# Effect of Adoptive Transfer of Cloned *Actinobacillus actinomycetemcomitans*-Specific T Helper Cells on Periodontal Disease

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Previously we isolated several *Actinobacillus actinomycetemcomitans*-specific T-cell clones from the spleens and lymph nodes of immunized Rowett rats. These clones were characterized as W3/13[+], W3/25[+], OX8^{−}, and OX22^{−}, suggesting a T helper (Th) phenotype. In the current experiments, $10^6$ cells from a single *A. actinomycetemcomitans*-specific clone (A3) were adoptively transferred to a group (AaTh; $n = 13$) of normal heterozygous rats (*rnu/+*) at 28 days of age. A second group received no T cells (AaNT; $n = 15$), and a third group also received no T cells (NAaNT, $n = 11$). Beginning 1 day after transfer, the first and second groups were infected orally with *A. actinomycetemcomitans* for 5 consecutive days. The presence of infection was confirmed immediately after challenge and after 5 months, when the experiments were ended. Significantly higher numbers of lymphocytes were recovered from the gingival tissues of the first group than from those of either of the other groups. Also, this group showed significantly elevated ($P < 0.01$) serum immunoglobulin G and immunoglobulin M antibody to *A. actinomycetemcomitans* in an enzyme-linked immunosorbent assay when compared with both other groups. Bone loss was significantly lower ($P < 0.01$) in recipients of *A. actinomycetemcomitans*-specific cloned cells when compared with the other infected group and was approximately equal to the bone loss of the uninfected group. These results are consistent with the hypothesis that T-cell regulation can affect periodontal disease. In this regulation, T helper cells appear to interfere with periodontal bone loss.

Periodontal disease is characterized by a localized inflammation related to bacterial infection (18) and is associated with infiltrations of lymphocytes, macrophages, neutrophils, and plasma cells (21, 31). Periodontal disease is not a single entity but comprises a spectrum of clinical conditions that correlate with host responses to oral bacteria including *Actinobacillus actinomycetemcomitans* (13) and *Bacteroides gingivalis* (12).

Early lesions in humans (31) and in rats (34) are characterized by a predominance of T lymphocytes, while B lymphocytes and plasma cells are prominant in more advanced lesions. Studies of other human chronic inflammatory diseases, such as leprosy (22) and lupus (24), and rodent periapical lesions (32) have indicated that the cells infiltrating the lesions manifest immune regulatory deficiencies. These studies have suggested that there is a local immunoregulatory imbalance in the more advanced forms of periodontal diseases (36).

A model of periodontitis has been developed by using gnotobiotic Rowett rats infected with *A. actinomycetemcomitans* Y4 (42). In earlier studies, we demonstrated that adoptive transfer of an enriched population of *A. actinomycetemcomitans*-specific T helper (Th) cells to *A. actinomycetemcomitans*-infected rats resulted in decreased bone loss compared with that in animals receiving nonspecific Th cells (35).

We have isolated several *A. actinomycetemcomitans*-specific T-cell clones from the spleens and lymph nodes of immunized Rowett rats. These clones were characterized as phenotypically and functionally Th (CD4^{+}) lymphocytes (9). The purpose of this study was to investigate the effects of adoptive transfer of cloned *A. actinomycetemcomitans*-specific Th cells on gingival inflammation and periodontal bone loss in gnotobiotic rats.

## MATERIALS AND METHODS

**Animals.** The animals used were male Rowett rats that harbored a restricted flora (40, 41). These animals were bred in plastic Trexlar-type germfree isolators and maintained under gnotobiotic, pathogen-free conditions in laminar flow cabinets. Experiments were started when the animals were 28 days of age.

**Bacteria.** *A. actinomycetemcomitans* Y4, the strain used in this study, was originally isolated from a gingival pocket of a juvenile patient with periodontitis (28). *A. actinomycetemcomitans* was grown in PPLO broth (glucose [3 g/liter], sodium bicarbonate [1 g/liter]) for 72 h at 37°C in a candle jar and used for oral inoculation. Antigens used for in vitro T-cell stimulation were whole bacterial cells fixed in 1% Formalin in phosphate-buffered saline (PBS) (25, 34). Bacterial numbers were estimated by spectrophotometric (Gilson) reading of a suspension in PBS at 580 nm.

**T helper clone for transfer.** The *A. actinomycetemcomitans*-specific Th clone A3 was established as previously described (9). Briefly, donor animals were injected intraperitoneally with $2 \times 10^5$ to $5 \times 10^5$ Formalin-killed *A. actinomycetemcomitans* bacteria. The spleen and mesenteric lymph nodes were removed and cultured in 24-well trays (Costar, Cambridge, Mass.) containing 1.5 ml of RPMI 1640

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TABLE 1. Characteristics of *A. actinomycetemcomitans*
Th clone A3[a]

| Characteristic | Response |
|---|---|
| Phenotype | |
| W3/13 (CD3).......................................................... | + |
| W3/25 (CD4).......................................................... | + |
| OX8 (CD8)............................................................. | − |
| OX22 (CD45R)........................................................ | − |
| Proliferation[b] | |
| A. actinomycetemcomitans Y4 (serotype b) ................. | + + |
| A. actinomycetemcomitans 29523 (serotype a)............. | + |
| A. actinomycetemcomitans 652 (serotype c)................ | + |
| A. actinomycetemcomitans Y4 LPS.......................... | − |
| H. aphrophilus....................................................... | + + |
| Other gram-negative bacteria[c].................................. | − |
| Protein antigens[d] .................................................. | − |
| | |
| Help for specific antibody......................................... | + + |
| IL-2 production....................................................... | − |
| DTH[e]................................................................... | − |
| ConA response[f]...................................................... | + + |

[a] Origin of clone is mesenteric lymph node cells; helper type is Th2 function. All data supporting these characteristics can be found in reference 9.
[b] Proliferation in vitro in presence of antigen-presenting cells and antigen listed.
[c] *Capnocytophaga* spp., *Bacteroides intermedius*, and *B. gingivalis* 381.
[d] *Streptococcus sobrinus* glucosyltransferase and ovalbumin.
[e] DTH, Delayed-type hypersensitivity.
[f] ConA, Concanavalin A.

(GIBCO, Grand Island, N.Y.) with *A. actinomycetemcomitans* (2 × 10^{7} cells per well), 10% fetal bovine serum (Hyclone Laboratory, Inc., Logan, Utah), 5 × 10^{5} M 2-mercaptoethanol, 12.5 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid), 100 U of penicillin per ml, and 100 μg of streptomycin per ml.

Five days later, the cells were harvested, and blast cells were centrifuged on Isolymph (Gallard-Schlesinger Chemical Manufacturing Corp., Carle Place, N.Y.) diluted 3:1 with RPMI 1640 at 2,000 rpm for 20 min. Cells from the interface were collected and washed twice, and cloning was performed under limiting-dilution conditions at various concentrations of blasts in 96-well flat-bottom microtiter plates (Costar). Each well contained 3 × 10^{5} irradiated syngenic spleen cells as antigen-presenting cells (3,300 rads from a cesium source) and 5 × 10^{7} *A. actinomycetemcomitans* per ml in RPMI 1640 supplemented with 10% fetal bovine serum. After expansion, the cloned T cells were separated from the antigen-presenting cells by gradient centrifugation, and 10[6] washed cloned cells were injected into the tail vein of each recipient rat.

**Characterization of A3 cloned cells. (i) Phenotype.** The phenotypic characteristics, specificity, and function of the A3 clone used in these experiments have been described previously (9) and are summarized in Table 1. These assays were performed to determine whether the A3 clone was of the Th1 or Th2 type (26). The A3 clone was phenotypically W3/13[+], W3/25[+], OX8^{−}, and OX22^{−}, indicating Th cells. This clone reacted to *A. actinomycetemcomitans* Y4 by in vitro proliferation but also responded to representative strains of other serotypes of *A. actinomycetemcomitans* and to the closely related *Haemophilus aphrophilus*. These responses were not related to the lipopolysaccharides (LPS) of the microorganisms, since the A3 clone was not activated by isolated *A. actinomycetemcomitans* Y4 LPS, LPS of other unrelated species of bacteria, or unrelated antigens. This

clone provided specific help for primed B cells (1) and did not produce interleukin-2 (IL-2) detectable in our assay system. The A3 clone gave a brisk response to concanavalin A (2) and was negative in delayed-type hypersensitivity induction (3, 23). These data suggested that the A3 clones were Th2-type cells.

**(ii) Experimental protocol.** Thirty-nine normal male rats were divided into three groups. The first group was called *Aa*Th (*n* = 13) and consisted of animals that received 10[6] A3 clone cells injected intravenously at 28 days of age (day 28). The second group, *Aa*NT (*n* = 15), received no T cells. These two groups were orally infected with live *A. actinomycetemcomitans* for 5 consecutive days beginning on day 29. The third group, N*Aa*NT (*n* = 11), was not infected and did not receive T cells. All analyses were for all available samples for each assay.

The animals were fed sterilized L356 diet (Teklad) and were given autoclaved water ad libitum. Five months after transfer, saliva was collected and the rats were exsanguinated. The rats' teeth and gingival areas were systematically swabbed with Calgiswabs, and the presence of infection with *A. actinomycetemcomitans* was determined by immunofluorescence. Rabbit anti-*A. actinomycetemcomitans* antibody prepared as described in reference 11, followed by goat anti-rabbit immunoglobulin G (IgG) conjugated with fluorescein isothiocyanate (Miles Laboratories), identified *A. actinomycetemcomitans* in heat-fixed smears on glass slides. *A. actinomycetemcomitans* was present in the oral cavities of all infected rats up to the end of the experiment. Gingival tissues were removed, and cervical lymph nodes and spleens were obtained. Finally, the rat heads were defleshed for the measurement of bone loss.

**(iii) Gingival cell preparation and flow cytofluorometry analyses.** Gingival tissue was prepared as previously described (34, 42). Briefly, after surgical removal of 1 mm of gingiva from the margin, the tissues were placed in a 60-mm-diameter petri dish containing 2 ml of RPMI 1640 with 12.5 mM HEPES and 10 U of heparin per ml, cut into 1-mm[3] pieces, and centrifuged. The supernatant was termed gingival cell wash fluid and was used for determination of anti-*A. actinomycetemcomitans* IgG antibody and IL-2 level. The segments were incubated at 37°C for 1 h in 1 mg of collagenase (Worthington) in 1 ml of RPMI 1640 containing 0.005 M $CaCl_2$. After treatment, the tissues were expressed through 60-gauge stainless steel screens to obtain a single-cell suspension in 1 ml of RPMI 1640. The cells were stained with acridine orange-ethidium bromide and were counted to determine the number of gingival lymphocytes relative to total gingival cells. For immunocytofluorometric analyses, the gingival cells were incubated with 50 μl of the appropriately diluted mouse anti-rat T cell monoclonal antibodies W3/25 (CD4) and OX8 (CD8) and fluorescein isothiocyanate-conjugated anti-B cell reagent (34) in a 96-well vinyl round-bottom microtiter plate (Costar) for 30 min on ice. Fluorescein isothiocyanate-conjugated F(ab')₂ fragment rat anti-mouse IgG (Jackson Immunoresearch, Cedar Park, Pa.) was used as the second antibody.

**(iv) Lymphocyte cell preparation and proliferation assay.** Spleens and cervical lymph nodes were removed under sterile conditions. Lymphocytes were prepared for culture by gently expressing tissues through 60-gauge stainless steel mesh. Viable cells (5 × 10^{5} cells per well) were suspended in RPMI 1640. The optimal amount of Formalinized *A. actinomycetemcomitans* (10[7]) was added to test cultures. All cells from individual rats were tested in triplicate. The cultures were incubated at 37°C in an atmosphere of 5% $CO_2$ in air for

5 days. Twenty-four hours before harvesting, 0.5 µCi of tritiated thymidine (New England Nuclear Corp.; specific activity, 6.7 Ci/mmol) was added to each well. The cells were harvested on glass fiber filters, dried, and counted in a liquid scintillation spectrometer (Beckman LS 100) with a xylene-based scintillation fluid (Scintilene; Fisher). All results are reported as mean counts per minute ± standard error.

**(v) Determination of levels of antibody to A. actinomycetemcomitans in serum, saliva, and gingival wash fluid.** Blood was obtained by cardiac puncture. Saliva was collected after subcutaneous administration of pilocarpine nitrate (Sigma; 1 mg/100 g of body weight). Gingival wash fluid was obtained as described above. An adaptation of an indirect enzyme-linked immunosorbent assay (ELISA) (10) was used for antibody measurement. In brief, Formalinized A. actinomycetemcomitans in 0.1 M sodium carbonate buffer (pH 9.6; 0.02% NaN$_3$) was bound to polystyrene microtiter plates (Linbro) at 37°C for 3.5 h. The procedure consisted of the following steps: (i) incubation of antigen-coated plates with rat serum (dilution, 1:200), saliva (undiluted), or gingival wash fluid (undiluted) in PBS containing 0.05% Tween 20 for 2 h at room temperature; (ii) incubation with monospecific rabbit anti-rat IgG (1:200), IgM (1:200), or IgA (1:50) for 2 h; (iii) incubation with affinity-purified goat anti-rabbit IgG conjugated to alkaline phosphatase (TAGO; 1:6,000) overnight; and (iv) addition of p-nitrophenyl phosphate (Sigma; 1 mg/ml) as a substrate. Reactivity was determined spectrophotometrically at 405 nm, and antibody levels were expressed as ELISA units related to a serum reference standard.

**(vi) IL-2 assay with gingival wash fluid.** IL-2 levels in gingival wash fluid supernatants were determined by an ELISA (5) performed with an automated system (Cetus/Perkin Elmer). Guinea pig anti-rat IL-2 (immunization with IL-2 [Collaborative Research, Waltham, Mass.] after preparative sodium dodecyl sulfate-polyacrylamide gel electrophoresis purification [38]), was plated onto "high-binding" ELISA plates (Nunc, Thousand Oaks, Calif.) at 30°C for 1 h; the sample was then added, and the plates were incubated for another hour. Rabbit anti-rat IL-2 (prepared as described above) was then added to the plates and incubated for 1 h, and then biotin-labeled goat anti-rabbit IgG (Vector Laboratories, Burlingame, Calif.) was added and incubated for 4 min. A mixture of Avidin-DH and biotinylated alkaline phosphatase (Vector) was then added and incubated for 20 minutes. p-Nitrophenyl phosphate was added (1 mg/ml), and the optical density at 405 nm was determined relative to an IL-2 standard (Collaborative Research). Half-maximal activity (in units per milliliter) in the standard was determined in a CTLL-2 cell proliferation assay (16).

**(vii) Horizontal bone loss.** After the mandibular and maxillary jaws were defleshed, a recticule eyepiece (4.1 grid squares = 1 mm) was used to measure the distance from the cementoenamel junction to the alveolar crest at a magnification of ×25. Recordings were made with the long axis of both buccal and lingual root surfaces of all molar teeth as previously described (41, 42). There were six recordings for the first molar, which has three roots, and four recordings for the second and third molars, each of which has two roots. The sum of the recordings for each tooth surface was used as a measure of the total bone loss expressed in millimeters. Measurements were made without prior knowledge of the group designation of the animals.

TABLE 2. Proliferative response to A. actinomycetemcomitans Y4

| Group | Response (mean ± SE, cpm) in: | |
| | Spleen cells | CLN[a] |
| --- | --- | --- |
| AaTh (n = 12) | 11,062 ± 1,235[b] | 2,828 ± 1,124 |
| AaNT (n = 12) | 7,781 ± 2,568[b] | 1,537 ± 423 |
| NAaNT (n = 10) | 6,345 ± 1,177[b] | 1,159 ± 666 |

[a] CLN, Cervical lymph node lymphocytes.
[b] Differences between AaNT and the other two groups were statistically significant (P < 0.039 by one-way analysis of variance).

## RESULTS

**Proliferative responses of recipient cervical lymphocytes and spleen cells to A. actinomycetemcomitans.** The spleens and cervical node cells of A3 clone recipient rats were investigated to determine whether the clone cells still resided in these organs at the termination of the experiment (Table 2). Proliferation with A. actinomycetemcomitans Y4 by spleen cells from animals receiving the A3 clone was significantly greater than proliferation by spleen cells from animals in the other groups. Although there were no significant differences in proliferative responses to A. actinomycetemcomitans by cervical node lymphocytes among the three groups, blastogenesis in the AaTh group animals was elevated above the responses of AaNT and NAaNT groups (Table 2). These data suggested that some cloned T cells could be found in these organs.

**Serum, salivary, and gingival wash fluid antibody to A. actinomycetemcomitans.** Specific antibody responses of the recipients to A. actinomycetemcomitans in serum, saliva, and gingival cell wash fluid were assayed (Table 3). Elevations in serum IgG, salivary IgA, and gingival IgG antibody in the AaTh group were highly significant. This finding also suggested that the transferred cells could be retained and remain functional in the recipient animals.

**Gingival cells.** We extracted gingival cells from all of the animals at the termination of the experiment. The average levels of cells ranged from approximately 10 × 10$^4$ to 12 × 10$^4$ gingival cells per mg of tissue, with lymphocytes representing from 1 to 4% of the cells recovered (Table 4). Although the numbers of total gingival cells recovered from the uninfected animals (NAaNT) were higher, elevated numbers of lymphocytes were recovered from the AaTh group, which had received the T-cell clone, suggesting that

TABLE 3. Serum, salivary, and gingival wash fluid antibody to A. actinomycetemcomitans in AaTh, AaNT, and NAaNT rat groups

| Group | Amt of antibody (EU)[a] | | | |
| | Serum antibody | | Saliva IgA | Gingival IgG |
| | IgG | IgM | | |
| --- | --- | --- | --- | --- |
| AaTh (n = 12) | 143 ± 55[b] | 47 ± 32 | 5 ± 2[c] | 12 ± 0.4[d] |
| AaNT (n = 14) | 4 ± 0.4[b] | 4 ± 0.2 | 2 ± 0.5[c] | 2 ± 0.3[d] |
| NAaNT (n = 10) | 4 ± 0.5[b] | 4 ± 0.4 | 2 ± 0.4[c] | 3 ± 0.1[d] |

[a] Mean ELISA units (EU) ± standard error (units not comparable among isotypes). Gingival IgG, 10$^{-2}$ EU.
[b] Significant differences at P < 0.01 in comparison with the value of the AaTh group.
[c] Differences statistically significant (P < 0.03 by one-way analysis of variance).
[d] Differences statistically significant (P < 0.007 by one-way analysis of variance).

TABLE 4. Gingival cells and lymphocytes recovered from AaTh, AaNT, and NAaNT rat groups[a]

| Group | Total no. of gingival cells ($10^4$/mg of tissue) | No. of lymphocytes ($10^3$/mg of tissue) | No. of lymphocytes with indicated phenotype/mg of tissue | | |
|---|---|---|---|---|---|
| | | | W3/25+ | OX8+ | B cells |
| AaTh | 10.8 ± 0.5[b] | 3.8 ± 0.6[c] | 454 ± 94[d] | 432 ± 109[e] | 2,264 ± 261[f] |
| AaNT | 10.3 ± 0.7[b] | 1.4 ± 0.3[c] | 138 ± 25[d] | 186 ± 43[e] | 1,372 ± 310[f] |
| NAaNT | 12.7 ± 0.6[b] | 1.8 ± 0.3[c] | 125 ± 45[d] | 137 ± 32[e] | 1,219 ± 294[f] |

[a] Data are means ± standard errors. For AaTh, n = 10 to 13; for AaNT, n = 6 to 15; and for NAaNT, n = 7 to 11.
[b] Differences statistically significant (P < 0.04 by one-way analysis of variance).
[c] Differences statistically significant (P < 0.0005 by one-way analysis of variance).
[d] Differences statistically significant (P < 0.0007 by one-way analysis of variance).
[e] Differences statistically significant (P < 0.05 by one-way analysis of variance).
[f] Differences statistically significant (P < 0.02 by one-way analysis of variance).

the gingiva was infiltrated with increased numbers of Th cells (presumably antigen specific). The characteristics of the gingival lymphocytes were investigated after the cells were stained with monoclonal antibodies to Th (W3/25)- and Ts (OX8)-type cells of rats and also with polyclonal antibody to rat B cells. The phenotypes of the gingival cells were determined by flow cytofluorometry (Table 4). There were significant elevations in levels of gingival T and B cells from rats receiving the Th clone. The most pronounced differences were observed in cells that had the A3 clone phenotype (W3/25), suggesting that the cloned cells could be residing in the gingival tissues.

**IL-2 level in gingival wash fluid.** IL-2 levels in gingival wash samples were measured by an avidin-biotin modification of an ELISA and are reported in half-maximal activity units. There was no significant difference in the levels of IL-2 between the control group (NAaNT) and the group that was infected but received no T cells (AaNT). However, there was a significant difference in the IL-2 levels obtained from gingival washes between the two control groups (AaNT, 158 ± 35 half-maximal units per ml; NAaNT, 152 ± 38 half-maximal units per ml [mean ± standard error]) and the group which received the cloned cells and was infected (AaTh; 330 ± 54 half-maximal units per ml; P < 0.008 by one-way analysis of variance). This may have been a consequence of the pronounced infiltration of T lymphocytes observed in the group receiving specific Th clone cells.

**Horizontal bone loss.** Bone loss was assessed as an indication of disease (Fig. 1). Infected normal animals (AaNT) demonstrated significantly more bone loss than animals in the group that received the Th cloned cells (AaTh). In fact, the group receiving cloned cells demonstrated a level of bone loss which was virtually identical to that of the group of animals that were not infected with A. actinomycetemcomitans.

## DISCUSSION

In an earlier study we demonstrated that adoptive transfer of enriched A. actinomycetemcomitans-specific Th cells to A. actinomycetemcomitans-infected rats resulted in decreased bone loss compared with that in animals receiving nonspecific Th cells (35). In the present study, we adoptively transferred an A. actinomycetemcomitans-specific Th clone (A3) with the phenotypic characteristics W3/13+, W3/25+, OX8−, and OX22− into normal rats that were infected with A. actinomycetemcomitans Y4. Proliferative responses of recipient splenocytes were elevated, suggesting survival of the cloned cells. Levels of serum IgG and IgM, salivary IgA, and gingival wash fluid IgG antibodies were dramatically elevated in recipients of the cloned Th cells. Numbers of

gingival lymphocytes and amounts of IL-2 recovered from rats that had received the cloned cells were also significantly elevated compared with control groups. Both T-lymphocyte and B-lymphocyte cell numbers in gingival tissue were significantly elevated in A3 recipient rats. Bone loss in A3 recipients was at the level of that in uninfected rats and significantly less than that found in infected control rats. These results are consistent with the hypothesis that T-cell regulation can affect periodontal disease (33, 35, 37). On the basis of the current experiments, we believe that the administration of antigen-specific Th cells to rats can interfere with periodontal bone loss.

The A3 clone used in this study had specificity for antigenic components of A. actinomycetemcomitans that are apparently shared by representative strains of other A. actinomycetemcomitans serotypes and the closely related species H. aphrophilus. The A3 clone did not react with isolated LPS from A. actinomycetemcomitans Y4 (19), although LPS is a significant B-cell mitogen in these animals (8, 40). Also, clone A3 did not respond to unrelated species of bacteria or to two protein antigens tested. The A3 clone bearing the helper phenotype (W3/25+ OX8− OX22−) pro-
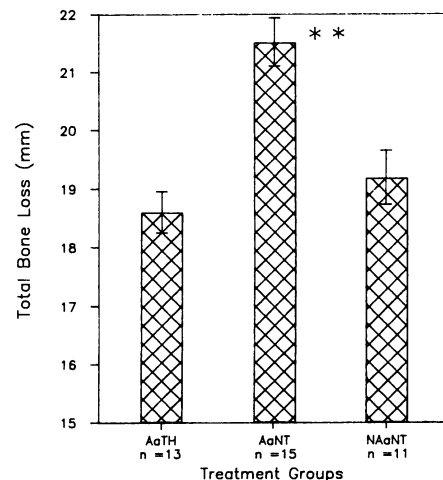


FIG. 1. Periodontal bone loss in infected rats receiving the Th clone cells (AaTh) compared with that in infected rats receiving no T cells (AaNT) and uninfected rats receiving no T cells (NAaNT). The bars show the means of the total bone loss in millimeters for the treatment groups. The standard errors of the means are shown by the error bars. The asterisks indicate statistical significance at P < 0.01 when AaNT is compared with the other two groups by one-way analysis of variance. The numbers of animals in each group are shown beneath the bars.

vided specific help for primed B cells and did not produce IL-2. Also, the A3 clone proliferated well in response to concanavalin A and was not involved in a delayed-type hypersensitivity response.

Recently, several laboratories have shown that murine $CD4^+$ T lymphocytes can be divided into at least two distinct subsets (Th1 and Th2) on the basis of the function and secretion of lymphokines (15, 26). The Th1 cells provide helper activity for polyclonal B-cell differentiation, have been involved in a delayed-type hypersensitivity response, have cytotoxic capacity, and release IL-2, gamma interferon, and tumor necrosis factor $\beta$. On the other hand, Th2 cells provide antigen-specific helper activity for B-cell Ig synthesis (6) and release IL-4, IL-5, and IL-6 (25, 26). In this context, our A3 clone was provisionally assumed to function as a Th2-type cell. Different functions have been assigned to $OX22^+$ ($CD45R^+$) and $OX22^-$ T helper cells in rats (17). Some evidence suggests that the $OX22^+$ $CD4^+$ subset behaves like Th1-type cells, while the $OX22^-$ $CD4^+$ subset more closely resembles Th2-type cells (17, 30). However, at present the relationship between the Th1 and Th2 types of T cells defined by OX22 antibody is not clear because few rat T-cell clones have been reported (29, 30) and attempts to find human T-cell clones that fall into the Th1/Th2 classification have generally failed (39).

The increased amount of IgG in the gingival wash fluid might especially suggest that a local gingival activation of the Th clones could take place in vivo. In fact, significantly increased gingival lymphocyte recoveries from A3-transferred animals were observed compared with the control groups. The phenotypic characteristics of these gingival lymphocytes has revealed that the most pronounced increases in cells in gingiva were of the W3/25 phenotype. However, levels of both $OX8^+$ and B cells were also increased in the rats receiving the Th clone (Table 4). It is conceivable that we have observed long-lived effects of the clone cells on B cells and other T cells which would account for these data.

The findings of elevated numbers of both $OX8^+$ and B cells in the gingiva from the A3-transferred group were consistent with our previous study (42) and also might explain the high level of IL-2, although the A3 clone was found to produce little or no IL-2 (Table 1). This finding supports the notion that the A3 clone cells can act in concert with other T cells. Furthermore, we have also injected the A3 clone into congenitally athymic rats. There was no indication of long-term survival of these cells (unpublished results), suggesting that other cell types were required for the survival or stimulation of the A3 clone. IL-2 is synthesized predominantly by stimulated helper T cells (14) and has a very short half-life in rat serum (7, 27). The IL-2 which is present in the gingiva of rats indicates that the cloned Th transferred cells may be locally stimulated by A. actinomycetemcomitans and locally involved in the synthesis of IL-2 in the gingival tissue.

It has been reported that decreased levels of IL-2 were eluted from the tissue around a ligature site associated with increased periodontal bone loss in an LBNF1 rat (35). That finding is consistent with this study, which demonstrated a negative correlation between bone loss and IL-2 levels obtained from the gingival tissue. In some cases, decreased levels of IL-2 obtained from the tissue may be due to increased IL-2 function, the resultant generation of IL-2 receptors on cells in the gingiva, and the subsequent reduction in the ability to elute IL-2 (4).

In addition to the periodontal role of IL-2 and the potential

involvement of antibody-mediated events in bone loss, the mechanism may involve direct helper effects of the Th2 cells on antibody synthesis and subsequent antibody-mediated effects on the disease-associated bacteria. A second potential mechanism may derive from the regulation, production, or utilization of other cytokines; e.g., IL-1$\beta$ can be responsible for osteoclastic resorption of bone. Since most Th2-type cell clones react with IL-1, these cells can serve to remove IL-1 from exerting effects on osteoclasts (20). Although the mechanism of periodontal destruction in this model is still unknown, it is clear that helper T cells have a role to play in the bone loss process.

In our studies of humans (33), elevated numbers of mononuclear cells were recovered from the gingival tissues of patients with periodontal disease. Normal tissues exhibited CD4/CD8 ratios almost identical to those in peripheral blood, whereas the diseased tissue cell ratios were significantly reduced, indicating alterations in the T-cell subset distribution in the tissue. The decreases in subset ratios could be attributed to statistically significant reduction in $CD4^+$ lymphocytes and also to slight relative increases in $CD8^+$ lymphocytes. These data as well as those from other studies (33, 37) support the concept of a local immunoregulatory imbalance in diseased periodontal tissues which could account for some destructive aspects of periodontal lesions. In the present study, an excess of Th, Ts, and B cells was found in the gingivae of adoptively transferred ($AaTh$) rats. The animals with the lowest $CD4^+/CD8^+$ ratios (not shown) were found in the $Aa$NT group, which also demonstrated the most bone loss. The elevated numbers of cells in the gingivae of $Aa$Th rats appear to indicate that the presence of A. actinomycetemcomitans antigen in the oral cavity can recruit antigen-specific Th cells to the area. This may be followed by a subsequent influx of $OX8^+$ and B cells. While the mechanisms and temporal sequence of this cellular infiltration are unknown, we infer that the following three features are foremost. (i) Th cells, presumably antigen specific, can be found in gingival tissues in high numbers. (ii) Antibody is formed in this area. (iii) This combination of factors seems to be protective with respect to periodontal bone loss. While further studies are required, the current data support the concept of Th cell-mediated protection with respect to experimental periodontal disease.

## ACKNOWLEDGMENTS

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