INFECTION AND IMMUNITY, Dec. 1982, p. 1130–1136
0019-9567/82/121130-07$02.00/0

Vol. 38, No. 3

# Effect of Neonatal Thymectomy on Dental Caries in Rats

JEFFREY L. EBERSOLE,* MARTIN A. TAUBMAN, AND DANIEL J. SMITH
*Department of Immunology, Forsyth Dental Center, Boston, Massachusetts 02115*

Received 7 June 1982/Accepted 23 August 1982

The effect of T-cell depletion on susceptibility to dental caries after infection with *Streptococcus mutans* was studied. Rats were neonatally thymectomized (Tx) and infected with *S. mutans* 6715 or locally immunized with the homologous organism before infection. The Tx rats uniformly exhibited a higher level of infection with *S. mutans* and subsequently showed a greater extent of carious activity. Correlation studies were performed comparing the level of salivary and serum anti-*S. mutans* antibodies and the relative amount of dental caries. The results demonstrated that salivary immunoglobulin A antibody after immunization and infection, or infection only, showed a significant negative correlation with dental caries. Also, after local immunization, serum immunoglobulin G antibody showed a negative correlation with dental caries in the rats. These findings further support a major protective role for salivary immunoglobulin A in experimental dental caries in rats.

The role of *Streptococcus mutans* in the pathogenesis of dental caries appears to be a result of accumulation on tooth surfaces (16) and production of large amounts of acid (24). It has been suggested that interference with adherence of the microorganisms would result in a diminished level of dental caries (6, 15). Experiments have shown that antibodies to *S. mutans* inhibit accumulation in vitro (15, 23) and can provide protection against dental caries in vivo (22, 30, 31). Studies using rats as a model of caries immunity have shown that actively induced salivary immunoglobulin A (IgA) antibodies are often associated with reduced numbers of *S. mutans* recovered from infected rats and a lower incidence of caries (22, 30, 31). Investigators from different laboratories have thus concluded that local administration of formalinized *S. mutans* in the salivary gland vicinity of germfree rats and conventional rats, hamsters, and monkeys elicits protective levels of salivary IgA antibody (13, 14, 22, 25, 30, 31).

We have reported that rats that were thymectomized at birth (Tx) to deplete T-lymphocyte numbers manifest various immunological abnormalities (8–10). In particular, salivary IgA and serum IgG antibody levels induced by local immunization or infection with *S. mutans* or both are significantly decreased in the Tx rats (11). Recent studies of humans with various immunological abnormalities have shown that these individuals have decreased salivary and serum antibodies to *S. mutans* (1, 2, 5). Accompanying this decreased immune response has been a generally increased level of dental caries (2, 5). The purpose of the present study is to determine whether experimentally induced specific immunological dysfunction in rats infected with *S. mutans* would affect dental caries activity.

## MATERIALS AND METHODS

**Bacteria.** *S. mutans* 6715, serotype g, is cariogenic in rats and hamsters and is resistant to streptomycin at concentrations of 2 mg/ml. The organisms were grown anaerobically (10% $CO_2$, 90% $N_2$) for 18 to 36 h at 37°C in 6 to 10 liters of a chemically defined medium (27). The cells were removed by centrifugation (13,000 × *g*) and washed three times in phosphate-buffered saline (PBS; 0.02 M phosphate) (pH 7.5) containing 1 mM EDTA. The bacteria were killed with 0.5% buffered formal saline (16 to 18 h at room temperature) and plated on mitis salivarius (Difco) and 5% sheep blood (GIBCO) agar plates to assure that the bacteria were nonviable. The organisms were again washed three times in PBS-EDTA, subsequently suspended in PBS-EDTA to a concentration of $7 \times 10^{10}$/ml, and stored at 4°C.

**Experimental protocol.** Four experiments were performed in outbred Sprague-Dawley (CD-1) rats, and one experiment was done in inbred Fischer rats (CDF/CrBl; Charles River, Wilmington, Mass.) (Table 1). In each experiment, values for a group of Tx rats and control (normal [N] or sham-thymectomized [STx]) rats were compared. The purpose of experiments A1 and A2 was to determine the effect of neonatal thymectomy on infection with *S. mutans* 6715 and caries after 45 or 65 days of infection. Experiments A3, A4, and A5 included groups of immunized Tx or N rats that received four weekly injections of $10^9$ formalinized *S. mutans* organisms, emulsified in complete Freund adjuvant, in the vicinity of the major salivary glands. Experiments A3, A4, and A5 also used sham-immunized (Tx and N) rats injected with PBS in complete Freund adjuvant and a nonimmunized nor-

mal group (only in A3) injected with PBS. All rats were subsequently infected, and the experiments were terminated at 65 or 67 days postinfection. The *S. mutans*-injected groups in these experiments provided a comparison of the effects of immunization before infection with those of infection alone on the extent of disease in the Tx and N rats. The sham-immunized groups in experiments A3, A4, and A5 can also be analyzed to determine the ability of older rats to manifest protection against *S. mutans*-induced caries following an infection, similar to experiments A1 and A2. At sacrifice, the whole jaws were defleshed, and all caries and lesions were scored by a modified Keyes method (30). Statistical differences in the data were determined by an analysis of variance.

**Infection.** *S. mutans* 6715 was cultured anaerobically in Trypticase soy broth (Difco) for 16 to 18 h; 0.4 ml (approximately $10^8$ colony-forming units) was used for infection. All animals from experiments A1 and A2 were infected on 3 consecutive days beginning on day 21 postparturition. All rats in experiments A3, A4, and A5 were also infected for 3 consecutive days, 7 to 10 days after completion of the immunization regimen (A3, days 46 to 48 of age; A4, days 43 to 45 of age; A5, days 44 to 46 of age) (Table 1). During the experiment and at termination, the molar and incisor teeth were swabbed (Calgiswab; Inolex), and the recovered organisms were dispersed by blending in a Vortex mixer in 2 ml of ¼-strength Ringer solution. Appropriate dilutions were plated on mitis salivarius agar for total streptococci and mitis salivarius agar containing 200 μg of streptomycin per ml for *S. mutans* 6715.

**Antibody analysis.** Antibody in saliva (IgA and IgG) and serum (IgG) was determined by a modification of the indirect enzyme-linked immunosorbent assay (10, 11). Formalinized *S. mutans* 6715 organisms were attached to polystyrene microtiter plates, and after incubation with saliva or serum and antiglobulin reagents, the antibody activity to the microorganism was determined spectrophotometrically as described previously (11).

**Verification of T-cell depression resulting from neonatal thymectomy.** We have previously shown that Tx rat spleens contain approximately 10% T lymphocytes, whereas normal rat spleens have 50 to 60% T cells (8). To verify that Tx rats exhibited decreased levels of T lymphocytes, two analyses were used. First, spleen cells from all Tx rats and representative control animals were prepared at the termination of each experiment. Spleens were excised from rats and placed in 10 ml of chilled Alsever solution. The spleens were minced and expressed through 60-gauge stainless-steel wire mesh. The cell suspensions were then forced through a 26-gauge needle and washed two times in Hanks basic salt solution containing 5% bovine serum albumin (Sigma Chemical Co., St. Louis, Mo.). After centrifugation (800 × *g*, 4°C), the cell pellets were resuspended in Hanks basic salt solution–bovine serum albumin, and lymphocytes were isolated by centrifugation into Ficoll-Hypaque (4). The isolated lymphocytes were washed two times in Hanks basic salt solution–bovine serum albumin, and viability was assessed by trypan blue dye exclusion (90 to 95% viable). A sample of $10^6$ cells was then suspended in 25 μl of an appropriate dilution of fluorescein-conjugated anti-rat T lymphocyte globulin (29). The suspension was incubated at 4°C for 20 min and washed three times in Hanks basic salt solution–bovine serum albumin. The cells were then diluted, and the percentage of positively staining cells was determined (200 to 300 lymphoid cells were counted in each preparation) by using a Leitz Ortholux fluorescence microscope with Ploem illumination and an XBO 75 lamp. The percentage of T lymphocytes in the groups of rats from all experiments showed that normal spleen T cell levels were 56.3 ± 3.5% ($n$ = 85), whereas Tx levels were 8.9 ± 1.5% ($n$ = 58). Although Tx rats are not devoid of T lymphocytes, T-cell levels in the spleens of these rats are decreased by 70 to 90% compared to control animals.

The second analysis of T-cell deprivation determined the functional capability of the residual T-cell population in the Tx rats to reject histoincompatible tail skin grafts (3). Two to four grafts from CDF-1 rats (inbred Fisher 344-derived strain) were placed on the dorsal tail surface of CD-1 (Sprague-Dawley) rats. The grafts were covered with a protective polystyrene tube for approximately 48 h, after which time the survival of the grafts was monitored every 2 days. Any graft not attached at 7 days was eliminated as a technical failure. The grafts were scored for evidence of rejection on a 0 to +++ basis for loss of hair, epithelial texture, and pigmentation. When two of three parameters were scored as +++, the graft was considered rejected. The rejection of histoincompatible skin grafts was studied in rats from experiment A4. The normal CD-1 rats rejected CDF-1 skin grafts in 10.8 ± 1.1 days. In contrast, the Tx rats showed a complete inability to reject the foreign tissue, and all grafts

TABLE 1. Experimental protocols for determining the effects of neonatal thymectomy on dental caries in rats

| Expt | Group ($n$) | Rat strain | Injection[a] | Duration of infection (days)[b] |
|---|---|---|---|---|
| A1 | Tx (5) | CD-1 | None | 45 |
| | N (12) | | None | |
| A2 | Tx (6) | CDF/BRL | None | 65 |
| | STx (10) | | None | |
| A3 | Tx (7) | CD-1 | 6715 + CFA | 65 |
| | Tx (5) | | PBS + CFA | |
| | N (10) | | 6715 + CFA | |
| | N (9) | | PBS + CFA | |
| | N (7) | | PBS | |
| A4 | Tx (9) | CD-1 | 6715 + CFA | 65 |
| | Tx (9) | | PBS + CFA | |
| | N (10) | | 6715 + CFA | |
| | N (11) | | PBS + CFA | |
| A5 | Tx (10) | CD-1 | 6715 + CFA | 67 |
| | Tx (7) | | PBS + CFA | |
| | N (10) | | 6715 + CFA | |
| | N (10) | | PBS + CFA | |

[a] Four weekly injections of this material in the vicinity of the major salivary glands. 6715, *S. mutans* 6715.

[b] Rats in all experiments were infected with *S. mutans* 6715.

TABLE 2. Bacterial recoveries from thymectomized and control rats during infection and at termination of experiment

| Expt | Treatment[a] | *S. mutans* in Tx vs N rats[b] | |
|---|---|---|---|
| | | During infection | At termination |
| A1 | None | + | + |
| A2 | None[c] | | |
| | 1 | + | + |
| | 2 | + | |
| A3 | I | + | + |
| | SI/NI[d] | + | − |
| A4 | I | − | + |
| | SI | + | + |
| A5 | I | + | + |
| | SI | + | + |

[a] I, Immunized; SI, sham-immunized; NI, nonimmunized.

[b] +, Geometric mean *S. mutans* was greater in Tx than in comparably treated N rats; −, no difference in *S. mutans* between Tx and N rats.

[c] Rats in experiment A2 were swabbed twice during the period of infection.

[d] Results are a composite of a group of N rats that were sham-immunized and a group of N rats that were nonimmunized.

survived until the termination of the experiment. Therefore, not only are T-lymphocyte numbers decreased in the Tx rats, but the graft rejection assay suggests that cell-mediated immune functions are drastically altered in these rats.

## RESULTS

**Bacteriological studies.** Tx rats produced levels of salivary antibodies, after infection or active immunization with *S. mutans* 6715, that were below normal (10). It was of interest to determine the effect of thymectomy on *S. mutans* infection and dental disease associated with these microorganisms. The extent of infection was monitored by systematically swabbing molar and anterior tooth surfaces of the infected rats and plating the swabs for *S. mutans* on mitis salivarius agar plus 200 μg of streptomycin per ml (Table 2). Statistically significant differences between the levels of recoverable *S. mutans* were not observed due to variations in infection or recovery of the organism among the rats. However, in 10 of 11 swabbing periods higher geometric mean levels of *S. mutans* were recovered from the nonimmunized or sham-immunized Tx rats than from N rats. Similar results were noted in groups of Tx and N rats that were actively immunized before infection with *S. mutans*. In five of six sampling intervals the immunized Tx rats harbored greater geometric mean numbers of *S. mutans* than comparably injected N rats.

**Lesions and caries.** To assess the effect of T-cell reduction on caries immunity, the numbers of carious lesions and caries scores of rats from experiments A1 through A5 were determined.

Groups of infected, nonimmunized or sham-immunized rats from each experiment provided data concerning the innate susceptibility of control (N or STx) or Tx rats to dental caries upon *S. mutans* infection (Table 3). In each experiment, Tx rats showed increased numbers of carious lesions as compared to N or STx animals. These differences were statistically significant ($P < 0.01$ to $P < 0.05$) in three of five experiments. The distribution of carious lesions, as shown by percent change in comparably treated Tx versus N or STx groups, is shown in Table 3. A greater difference was seen on smooth (buccal and lingual) than on occlusal surfaces in immunized, sham-immunized, and nonimmunized rats infected with *S. mutans* in 9 of 10 instances. In every experiment, the mean caries scores of the Tx rats (nonimmunized or sham-immunized) were greater than those of comparable N or STx rats (A1, A3, A4, A5; $P > 0.05$) after infection with *S. mutans* (Table 4).

Immunized groups of rats in experiments A3, A4, and A5 were used to examine the ability of N and Tx rats to manifest a protective response upon active local immunization with *S. mutans*. In each experiment, local immunization with Formalin-killed *S. mutans* resulted in significantly decreased numbers of lesions (Table 3) and caries scores (Table 4) in N rats as compared to their sham-immunized counterparts. A comparison of lesions (Table 3) and caries scores (Table 4) between immunized Tx and N rats showed that in all experiments the Tx rats exhibited significantly greater disease. The increase in caries scores was most pronounced on buccal and lingual surfaces of the immunized Tx versus the immunized N rats (Table 4). When the extent of disease was compared in Tx rats that were either immunized or sham-immunized (Tables 3 and 4), in 11 of 12 instances, the number and severity of carious lesions were lower in immunized Tx rats than in sham-immunized Tx control animals. These results suggest that although the Tx rats exhibit an inability to manifest a protective response compared to similarly treated N rats, local immunization of the Tx animals can elicit a level of protection resulting from the remaining immune capabilities of the rats.

**Correlations between antibody levels and caries protection.** In a previous study, we emphasized immunological differences between the responses of Tx and N rats to *S. mutans* (10). The present study examined differences in disease susceptibility between these groups of rats. To determine the relationships among these findings, correlation studies were performed. Caries scores were compared with salivary and serum antibody levels (Table 5). In six of eight experi-

TABLE 3. Mean numbers of carious lesions of control (N or STx) and Tx rats after immunization and infection

| Expt | Treatment[a] | Group | Mean no. (±SE) of carious lesions[b] | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Occlusal[c] | % Increase[d] | Buccal-Lingual[c] | % Increase[d] | Total lesions | % Increase[d] |
| A1 | None | Tx | 12.2 ± 1.9 | | 12.6 ± 3.6 | | 24.8 ± 6.9[e] | |
| | | N | 5.8 ± 1.1 | 52.5 | 4.0 ± 1.1 | 68.3 | 9.8 ± 1.9[e] | 60.5 |
| A2 | None | Tx | 16.0 ± 1.0* | | 19.2 ± 2.5* | | 35.2 ± 3.7[f] | |
| | | STx | 12.1 ± 1.1* | 24.3 | 12.6 ± 1.3* | 34.4 | 24.7 ± 2.4[f] | 29.8 |
| A3 | I | Tx | 8.0 ± 2.2 | | 9.2 ± 3.2 | | 17.2 ± 5.4[g] | |
| | | N | 3.8 ± 0.6 | 52.5 | 0.7 ± 0.3 | 92.4 | 4.5 ± 0.8[gh] | 73.8 |
| | SI | Tx | 13.5 ± 2.0 | | 12.3 ± 2.2 | | 25.8 ± 4.0[i] | |
| | | N | 9.3 ± 1.1 | 31.1 | 6.4 ± 1.4 | 48.0 | 15.7 ± 2.3[hi] | 39.1 |
| | NI | N | 8.8 ± 2.8 | 34.8 | 10.3 ± 1.5 | 16.3 | 19.1 ± 4.2[h] | 26.0 |
| A4 | I | Tx | 7.2 ± 0.8* | | 7.9 ± 1.3 | | 15.1 ± 2.0[jk] | |
| | | N | 6.3 ± 1.5* | 12.5 | 1.6 ± 0.8 | 79.7 | 7.9 ± 2.8[jl] | 47.7 |
| | SI | Tx | 14.4 ± 1.2* | | 16.0 ± 2.0* | | 30.4 ± 3.0[km] | |
| | | N | 11.2 ± 1.2* | 22.2 | 11.8 ± 2.3* | 26.3 | 23.0 ± 3.5[lm] | 24.3 |
| A5 | I | Tx | 9.7 ± 2.1 | | 10.3 ± 1.4 | | 20.0 ± 3.2[no] | |
| | | N | 4.1 ± 1.0 | 42.3 | 1.3 ± 0.3 | 87.4 | 5.4 ± 1.3[np] | 73.0 |
| | SI | Tx | 16.1 ± 3.7 | | 16.3 ± 4.5 | | 32.4 ± 7.6[oq] | |
| | | N | 9.7 ± 1.6 | 39.8 | 9.1 ± 2.5 | 44.2 | 18.8 ± 4.0[pq] | 42.0 |

[a] I, Immunized; SI, sham-immunized; NI, nonimmunized.
[b] Group means and standard errors: each mean represents the scores of at least five rats.
[c] All occlusal and buccal-lingual differences are statistically different as represented by the total caries pairings except those designated by the asterisk.
[d] 100 − (mean number of lesions of control group/mean number of lesions of Tx group) × 100.
[e–q] Corresponding pairs statistically significant ($P < 0.01$ to $P < 0.05$).

mental pairings there was a significant negative correlation ($P < 0.01$ to $P < 0.05$) between salivary IgA antibody levels and the extent of caries. In the other two cases a negative correlation was noted that nearly reached statistical significance. In contrast, salivary IgG antibody levels never showed a significant correlation with caries incidence. After infection of rats with *S. mutans*, serum IgG antibodies are induced. In all five experiments, this antibody level did not correlate with dental caries in the rats. However, immunization of rats with *S. mutans* induced substantial levels of serum IgG antibodies that generally remained unchanged after the infection (10), and in each experiment (A3, A4, and A5) serum IgG antibody levels showed a significant negative correlation to caries incidence in the immunized Tx and N rats.

## DISCUSSION

Previous studies have shown that local immunization of conventional rats by either injection (22, 30) or feeding (22) of *S. mutans* gives rise to a salivary IgA antibody response. In normal rats this salivary antibody is associated with decreased levels of disease associated with *S. mutans* infection (22, 30). We have shown that neonatal thymectomy of rats produces a prolonged and significant diminution in salivary IgA levels (8) and also a decrease in specific antibodies of this isotype (9). In addition, Tx rats showed an inability to manifest normal salivary IgA and serum IgG antibody responses to local administration of formalinized *S. mutans*, to infection with the microorganism, or to both (10).

In this report, we have investigated the resultant caries activity in immunologically deficient rats. Bacteriological studies showed that in 15 of 17 instances, Tx rats harbored more *S. mutans* than similarly treated control animals. Antibodies have been shown to be capable of affecting accumulation of *S. mutans* (14, 15, 23), possibly as a result of inhibition of the enzyme responsible for glucan production (glucosyltransferase). Theoretically, any alteration in the level or quality of antibody to antigens associated with accumulation in the oral cavity could presumably affect *S. mutans* accumulation. Thus, the significantly decreased immune responses to *S. mutans* in the Tx rats may have resulted in an increased bacterial burden.

A previous report (18) examined caries incidence in rats that were thymectomized as neonates. No difference in the extent of caries

TABLE 4. Mean caries scores of control (N or STx) and Tx rats after immunization and infection

| Expt | Treatment[a] | Group | Mean (±SE) caries scores[b] | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Occlusal[c] | % Increase[d] | Buccal-Lingual[c] | % Increase[d] | Total caries | % Increase[d] |
| A1 | None | Tx | 10.4 ± 3.3 | | 9.5 ± 4.4 | | 19.9 ± 7.6[e] | |
| | | N | 3.5 ± 0.7 | 66.3 | 1.8 ± 0.5 | 81.1 | 5.4 ± 1.1[e] | 72.9 |
| A2 | None | Tx | 24.6 ± 4.6* | | 19.4 ± 2.4* | | 43.8 ± 6.7 | |
| | | STx | 18.9 ± 2.9* | 23.2 | 17.3 ± 2.7* | 10.8 | 36.3 ± 5.5 | 17.1 |
| A3 | I | Tx | 19.9 ± 8.0 | | 15.0 ± 6.3 | | 36.5 ± 14.4[f] | |
| | | N | 6.7 ± 1.2 | 93.3 | 1.0 ± 0.2 | 76.3 | 7.5 ± 1.3[fg] | 75.9 |
| | SI | Tx | 22.1 ± 4.1 | | 11.4 ± 5.0 | | 35.2 ± 8.7[h] | |
| | | N | 11.0 ± 1.8 | 62.2 | 4.2 ± 1.1 | 50.2 | 15.2 ± 2.7[gh] | 56.8 |
| | NI | N | 19.9 ± 6.8 | 37.0 | 7.2 ± 3.9 | 10.0 | 27.1 ± 10.6[g] | 23.0 |
| A4 | I | Tx | 8.0 ± 1.6 | | 5.0 ± 1.2 | | 13.1 ± 2.5[ij] | |
| | | N | 4.3 ± 1.5 | 46.2 | 1.7 ± 0.7 | 66.0 | 6.0 ± 2.1[ik] | 54.2 |
| | SI | Tx | 23.6 ± 3.4 | | 12.4 ± 1.5 | | 36.0 ± 4.5[il] | |
| | | N | 14.2 ± 2.9 | 39.8 | 8.3 ± 1.9 | 33.1 | 22.5 ± 4.7[kl] | 37.5 |
| A5 | I | Tx | 23.4 ± 6.9 | | 18.1 ± 7.7 | | 41.5 ± 13.3[m] | |
| | | N | 8.5 ± 2.4 | 63.7 | 3.3 ± 1.3 | 81.8 | 11.8 ± 3.4[mn] | 71.6 |
| | SI | Tx | 32.5 ± 10.7* | | 21.0 ± 4.9 | | 53.5 ± 14.9 | |
| | | N | 18.9 ± 5.6* | 41.8 | 11.3 ± 3.6 | 46.2 | 30.2 ± 8.8[n] | 43.6 |

[a] I, Immunized; SI, sham-immunized; NI, nonimmunized.
[b] Group means and standard errors: each mean represents the scores of at least five rats.
[c] All occlusal and buccal-lingual differences are statistically different as represented by the total caries pairings except those designated by the asterisk.
[d] 100 − (mean caries score of control group/mean score of Tx group) × 100.
[e–n] Corresponding pairs statistically significant ($P < 0.01$ to $P < 0.05$).

activity between the thymectomized and control groups was found. In our experiments, Tx rats showed an increase in caries. These increases were apparent on both occlusal and smooth surfaces and accompanied decreased antibody responses to the infecting bacteria. The disparity could possibly be accounted for by differences in the methodologies employed. In particular, no attempt was made to monitor the presence or absence of *S. mutans* in the oral flora in the previous study, whereas rats in our study were specifically infected with a cariogenic *S. mutans*.

A recent report (17) used gnotobiotic rats treated with an immunosuppressive agent, cyclosporin A, to examine immune functions and

TABLE 5. Correlation coefficients comparing serum or salivary antibody levels with caries scores in Tx and control rats after immunization or infection with *S. mutans* or both

| Expt | Treatment | Correlation coefficient | | |
|---|---|---|---|---|
| | | Salivary IgA | Salivary IgG | Serum IgG |
| A1 | Infected | −0.550[a] | −0.165 | −0.017 |
| A2 | Infected | −0.538 | 0.145 | −0.194 |
| A3 | Immunized, infected | −0.495 | −0.095 | −0.560[a] |
| | Sham-immunized, infected | −0.865[b] | −0.293 | −0.293 |
| A4 | Immunized, infected | −0.695[b] | −0.387 | −0.546[a] |
| | Sham-immunized, infected | −0.893[b] | −0.407 | −0.361 |
| A5 | Immunized, infected | −0.603[a] | −0.111 | −0.588[a] |
| | Sham-immunized, infected | −0.852[a] | −0.209 | −0.325 |

[a] Statistically significant, $P < 0.05$.
[b] Statistically significant, $P < 0.01$.

the relationship to dental caries. These authors indicated that the agent primarily affected T-cell function and that after challenge with *Actinomyces viscosus* Ny1, the immunosuppressed rats had fewer dental caries than untreated controls. The interpretation of these findings was that if immune responses were important in protection from dental caries, then antibodies directed to T-independent antigens may be the most significant. Our results seem to contrast with these, in that T-cell deprivation of rats by neonatal thymectomy led to a significant increase in disease. Two important differences in these systems must be taken into account when comparing these findings. First, we utilized conventional rats specifically infected with *S. mutans*, whereas the other study used gnotobiotic rats monoinfected with *A. viscosus*. The importance of the difference lies in the evidence that generally associates *Actinomyces* sp. only with root surface caries in the rodent system (17), whereas *S. mutans* has been identified as a causative agent in both smooth-surface and occlusal disease (16). Secondly, the method of the T-cell depression in the rats was quite different. The actual cell populations affected by the immunosuppressive agent (cyclosporin A) in the previous study were only defined by the effect on serum agglutinins to *A. viscosus*. We have shown significant decreases in T-cell numbers in the blood and spleen of the Tx rats as well as a dysfunction in a cell-mediated immune correlate (graft rejection). Also, in contrast to the *A. viscosus* report, we identified significant decreases in salivary antibodies to the cariogenic agent in the Tx rats. Thus, whereas both of these studies attempted to examine regulation of immune responses in relationship to dental caries incidence, the differences in methodology do not allow a direct comparison of the findings.

IgA-deficient Tx rats were also actively immunized with *S. mutans*. Tx rats showed significantly less IgA antibody after the immunization regimen than N rats (10). Also, serum IgG antibody responses to the injected *S. mutans* were significantly decreased (10). These experiments also showed that immunized Tx rats had significantly greater disease after *S. mutans* infection than the comparably treated N rats. A pattern of disease susceptibility was also noted between the Tx and N rats (Tables 3 and 4). In general, the percentage of increase in lesions and caries in the Tx rats was greater on smooth surfaces than on occlusal surfaces. This finding may indicate that the Tx rats manifest a specific inability to respond to *S. mutans* infection which is reflected by the increased smooth-surface disease. However, the increase in extent of lesions (defined by the caries score) on occlusal surfaces in the Tx rats may be an additive effect of the *S. mutans* infection and an increase in caries activity as a result of immune alterations to the indigenous cariogenic flora.

Numerous studies using rat (21, 30, 31), hamster (25), or monkey (13, 19) model systems have indicated that increased salivary IgA antibodies to *S. mutans* are associated with decreased numbers of organisms or disease activity. Thus, correlation studies between antibody levels and disease were performed in this investigation. In general, salivary IgA antibody showed a significant negative correlation with the extent of caries. Therefore, in this system using immunocompromised as well as normal rats, the increased levels of salivary IgA anti-*S. mutans* antibody elicited via infection or active immunization resulted in decreased caries activity. Also, local immunization of the rats resulted in serum IgG antibody levels which showed a significant negative correlation to dental caries. These results are similar to those reported with a primate model (19, 20). In the primate studies, serum IgG antibody, presumably reaching the oral cavity via the gingival crevice, correlated with protection against dental caries. In our studies, only those groups of rats injected with antigen showed an IgG correlation, whereas infected rats showed no correlation. This may suggest that much greater levels of serum IgG are necessary for protection as compared to salivary IgA antibodies, with which all groups in the experiments showed a strong negative correlation.

These findings support the concept that salivary IgA antibodies are of considerable importance in the protection of rats against *S. mutans*-caused dental caries. However, it does not eliminate the possibility that other humoral and cellular immune factors are involved in protection. Experiments with rats deprived of T-cells, which results in a severe IgA deficiency, suggest that the protective responses to *S. mutans* are dependent upon thymic-derived factors. Although T-cell functions (cell-mediated immunity) are depressed in these rats, the mechanism of cell-mediated immune protection from *S. mutans*-induced disease is difficult to envisage in the milieu of the oral cavity. Thus, the immunological deficiency in these rats that may lead to increased disease is probably via T-cell interactions in the elicitation of protective antibody responses.

ACKNOWLEDGMENTS

This research was supported by Public Health Service grant DE-04733 and contract DE-42438 from the National Institute of Dental Research. J.L.E. (DE-00075) is a recipient of Public Health Service Research Career Development Awards from the National Institute of Dental Research.

We thank D. E. Frey, D. Louie, and J. M. Buckelew for expert technical assistance. We thank B. Connolly for secretarial assistance.

## LITERATURE CITED


1. **Arnold, R. R., M. F. Cole, S. Prince, and J. R. McGhee.** 1977. Secretory IgM antibodies to *Streptococcus mutans* in subjects with selective IgA deficiency. Cell. Immunol. **8**:475–486.
2. **Arnold, R. R., S. J. Prince, J. Mestecky, D. Lynch, M. Lynch, and J. R. McGhee.** 1978. Secretory immunity and immunodeficiency. Adv. Exp. Biol. Med. **107**:401–410.
3. **Bailey, D. W., and B. Usama.** 1960. A rapid method of grafting skin on tails of mice. Trans. Bull. **7**:424.
4. **Boyum, A.** 1968. Isolation of mononuclear cells and granulocytes from human blood. Isolation of mononuclear cells by one centrifugation. Scand. J. Clin. Lab. Invest. **21**(Suppl.):27–50.
5. **Cole, M. F., R. R. Arnold, M. J. Rhodes, and J. R. McGhee.** 1977. Immune dysfunction and dental caries: a preliminary report. J. Dent. Res. **56**:198–204.
6. **DeStoppelaar, J. D., K. G. Konig, A. J. N. Plosschaut, and J. S. van der Hoeven.** 1971. Decreased cariogenicity of a mutant of *Streptococcus mutans*. Arch. Oral Biol. **16**:972–975.
7. **Ebersole, J. L., M. A. Taubman, and D. J. Smith.** 1979. The effect of neonatal thymectomy on the level of salivary and serum immunoglobulins in rats. Immunology **36**:649–657.
8. **Ebersole, J. L., M. A. Taubman, and D. J. Smith.** 1979. Thymic control of secretory antibody responses in the rat. J. Immunol. **123**:19–24.
9. **Ebersole, J. L., M. A. Taubman, and D. J. Smith.** 1979. The neonatally thymectomized rat: a model of compensatory IgM antibody formation in exocrine secretions. Immunology **38**:33–39.
10. **Ebersole, J. L., M. A. Taubman, D. J. Smith, and D. E. Frey.** 1982. Effect of neonatal thymectomy on immune responses of rats to *Streptococcus mutans*. Infect. Immun. **37**:993–1000.
11. **Ebersole, J. L., M. A. Taubman, D. J. Smith, and J. M. Crawford.** 1978. Characterization of immunoglobulin-containing cells in the submandibular gland of the rat after local immunization. Adv. Exp. Biol. Med. **197**:155–164.
12. **Emmings, F. G., R. T. Evans, and R. J. Genco.** 1975. Antibody response in the parotid fluid and serum of irus monkeys (*Macaca fascicularis*) after local immunization with *Streptococcus mutans*. Infect. Immun. **2**:281–292.
13. **Evans, R. T., F. G. Emmings, and R. J. Genco.** 1975. Prevention of *Streptococcus mutans* infection of tooth surfaces by salivary antibody in irus monkeys (*Macaca fascicularus*). Infect. Immun. **12**:293–302.
14. **Genco, R. J., R. T. Evans, and M. A. Taubman.** 1974. Specificity of antibodies to *Streptococcus mutans*: significance in inhibition of adherence, p. 327–336. *In* J. Mestecky and A. R. Lawton (ed.), The immunoglobulin A system. Plenum Publishing Corp., New York.
15. **Gibbons, R. J., and J. van Houte.** 1975. Bacterial adherence in oral microbiol ecology. Annu. Rev. Microbiol. **29**:19–44.
16. **Gibbons, R. J., and J. van Houte.** 1978. Bacteriology of dental caries, p. 975–991. *In* J. H. Shaw, E. A. Sweeney, C. C. Capuccino, and S. M. Miller (ed.), Textbook of oral biology. W. B. Saunders Co., Philadelphia.
17. **Guggenheim, B., A. Hefti, and J. J. Burckhardt.** 1978. The effect of cyclosporin A on dental caries in rats monoassociated with *Actinomyces viscosus* Ny1. Adv. Exp. Biol. Med. **107**:293–301.
18. **Herzog, E., M. Just, and K. G. Konig.** 1966. Effect of early thymectomy on caries incidence, eating pattern, and masticatory performance in Osborne-Mendel strain rats. J. Dent. Res. **45**:974.
19. **Lehner, J., S. J. Challacombe, and J. Caldwell.** 1976. Immunologic basis of vaccination against dental caries in Rhesus monkeys. J. Dent. Res. **55**:C166–C180.
20. **Lehner, T., S. J. Challacombe, J. M. A. Wilton, and J. Caldwell.** 1976. Cellular and humoral immune responses in vaccination against dental caries in monkeys. Nature (London) **264**:69–72.
21. **McGhee, J. R., S. M. Michalek, J. Webb, J. M. Navia, A. F. R. Rahman, and D. Legler.** 1975. Effective immunity to dental caries: protection of gnotobiotic rats by local immunization with *Streptococcus mutans*. J. Immunol. **114**:300–305.
22. **Michalek, S. N., J. R. McGhee, J. Mestecky, R. R. Arnold, and L. Bozzo.** 1976. Ingestion of *Streptococcus mutans* induces secretory IgA and caries immunity. Science **192**:1238–1240.
23. **Olson, G. A., A. S. Bleiweis, and P. A. Small, Jr.** 1972. Antibody mediated inhibition of dextransucrase-induced agglutination of *Streptococcus mutans*. Infect. Immun. **9**:273–278.
24. **Robrish, S. A., and M. I. Krichevsky.** 1972. Acid production from glucose and sucrose by growing cultures of caries-conductive streptococci. J. Dent. Res. **51**:734–739.
25. **Smith, D. J., M. A. Taubman, and J. L. Ebersole.** 1978. Effects of local immunization with glucosyltransferase fractions from *Streptococcus mutans* on dental caries in hamsters caused by homologous and heterologous serotypes of *Streptococcus mutans*. Infect. Immun. **21**:843–851.
26. **Smith, D. J., M. A. Taubman, and J. L. Ebersole.** 1980. Local and systemic antibody response to oral administration of glucosyltransferase antigen complex. Infect. Immun. **28**:441–450.
27. **Socransky, S. S., C. Smith, and A. Manganiello.** 1973. Defined media for the cultivation of oral gram-positive rods. J. Dent. Res. **52**:88.
28. **Taubman, M. A.** 1973. Role of immunization in dental disease, p. 138–158. *In* S. Mergenhagen and H. W. Scherp (ed.), Proceedings of the AAAS Symposium on Comparative Immunology of the Oral Cavity. U.S. Government Printing Office, Washington, D.C.
29. **Taubman, M. A., J. M. Buckelew, J. L. Ebersole, and D. J. Smith.** 1981. Periodontal bone loss and immune response to ovalbumin in germfree rats fed antigen-free diet with ovalbumin. Infect. Immun. **32**:145–152.
30. **Taubman, M. A., and D. J. Smith.** 1974. Effects of local immunization with *Streptococcus mutans* on induction of salivary immunoglobulin A antibodies and experimental dental caries in rats. Infect. Immun. **9**:1079–1091.
31. **Taubman, M. A., and D. J. Smith.** 1977. Effects of local immunization with glucosyltransferase fractions from *Streptococcus mutans* on dental caries in rats and hamsters. J. Immunol. **118**:710–720.