of four to six mice/group. Animals were given OVA or PBS IG, and 7 d later challenged subcutaneously with 100  $\mu$ g OVA in H37 Ra complete adjuvant. 8 d later (day 15), samples of serum and saliva were taken and the proliferative assay performed. Salivary antibody titers were assayed by hemagglutination as described in Materials and Methods. No serum antibodies were detected.

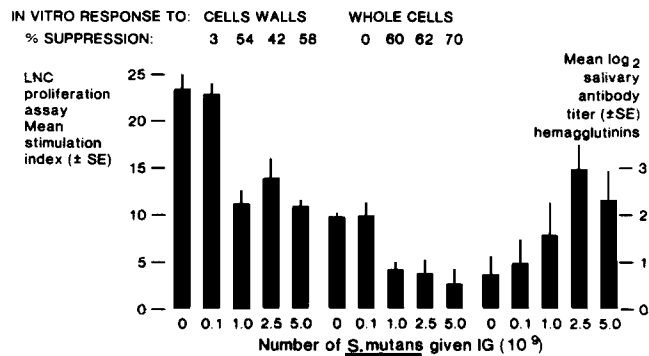


FIG. 3. Salivary antibodies and systemic suppression after intragastric administration of *S. mutans* in CBA/J mice. Results expressed as mean  $\pm$  SE of three animals/group. Mice given *S. mutans* or PBS IG and 7 d later challenged subcutaneously with  $5 \times 10^8$  *S. mutans* in H37 Ra complete adjuvant. 8 d later (day 15) samples of serum and saliva obtained and the proliferative assay performed. Antibody titers assayed by hemagglutination. No serum antibodies detectable.

with defined concentrations of antigen, gives rise to salivary antibodies and systemic suppression concurrently.

*Effect of Intragastric Immunization with S. Mutans.* Mice given  $10^8$  *S. mutans* IG did not show significant suppression of the proliferative response to *S. mutans* when challenged 7 d later. However, a dose of  $10^9$  or greater induced suppression as revealed by the proliferative assay against cell wall antigen or whole cells (Fig. 3). The suppression was not as great as that found with OVA. Suppression was somewhat greater for whole cells (maximum 70%) than for cell walls (maximum 58%). This was just the reverse of their potency in stimulating proliferation in the regional lymph node when given subcutaneously in the tail (compare Fig. 3 with Fig. 1). Significant salivary antibody titers were detectable in animals given 2.5 or  $5 \times 10^9$  *S. mutans* IG with mean titers of  $3.2 \pm 0.4$  and  $2.5 \pm 0.5$ , respectively. No serum antibodies were detected after

intra-gastric immunization nor after subcutaneous challenge in the LNC proliferative assay. Two additional experiments showed very similar results.

*Kinetics of Suppression of Proliferative Response after Intra-gastric Immunization.* Mice challenged subcutaneously with OVA 2 d after intra-gastric administration of 20 mg OVA showed suppression of the LNC proliferative response by ~85%, compared with mice given PBS (Fig. 4). Maximum suppression was found when mice were challenged subcutaneously 4–7 d after IG administration, but suppression of 60% was still evident when animals were challenged 60 d later.

With *S. mutans*, the suppression was not as profound as with OVA, and the maximum suppression was ~70% at 7 d after intra-gastric administration (Fig. 5). After day 14, suppression rapidly diminished and by day 30 was no longer evident.

*Specificity of Suppression.* Induction of suppression appeared to be specific because mice that had received 20 mg OVA or 15 mg KLH were challenged 7 d later; only those animals challenged with the homologous antigen showed significant suppression (Fig. 6). Mice primed with OVA showed suppression when challenged with OVA but not with KLH, and vice versa. Although the induction of suppression was specific, a

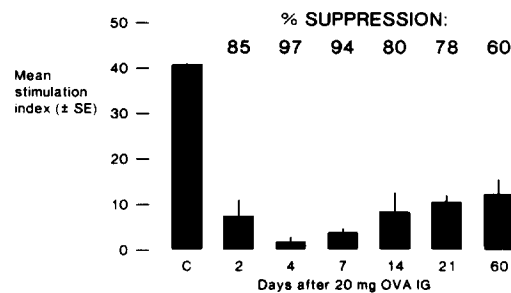


FIG. 4. Kinetics of suppression of the proliferative response after intra-gastric administration of OVA. CBA/J mice challenged subcutaneously with 100  $\mu$ g OVA in H37 Ra complete adjuvant 2–60 d after single intra-gastric administration of 20 mg OVA. Results indicate mean  $\pm$  SE of in vitro proliferative response (measured as SI) in three to five mice/group.

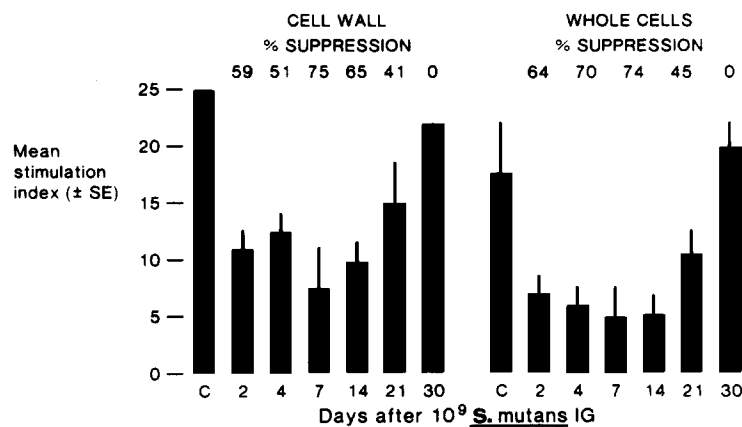


FIG. 5. Kinetics of suppression of the proliferative response after intra-gastric administration of *S. mutans*. CBA/J mice challenged subcutaneously with  $5 \times 10^8$  *S. mutans* in H37 Ra complete adjuvant 2–30 d after single intra-gastric administration of  $10^9$  *S. mutans*. Results indicate mean  $\pm$  SE of in vitro proliferative response (measured as SI) in four to five mice/group.

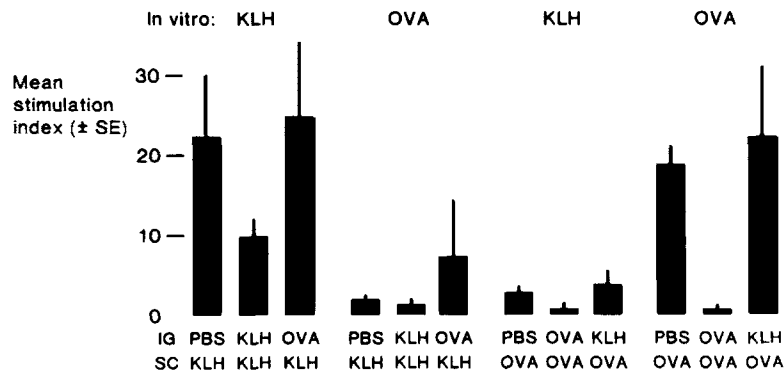


FIG. 6. Specificity of suppression of proliferative response by prior intragastric administration of antigen. Mice received either PBS, 15 mg KLH, or 20 mg OVA by intragastric intubation. Animals were immunized subcutaneously 7 d later with 100  $\mu$ g of OVA or KLH in H37 Ra complete adjuvant. Proliferative responses were assayed 8 d later. Mean  $\pm$  SE of three to four mice/group.

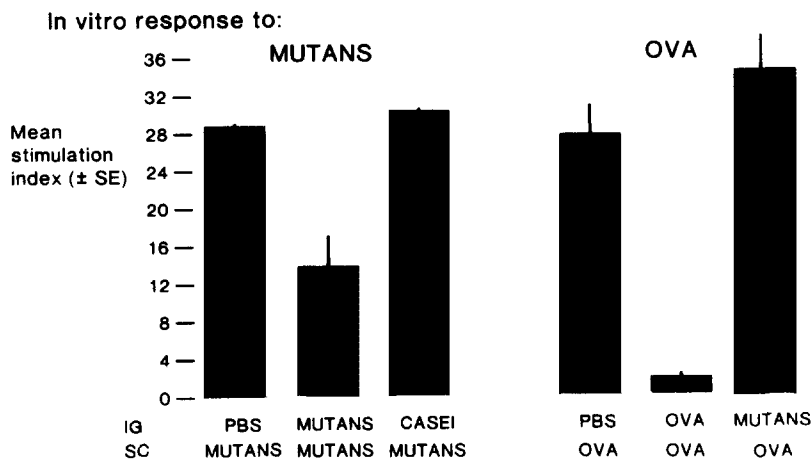


FIG. 7. Specificity of suppression induced by intragastric immunization with *S. mutans*. Mice received  $10^9$  *S. mutans* or *Lactobacillus casei*, or 20 mg OVA IG, and were challenged subcutaneously 7 d later with  $5 \times 10^8$  *S. mutans* or 100  $\mu$ g OVA in H37 Ra complete adjuvant. Mean  $\pm$  SE of SI in groups of four to five mice.

nonspecific component was also noted. When suppression was induced with the specific antigen, not only was the proliferative response to that antigen suppressed, but also the response to PPD and LPS ( $P < 0.05$ ). Some suppression of the mean Con A response was noted, but this was not statistically significant.

Suppression induced by *S. mutans* was also found to be specific. Intragastric administration of *S. mutans* did not affect the response to a subcutaneous challenge with OVA, but suppressed the response to *S. mutans* (Fig. 7). Conversely, intragastric administration of OVA led to suppression of the response to OVA but not to *S. mutans*. IG administration of  $10^9$  *Lactobacillus casei* (ATCC 393) did not affect the proliferative response to *S. mutans*.

*Class of Salivary Antibodies.* Absorption of saliva samples with anti-IgA consistently reduced the hemagglutinating antibody titer to either OVA or *S. mutans* by two log<sub>2</sub>

steps or greater. Absorption with anti-IgG had no effect, whereas with anti-IgM a slight (one log<sub>2</sub>) but consistent reduction in the antibody titer was found (Table II).

*Transfer of Suppression.* Transfer of  $40 \times 10^6$  PP cells from mice given 20 mg OVA IG to syngeneic recipients resulted in slight suppression of the proliferative response to OVA, but this did not prove to be statistically significant (Fig. 8). However, transfer of MLN cells resulted in ~70% suppression of the OVA response in comparison with animals who had been given cells from PBS control animals ( $P < 0.03$ ). The degree of suppression was similar with cells taken 2 or 4 d after intragastric administration of OVA. Suppression was antigen-specific because the response to KLH, PPD,

TABLE II  
Class Specificity of Salivary Antibodies Induced by IG Immunization with OVA or *S. mutans*

	Log <sub>2</sub> hemagglutination titer after absorption with:			
	Saline	Anti-IgG	Anti-IgA	Anti-IgM
OVA	2	2	0	1
	3	3	0	2
	3	3	1	1
	2	2	0	2
	$2.5 \pm 0.3^*$	$2.5 \pm 0.3$	$0.2 \pm 0.3$	$1.5 \pm 0.3$
<i>S. mutans</i>	3	3	1	2
	3	2	1	2
	3	3	0	1
	2	2	0	2
	$2.8 \pm 0.3$	$2.5 \pm 0.3$	$0.5 \pm 0.3$	$1.8 \pm 0.3$

Samples incubated with equal volumes of anti-serum or saline 1 h at 37°C; centrifuged at 15,000 rpm for 30 min. Antisera absorbed previously with *S. mutans* or albumin-coated SRBC; diluted 1:5 before use. Mice immunized with 20 mg OVA or  $2.5 \times 10^9$  *S. mutans* IG.

\*  $\bar{x} \pm SE$ .

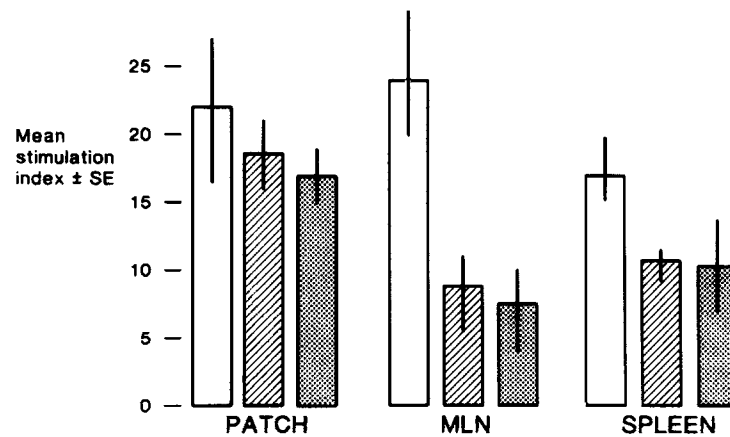


FIG. 8. Proliferative response to OVA after adoptive transfer of cells from CBA/J mice given OVA or PBS IG.  $40 \times 10^6$  cells transferred to syngeneic mice from animals given PBS (open bar) or 20 mg OVA 2 d (hatched bar) or 4 d (stippled bar) previously. Mice were challenged with 100  $\mu$ g OVA in H37 Ra complete adjuvant subcutaneously 1 h after transfer of cells (IV). Mean  $\pm$  SE of SI of LNC proliferative assay of four to five animals/group. Significant suppression ( $P < 0.05$ ) was noted on transfer of MLN cells from day-2 and -4 animals. Transfer of PP cells or spleen cells did not produce statistically significant suppression.



and *S. mutans* was not affected. Transfer of spleen cells led to less suppression than with MLN cells, and this was not statistically significant ( $P = 0.06$  at 2 d).

Transfer of cells from mice given  $10^9$  *S. mutans* IG was performed at 1 and 3 d. Transfer of PP or spleen cells did not lead to significant suppression. With MLN cells, suppression was found with cells taken at 3 d ( $P < 0.02$ ). Feeding *S. mutans* did not alter the proliferative response to PPD or OVA.

An attempt was also made to suppress the in vitro proliferative response of sensitized LNC. MLN cells were taken 4 d after IG immunization with OVA because cells taken at this time had been shown to transfer suppression to syngeneic recipients. Cells were incubated at various ratios (1:3, 1:1, and 3:1) with sensitized LNC taken from animals immunized 8 d previously subcutaneously in the tail. No significant suppression was detected under these conditions.

*Sensitization of PP by Antigen Given IG.* During experiments that showed the induction of secretory antibodies after intragastric immunization, it was noted that mice given OVA IG showed no proliferative response when PP taken at 2 and 6 d after intragastric administration were challenged in vitro with OVA (Table III). At 13 d after feeding, no proliferative response was found with 4 or 10 mg of OVA, whereas 20 mg elicited a slight but questionably significant response (stimulation index [SI] = 2.2). In contrast, PP and MLN responded strongly to the intragastric immunization with *S. mutans* at 6 and 13 d, and a slight response (SI = 2.5) was noted in MLN at 2 d (Table III). A maximum response in PP (media:  $3,826 \pm 1,610$  cpm; cell wall:  $59,941 \pm 10,704$  cpm; SI: 15.7) was obtained when  $1.5 \times 10^9$  organisms were given IG 13 d previously. However, significant stimulation was also obtained with concentrations as low as  $3 \times 10^8$  (SI = 9.9). OVA and *S. mutans* are both excellent antigens when administered subcutaneously, and in general induce approximately equivalent degrees of proliferation in regional LNC (as measured by counts per minute and SI). Thus, the differences noted here between these antigens on the proliferative response of PP after oral immunization stands in striking contrast to their effect on regional lymph nodes when these same antigens are given subcutaneously with adjuvants.

### Discussion

The phenomenon of systemic hyporesponsiveness after oral immunization appears to be well established and common to various species of experimental animals as well

TABLE III  
*Proliferative Response after IG Immunization with OVA and S. mutans*

	SI*						
	2 d			6 d			13 d
	PP	MLN	Spleen	PP	MLN	Spleen	PP
OVA	1.0	1.0	1.1	1.0	1.0	1.1	2.2
<i>S. mutans</i>	1.0	2.5	1.0	11.1	4.4	2.1	15.7

In vitro proliferation response of PP, MLN, and spleen at 2, 6, and 13 d after IG administration of 20 mg OVA and  $1.5 \times 10^9$  *S. mutans*. In vitro concentration: OVA 500  $\mu\text{g}/\text{ml}$ , *S. mutans* cell wall 250  $\mu\text{g}/\text{ml}$ .

\* SI =  $\frac{\text{cpm in presence of antigen}}{\text{cpm in medium}}$ .

as man (20, 21). Oral tolerance has been described to T dependent and not T independent antigens, and only the T cell appears to be tolerized (4). Oral immunization with OVA has been reported to suppress various systemic reactions including passive cutaneous anaphylaxis (3), T cell proliferation (9), and plaque-forming cells (9). A recent editorial (22) reviews the phenomenon of oral tolerance and its implications. Recently, Swarbrick et al. (23) showed that prior feeding of antigen may lead not only to suppression of the induction of serum antibodies, but also to a reduction in the subsequent absorption of antigen, presumably a function of local immunity as previously suggested by Walker et al. (24). The current findings extend this concept and suggest that the secretory system can be stimulated at the same time that systemic tolerance and suppressor cells are induced.

There is an apparent paradox in that PP have been postulated to play a central role both in the induction of suppressor cells, and in the production of antigen-sensitive cells. Thus, in other studies, suppression after intragastric immunization has been transferred by PP cells (7, 8, 10), and yet PP are also believed to be the source of the antigen-sensitive cells found in the secretory system (11, 12). The relative deficiency of antigen-presenting cells in PP (25), at least for certain antigens, could theoretically contribute to the ease with which suppression can be induced by this route because the absence of macrophages appears to favor the induction of suppressor cells (26-28), and responses to both OVA (18) and *S. mutans* (Table I) appear to be macrophage-dependent. Recently, IgA-specific helper T cells have been identified in PP (29) and, therefore, it appears that the PP can be a source of both suppressor and helper cells. It is possible that there is a relative predominance of T suppressors for IgG and IgM (as well as for delayed reactions) in PP, whereas suppressor cells specific for IgA are deficient compared with IgA helper cells. In our studies, suppression was not adoptively transferred with PP cells, but was transferred with MLN cells. This may indicate that in the system used, cells from the patches require a period of maturation in the mesenteric nodes to become fully effective in suppression. Alternatively, and perhaps more likely, if suppression is mediated by T cells, as other reports suggest, then the relative proportions of T cells in PP (37%) to mesenteric nodes (80%) (17) could explain the ease of transfer of suppression by mesenteric nodes in comparison with PP. Further studies are now in progress that attempt to define the sequence of appearance of suppressor cells of various isotypes in different tissues, the cell type(s) involved, and whether suppressor cells and soluble factors are responsible for the initiation and/or maintenance of oral tolerance.

Whereas both particulate and soluble antigens were shown to induce salivary IgA antibodies, the profound suppression achieved with OVA was in contrast to the weak suppression found with *S. mutans*. There are several possible explanations. The proliferative response to OVA is entirely a T cell response, whereas the response to *S. mutans*, although predominantly a T cell response (Table I), could contain a B cell component. It has been suggested that the response to T independent antigens is not suppressed after oral immunization, in contrast to T dependent antigens (4). The observation that intragastric immunization with *S. mutans*, but not with OVA, induced a proliferative response in PP (Table III) further suggests that the immunological response to certain antigens, perhaps more particulate and complex antigens, may be different from that induced by others such as soluble readily degradable protein antigens. Antigens appear to be introduced into PP via the specialized epithelial cells

overlying the patch (30). Although it is unclear how long soluble antigens may remain in the patch, particulate antigens may possibly persist for a considerable time because carbon particles have been found in subepithelial macrophages for as long as 2 mo after ingestion (31). Thus, differences in the distribution and macrophage uptake or presentation between particulate and soluble antigens in the patch may account for differences in immunological responses.

In this investigation, adoptive transfer experiments indicated that suppression of the proliferative response could be mediated by suppressor cells before *in vivo* sensitization, but, in common with other studies, (9) *in vitro* proliferation was not suppressed. This suggests that suppression may occur in the induction phase of the immune response, perhaps via an effect on the T helper cells (4, 32). The suppressor cells induced after intragastric immunization with either OVA or *S. mutans* appeared to be antigen-specific in that systemic challenge with the homologous and not a heterologous antigen was suppressed. Interestingly, the response to PPD was also suppressed, but only in those animals challenged with the same antigen that had been given IG. It seems likely, therefore, that after specific induction, the proliferation of suppressor cells may include some nonspecific suppressor cells (33) or factors (34, 35) that will suppress the response to a second antigen given concurrently. Although suppressor T cells (4, 9, 10) and suppressor B cells (8) have been demonstrated after oral feeding, serum factors may also play a role (5, 6). Serum suppressive factors were not examined in this study, and it remains possible that suppressor cells and serum factors could be produced simultaneously. It should also be stressed that the presence of suppressor cells was examined in this study only early in the response (2-4 d), and further studies are required to determine if the long-lasting (>60 d) tolerance after intragastric immunization results from active suppression or some alternative mechanism. There is evidence that in certain circumstances the presence of suppressor cells and tolerance may not be related as cause and effect. In tolerance induced by the systemic injection of human gammaglobulin, tolerance may occur in the apparent absence of suppressor cells; even when suppressor cells are present, they may not be entirely responsible for the maintenance of tolerance (36).

Although systemic suppression has been demonstrated after feeding or intragastric administration of a variety of soluble antigens, and in this study with whole bacterial cells, it is not necessarily a general phenomenon, and some antigens given orally induce a systemic immune response. Whether tolerance or immunity follows oral administration of an antigen depends not only on its chemical nature and dose but on whether the animal has had prior contact with the antigen (4, 37). *S. mutans* is not a normal component of the murine flora; bacteria, which are part of the intestinal flora, may induce different responses.

The demonstration that intragastric immunization may lead to simultaneous induction of secretory antibodies and systemic suppression raises intriguing speculation as to the relationship between the two systems and the biological relevance of these observations. The occurrence of a secretory immune response after mucosal contact with antigen would appear to be a useful mechanism for the exclusion of potentially harmful substances from the body (20), whereas the induction of systemic tolerance could act as a secondary defense to ensure that immunologically damaging reactions to antigens that escape exclusion mechanisms do not occur. This may be a

particularly appropriate response to various allergens (3, 10) and to antigens that cross-react with self and, therefore, might induce autoimmunity.

### Summary

Diminished systemic immune reaction after ingestion of antigen has been reported in several animal models. Conversely, it has been reported recently that oral immunization may lead to the production of secretory antibodies. To determine whether these events could occur concurrently, CBA/J mice were immunized intragastrically with varying doses of ovalbumin (OVA) and *Streptococcus mutans*. After 7 d, the animals were challenged systemically with antigen in complete adjuvant, and 8 d later serum and saliva taken, and the draining lymph nodes assayed for a proliferative response. Intragastric doses of 1 mg OVA or  $10^9$  *S. mutans* led to significant suppression of the proliferative response, and intragastric doses of 10 mg OVA or  $2.5 \times 10^9$  *S. mutans* led to the production of detectable salivary antibodies using hemagglutination. Serum antibodies were not detected after intragastric administration of OVA or *S. mutans*. Suppression of the proliferative response could be detected from 2–60 d after intragastric administration of OVA, and 2–21 d after *S. mutans*. Prior intragastric immunization with heterologous antigens did not suppress the response to OVA or *S. mutans*. Transfer of  $40 \times 10^6$  mesenteric lymph node cells from mice given 20 mg OVA or  $10^9$  *S. mutans* led to suppression of the proliferative response in syngeneic recipients. Salivary antibodies were removed by absorption with anti-IgA, but not anti-IgG or IgM, indicating that they were of the IgA class. It appears that intragastric administration of soluble or particulate antigens in mice may lead to the concurrent induction of salivary antibodies and systemic suppression.

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