Functional human T-cell immunity and osteoprotegerin ligand control alveolar bone destruction in periodontal infection

Rapid PUBLICATION

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Periodontitis, a prime cause of tooth loss in humans, is implicated in the increased risk of systemic diseases such as heart failure, stroke, and bacterial pneumonia. The mechanisms by which periodontitis and antibacterial immunity lead to alveolar bone and tooth loss are poorly understood. To study the human immune response to specific periodontal infections, we transplanted human peripheral blood lymphocytes (HuPBLs) from periodontitis patients into NOD/SCID mice. Oral challenge of HuPBL-NOD/SCID mice with *Actinobacillus actinomycetemcomitans*, a well-known Gram-negative anaerobic microorganism that causes human periodontitis, activates human CD4$^+$ T cells in the periodontium and triggers local alveolar bone destruction. Human CD4$^+$ T cells, but not CD8$^+$ T cells or B cells, are identified as essential mediators of alveolar bone destruction. Stimulation of CD4$^+$ T cells by *A. actinomycetemcomitans* induces production of osteoprotegerin ligand (OPG-L), a key modulator of osteoclastogenesis and osteoclast activation. In vivo inhibition of OPG-L function with the decoy receptor OPG diminishes alveolar bone destruction and reduces the number of periodontal osteoclasts after microbial challenge. These data imply that the molecular explanation for alveolar bone destruction observed in periodontal infections is mediated by microorganism-triggered induction of OPG-L expression on CD4$^+$ T cells and the consequent activation of osteoclasts. Inhibition of OPG-L may thus have therapeutic value to prevent alveolar bone and/or tooth loss in human periodontitis.

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Introduction

Host inflammatory and immune responses to specific oral bacterial infections can result in periodontal disease, i.e., periodontitis (1). Human periodontitis is heterogeneous in etiology, but a common hallmark is alveolar bone destruction, one of the major causes of tooth loss in human (2, 3). Interestingly, human periodontitis has recently been implicated in the increased risks of certain systemic disorders such as pre-term low birth weight, bacterial pneumonia, congestive heart diseases, and stroke (4–8), possibly due to an underlying inflammatory trait (9). About 10–12 subgingival microorganisms have been implicated in the pathogenesis of periodontitis, including *Porphyromonas gingivalis*, *Prevotella intermedia*, *Bacteroides forsythus*, and mixed spirochetes (10). In particular, *Actinobacillus actinomycetemcomitans*, a Gram-negative facultative capnophilic rod bacterium, has been identified as the etiological agent of localized juvenile periodontitis (LJP) and of some rapidly progressing and severe forms of periodontitis (10–13). The prevalence of LJP is about 1–4% among teens and young adults, and 10% among insulin-dependent diabetic patients (10). LJP is characterized by advanced alveolar bone destruction in a molar-incisor pattern that often leads to tooth mobility and loss, resulting in functional and aesthetic deficits.

A. actinomycetemcomitans is able to invade the gingival epithelium (14) and releases several virulence factors such as cytotoxins, endotoxins, and a potent leukotoxin (15–17). *A. actinomycetemcomitans* infection is usually accompanied by local and systemic antigen-specific immune responses (18–19). Earlier studies demonstrated altered CD4$^+$/CD8$^+$ T-cell ratios and autologous mixed lymphocyte reactions in LJP patients (20, 21) and the ability of T helper cells to home to periodontal tissues in rat and mouse models of periodontitis (22–24). Further, we have previously demonstrated that *A. actinomycetemcomitans* infection in NOD/SCID mice engrafted with human peripheral blood leukocytes (HuPBLs) leads to periodontal inflammation characterized by the infiltration of CD4$^+$ T cells, CD8$^+$ T cells, CD20$^+$ B cells, and Mac1$^+$ macrophages into the fibrous connective tissues adjacent to the periodontal pockets (24). These results suggested that T cells could modulate bacterium-induced periodontal inflammation and/or alveolar bone destruction. However, the exact role of T-cell subtypes and B cells in periodontitis, the role of antibacterial

immunity in local alveolar bone destruction, and the mechanisms by which host immune responses contribute to alveolar bone destruction and/or tooth loss remain unclear.

To investigate the mechanism or mechanisms that regulate periodontal immunity and alveolar bone destruction, we transplanted HuPBLs from LJP patients into NOD/SCID mice (which lack endogenous T and B cells), generating HuPBL-NOD/SCID mice (24). Here we show that oral challenge of these "humanized" mice with *A. actinomycetemcomitans* (designated *Aa*-HuPBL-NOD/SCID) leads to functional activation of the human $CD4^+$ T cells in the periodontium and triggers local alveolar bone destruction. In vitro stimulation of $CD4^+$ T cells from these mice with antigens from *A. actinomycetemcomitans* leads to the expression of osteoprotegerin ligand (OPG-L, also known as TRANCE, ODF, and RANKL), a key mediator of osteoclastogenesis and osteoclast activation (25–31). Inhibition of OPG-L function via the decoy receptor osteoprotegerin (OPG) significantly reduces the alveolar bone destruction detected in *Aa*-HuPBL-NOD/SCID mice after bacterial inoculation, as well as the numbers of osteoclasts at the sites of local periodontal inflammation. These results identify for the first time a critical role for human $CD4^+$ T cells reactive to oral microorganisms in periodontal disease. Moreover, *A. actinomycetemcomitans*–triggered induction of OPG-L expression on T cells and OPG-L–mediated osteoclast activation and bone loss could provide one molecular explanation for the alveolar bone destruction observed in local periodontal infection.

Methods

Patients. Four LJP patients (3 males and 1 female, mean age 21 ± 4.4 years) were recruited as blood donors for the current study. Inclusion criteria, as defined previously (24), were: (a) positive anti–*A. actinomycetemcomitans* immunofluorescence reactions of subgingival plaque samples collected adjacent to advanced lesions, and (b) high serum Ab titers to *A. actinomycetemcomitans* (Y-4 or JP2 strains; see below) as detected by ELISA. Age-matched healthy subjects without clinical evidence of periodontal disease were recruited as controls. Informed consent was obtained from the patients, and all protocols used were approved by the Human Ethics Committee and the Animal Experimentation Committee of the University of Western Ontario.

Reagents. The following reagents were commercially purchased: Ficoll-Hypaque (Pharmacia Biotech Inc., Piscataway, New Jersey, USA); collagenase (Sigma Chemical Co., St. Louis, Missouri, USA); mAb's specific for human $CD45^+$ hematopoietic cells, human $CD45RO^+$ T cells, and human $CD19^+$ pan-B cells (Serotec Ltd., Kidlington, Oxford, United Kingdom); anti-human T-cell receptor (TCR-αβ), CD25, CD28, Fc receptor, CD3, CD4, CD8, and CD14 mAb's (PharMingen, San Diego, California, USA); rabbit and human complements (Cedarlane Laboratories Ltd., Hornby, Ontario, Canada); goat anti-mouse and mouse anti-human IgG-H+L FITC-conjugated Ab's (Bio-Rad Laboratories Inc., Hercules, California, USA, and R&D Systems Inc., Minneapolis, Minnesota, USA, respectively); and isotypic control Ab's (Sigma Chemical Co.; PharMingen). Human recombinant OPG-FITC conjugate and OPG-Fc fusion protein were prepared as previously described (25–27, 31). All tissue culture was performed in complete RPMI 1640 medium supplemented with 2–3% individual donor's serum and 10% FCS (GIBCO BRL, Burlington, Ontario, Canada).

Generation of HuPBL-NOD/SCID mice and A. actinomycetemcomitans inoculation. At day 0, 8- to 9-week-old female NOD/SCID ($H2^{d/d}$) mice were subjected to 2.5 Gy γ-irradiation prior to intraperitoneal injection of 10×10^6 to 35×10^6 mononuclear cells freshly prepared from HuPBLs by Ficoll-Hypaque gradient centrifugation (24, 32–33). On days 1, 3, and 5, the HuPBL-NOD/SCID mice were inoculated orally with live *A. actinomycetemcomitans* (100 μl of 10^{10} CFU/ml culture broth grown in an anaerobic chamber; Y-4 strain [no. 43781, American Type Culture Collection, Rockville, Maryland, USA] or JP2 strain [no. 29523, American Type Culture Collection]). Autologous HuPBL engraftment (weekly) and bacterial challenge (3 times per week) were repeated for 3–4 consecutive weeks. The infected mice were designated *Aa*-HuPBL-NOD/SCID mice. All *Aa*-HuPBL-NOD/SCID mice had serum levels of 0.1–0.85mg/ml IgG Ab's against *A. actinomycetemcomitans*. All mice were housed under specific pathogen-free conditions at the Animal Facility of the Faculty of Medicine and Dentistry, the University of Western Ontario, following institutional guidelines.

Measurement of alveolar bone loss in mice. Changes in alveolar bone mass in *Aa*-HuPBL-NOD/SCID mice were determined in maxillary molars as described (34). Briefly, horizontal bone loss, the distance between the cementum-enamel junction (CEJ) and the alveolar bone crest (ABC) along the long axis of either the buccal or lingual roots, was measured under a dissecting microscope on the mesiobuccal, midbuccal, distobuccal, mesiolingual, midlingual, and distolingual sites of the maxillary right and left molars (M1 and M2). The mean horizontal bone loss in each experimental group was calculated from the pooled data sets (the measurements of individual right and left molars, M1 and M2, per mouse). Baseline alveolar bone height was measured at day 1 (start point), whereas values at the fourth and eighth week were recorded as mid- and end-points, respectively. Results for each experimental group were normalized to the mean alveolar bone loss in control *A. actinomycetemcomitans*–inoculated BALB/c ($H2^{d/d}$) mice (100% ≈ 0.6 ± 0.12 mm per tooth in an 8-week period). Statistical significance of differences in alveolar bone loss between different groups was assessed by one-way ANOVA with a P value of less than 0.05.

T-cell activation. Periodontal $CD4^+$ T cells were prepared from *Aa*-HuPBL-NOD/SCID mice during weeks 4–6 and restimulated in 24-well plates with 100 μg/ml antigens from sonicated *A. actinomycetemcomitans* antigens (24) in the presence of irradiated (25 Gy) autologous HuPBL-derived monocytes/macrophages as antigen-pre-

senting cells (APCs) (24). After 48 hours, T-cell blasts were collected by Ficoll gradient centrifugation and sequentially immunostained with anti-human CD25 (IL-2 receptor-α chain) and FITC-conjugated goat anti-mouse IgG (H+L) Ab. The positive controls included human Jurkat T cells and HuPBL-derived $CD4^+$ T cells whose TCRs were cross-linked with anti-human TCR mAb (10 μg/ml for 10^6 cells) and anti–Fc receptor mAb. After 24 hours, 3×10^5 to 4×10^5 cells per sample were washed and CD25 expression was determined by flow cytometric analysis using a FACScan.

Immunization with A. actinomycetemcomitans in vivo. Systemic immune responses to *A. actinomycetemcomitans* were tested 6 weeks after the start of the experiment. *Aa*-HuPBL-NOD/SCID mice were immunized intraperitoneally with 30 μl *A. actinomycetemcomitans* sonicate antigens (1 μg/μl) mixed 1:1 with a 1:10 mixture of CFA/IFA adjuvants. After 14 days, human $CD4^+$ T cells were isolated from mouse spleens and purified by FACS (purity >85–95%). Purified T cells were subjected in triplicate to in vitro stimulation (1.5×10^4 T cells/well in U-bottom 96-well plates) using serial dilutions of *A. actinomycetemcomitans* sonicate antigens (1–100 μg/ml). After 48 hours, supernatants were collected for analysis of human IL-2 production by bioassay with IL-2–dependent CTLL-2 cells as described (24, 35). Periodontal $CD4^+$ T cells from *Aa*-HuPBL-NOD/SCID mice served as positive controls, while splenic $CD4^+$ T cells from nonimmunized HuPBL-NOD/SCID mice constituted the negative control.

Lymphocyte depletion and adoptive transfer experiments. For in vivo depletion of human lymphocytes in HuPBL-NOD/SCID mice, mAb's specific for human $CD4^+$ T cells, $CD8^+$ T cells, or B cells were injected (150–200 μg) intraperitoneally every 5–7 days from weeks 4–8 in the presence of human complement (24, 32–33). PBLs and splenocytes were stained with phycoerythrin-conjugated mAb's to human CD4, CD8, and CD20 (pan-B cells) to confirm the depletion of the corresponding cells by FACS analysis. The efficiency of immunodepletion was typically 95–98% without significant numbers of target cells remaining (data not shown) (24, 32–33). For adoptive transfer, 1×10^6 to 2×10^6 *A. actinomycetemcomitans*–reactive periodontal $CD4^+$ T cells were harvested from the margins of oral mucosal tissues of *Aa*-HuPBL-NOD/SCID mice during week 6–8; then these $CD4^+$ T cells (1×10^6 to 2×10^6) plus 3×10^6 to 5×10^6 irradiated (25 Gy) autologous HuPBL-derived monocytes/macrophages (as APCs) were adoptively transferred intravenously into naive NOD/SCID hosts. Adoptive transfer was performed weekly for 3–4 consecutive weeks and in each case immediately followed by oral inoculation with *A. actinomycetemcomitans as* described above.

OPG-L expression and inhibition of OPG-L activity in vivo. To detect OPG-L expression, periodontal $CD4^+$ T cells isolated from control or *Aa*-HuPBL-NOD/SCID mice were restimulated in vitro with *A. actinomycetemcomitans* sonicate antigens. OPG-L surface expression was detected using OPG-FITC (31). To investigate the in vivo inhibition of OPG-L by its natural decoy receptor OPG, recombinant human OPG-Fc fusion protein was injected into mice as described previously (25–27, 31). Briefly, *Aa*-HuPBL-NOD/SCID and control mice were treated intraperitoneally every other day with PBS or OPG-Fc (1 mg/kg) between weeks 4 and 8 for a total of 14 injections per mouse. By the end of the 8th week, all mice were sacrificed, and then alveolar bone loss was determined as described above and assessed by histology. Osteoclasts were identified in situ using tissue sections with osteoclast-specific tartrate-resistant acid phosphatase (TRAP) staining for determining the numbers of positive cells as described previously (31, 36).

Results

Construction of a model for A. actinomycetemcomitans–induced periodontal infection in HuPBL-NOD/SCID mice. To study the mechanism or mechanisms that regulate periodontal immunity and alveolar bone destruction, we transplanted HuPBL into NOD/SCID mice to generate HuPBL-NOD/SCID mice by using HuPBL from LJP patients and healthy donors, respectively (24). Four independent HuPBL-NOD/SCID chimeric mouse strains were generated using cells from four LJP patients. In all experiments, screening for the human $CD45^+$ hematopoietic cell marker revealed that the level of HuPBL engraftment in NOD/SCID mice detected over the 8-week period was 30–60% (24) (data not shown). Oral inoculation of HuPBL-NOD/SCID mice with *A. actinomycetemcomitans* resulted in periodontal inflammation characterized by the infiltration of human $CD4^+$ T cells, $CD8^+$ T cells, $CD20^+$ B cells, and $Mac1^+$ macrophages into the fibrous connective tissues adjacent to the periodontal pockets at the site of infection (24). Further, we have recently demonstrated the feasibility of generating human primary and secondary immune responses to exogenous antigens and putative pathogens isolated from human periodontal lesions in HuPBL-SCID and HuPBL-NOD/SCID animal models (32–33). As a result, both periodontal and splenic $CD4^+$ T cells from *Aa*-HuPBL-NOD/SCID mice were fully immunocompetent, as assessed by their significant upregulation of the high-affinity IL-2 receptor-α chain expression (CD25; data not shown) and production of the T-cell growth factor IL-2 (Figure 1a) in response to in vitro restimulation with sonicate antigens of *A. actinomycetemcomitans*, respectively. Splenic $CD4^+$ T cells from *Aa*-HuPBL-NOD/SCID mice failed to produce IL-2 in response to restimulation in vitro with sonicate antigens of *P. gingivalis* (Figure 1a). In addition, the TCR-Vα and TCR-Vβ repertoire of *A. actinomycetemcomitans*–reactive $CD4^+$ T cells from *Aa*-HuPBL-NOD/SCID mice significantly overlapped (>83%) that of cells isolated from periodontal surgical excisions from clinical LJP patients (data not shown). These results indicate that bacterial antigens can activate human T cells present in the periodontal tissues of the chimeric mice. Thus, *Aa*-HuPBL-NOD/SCID chimeric mice constitute a useful and specific model to investigate human oral pathogen-induced periodontal infection.

In humans, periodontal inflammation/infection results in destruction of

alveolar bone structure surrounding the teeth, ultimately followed by tooth loss (1–3). Thus, we analyzed whether alveolar bone loss occurs in *A. actinomycetemcomitans*–infected HuPBL-NOD/SCID mice. The results showed that periodontal and alveolar bone structures were comparable among sham-infected NOD/SCID (Figure 1b, group I) and HuPBL-NOD/SCID (Figure 1b, group II) mice, indicating that the transfer of HuPBL per se did not lead to tissue loss (see Figure 3a) (24). Neither did repeated inoculation of bacteria into nonchimeric NOD/SCID ($H2^{d/d}$) mice lead to significant levels of alveolar bone loss (Figure 1b, group III) compared with the positive control, *A. actinomycetemcomitans*–infected BALB/c mice (total bone loss was taken as "100%"). This is consistent with the findings of Baker et al. (37) that pathogen-induced alveolar bone loss in SCID mice is significantly less than that in immunocompetent hosts.

By week 8, groups I, II, and III showed significantly less alveolar bone loss ($P < 0.01$) than the positive control *A. actinomycetemcomitans*–infected BALB/c mice (taken as 100%), and about the same amount as occurred in noninfected BALB/c mice (data not shown). This background level of spontaneous alveolar bone loss (mean = 22.6 ± 3.4% of the positive control; see Figures 1b, 2, and 4e) has been shown to occur even under normal circumstances without bacterial infection. Accordingly, it has been recognized to represent a physiological alteration of the periodontium (38). However, oral inoculation of *A. actinomycetemcomitans* in NOD/SCID mice reconstituted with HuPBL from LPJ patients (Figure 1b, group IV) led, in time, to the development of alveolar bone destruction (mean = 82.2% of the positive control; see Figure 3, b and c). Chimeric mice constructed using HuPBL from each of four different LJP patients showed equivalent levels of alveolar bone destruction induced by *A. actinomycetemcomitans* (24) (data not shown). In contrast, NOD/SCID chimeric mice reconstituted with HuPBL from healthy human donors (Figure 1b, group V) followed by inoculation with bacteria developed only background levels of alveolar bone destruction by 8 weeks. These data indicate that a model of *A. actinomycetemcomitans*–specific periodontal infection can be produced in "humanized" NOD/SCID mice transplanted with HuPBL from LJP, but not from normal healthy subjects.

Human CD4⁺ T cells mediate alveolar bone destruction in periodontitis. We used our "humanized" mouse model to elucidate the exact role of human T-cell subtypes and B cells in periodontitis and the role of antibacterial immunity in local alveolar bone destruction. To assess the contribution of different lymphocyte subsets to alveolar bone destruction, we studied the effect of immunodepletion of $CD4^+$ T cells, $CD8^+$ T cells, or B cells from *Aa*-HuPBL-NOD/SCID mice. *Aa*-HuPBL-NOD/SCID mice depleted of $CD4^+$ T cells showed significantly less alveolar bone destruction by the end of 8 weeks (Figure 2, group III: mean = 37.6% of the positive control) compared with nondepleted *Aa*-HuPBL-NOD/SCID mice (Figure 2, group II). The relative reduction of alveolar bone destruction (Figure 2, group III) was significant (≈ 73.6% of that of *Aa*-HuPBL-NOD/SCID mice) after taking into account the background levels of spontaneous bone loss (≈ 22.6%; $P \leq 0.005$). The failure to achieve higher levels of reduction of alveolar bone loss was not due to

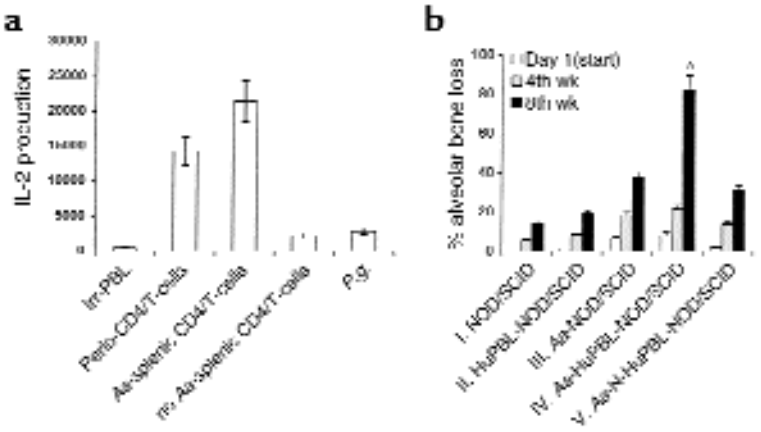

Figure 1

(**a**) Immunocompetence of $CD4^+$ T cells in *Aa*-HuPBL-NOD/SCID mice. IL-2 production of periodontal $CD4^+$ T cells (Perio-CD4/T-cells) and splenic $CD4^+$ T cells (*Aa*-splenic CD4/T-cells) isolated from *A. actinomycetemcomitans*–immunized HuPBL-NOD/SCID mice is shown. Irradiated (25 Gy) HuPBL-derived autologous monocytes/macrophages (irr-PBL) and $CD4^+$ T cells isolated from non–*Aa*-immunized HuPBL-NOD/SCID mice (no *Aa*-splenic CD4/T-cells) were included as the negative controls. Restimulation of *Aa*-immunized splenic $CD4^+$ T cells with a third-party antigen, *P. gingivalis* sonicate antigens served as the specificity control (*P.g.*). Serial dilution of *Aa* sonicate antigens did not result in significantly different patterns of the IL-2 responses measured. Differences in IL-2 production between the Perio-CD4/T-cell and *Aa*-splenic CD4/T-cell groups as compared with the irr-PBL, no *Aa*-splenic CD4/T-cell, and *P.g.* groups were statistically significant ($P < 0.008$, paired *t* test). Values shown are mean IL-2 production of triplicate samples ± SD. One result representative of three independent experiments is shown. (**b**) Increased alveolar bone loss in *Aa*-HuPBL-NOD/SCID mice. Groups of mice as indicated were either sham-infected or inoculated with *A. actinomycetemcomitans* (*Aa*). Data shown are the relative amounts of alveolar bone loss at the day of the first *Aa* inoculation (Day 1), by the end of 4 weeks (4th wk), and by the end of 8 weeks (8th wk), expressed as a percentage of the amount of bone loss in the positive control, *Aa*-infected BALB/c mice (100% = 0.6 ± 0.12 mm per tooth in 8 weeks). Group I, sham-infected NOD/SCID mice ($n = 10$); group II, sham-infected chimeric NOD/SCID mice engrafted with HuPBL from four LJP patients ($n = 12$); group III, NOD/SCID mice infected with *Aa* ($n = 16$); group IV, *Aa*-infected chimeric NOD/SCID mice engrafted with HuPBL from four LJP patients ($n = 32$); group V, *Aa*-infected chimeric NOD/SCID mice engrafted with HuPBL (N-HuPBL) from two healthy donors ($n = 12$). Data shown are mean values ± SD pooled from four independent experiments involving HuPBL engraftment from four LJP subjects, each of which gave comparable results. Alveolar bone loss was determined as described in Methods. [A]The extent of bone loss in group IV at 8 weeks was significantly increased as compared with all other groups ($P < 0.01$).

incomplete depletion of CD4+ T cells, as the efficiency of immunodepletion was typically 95–98% by FACS analysis (24, 32, 33). One conclusion would be that there are other cell type(s) involved in alveolar bone loss. However, histopathologically, only a mild inflammatory infiltrate was observed in the fibrous connective tissue adjacent to the periodontal pockets in seven of ten depleted mice studied (compare Figure 3, b and c, with Figure 3d). Alveolar bone loss in sham-infected CD4+ T cell–depleted HuPBL-NOD/SCID mice (Figure 2, group IV) was not significantly different from that in the sham-infected, nondepleted NOD/SCID mice (Figure 2, group I). By the end of 8 weeks, depletion of CD8+ T cells (Figure 2, group V) or B cells (Figure 2, group VI) from *Aa*-HuPBL-NOD/SCID mice did not significantly reduce alveolar bone loss ($P > 0.05$) compared with nondepleted *Aa*-HuPBL-NOD/SCID mice (Figure 2; Figure 3, e and f). Injection of either human complement alone or isotypic (IgG1a) control Ab into *Aa*-HuPBL-NOD/SCID mice resulted in the same level of alveolar bone loss as in nondepleted *Aa*-HuPBL-NOD/SCID mice (data not shown). These data show that specific removal of CD4+ T cells prevents inflammation and periodontal tissue damage in "humanized" animals in response to oral microbial infection, a result consistent with data from Baker et al., who reported that *P. gingivalis*–induced alveolar bone loss in mice required CD4+ T cells (39).

To obtain direct evidence that CD4+ T cells reactive to a specific microorganism can contribute to alveolar bone destruction, we performed adoptive transfer experiments using purified periodontal CD4+ T cells isolated from *Aa*-HuPBL-NOD/SCID mice. Purified periodontal CD4+ T cells (1×10^6 to 2×10^6 per mouse) were adoptively transferred to naive NOD/SCID hosts in the presence of irradiated autologous monocytes/macrophages followed by oral inoculation with *A. actinomycetemcomitans*. Transfer of these CD4+ T cells triggered significant alveolar bone destruction in the adoptive recipients by the end of 8 weeks (Figure 2, group VII: AT-CD4T), comparable with that observed in nondepleted *Aa*-HuPBL-NOD/SCID mice (Figure 2, group II). Adoptive transfer of irradiated autologous monocytes/macrophages alone as APCs did not yield any significant alveolar bone loss (Figure 2, group VIII: irr-APC). Thus, periodontal CD4+ T cells and/or CD4+ T cell–regulated immunity are critical for the pathogenesis of *A. actinomycetemcomitans*–induced periodontal disease and alveolar bone destruction in vivo (see also ref. 39).

A. actinomycetemcomitans stimulation triggers OPG-L expression on human CD4+ T cells. It remains unclear how CD4+ T cells mediate alveolar bone destruction in periodontal disease. We have previously shown that T cells stimulated via cross-linking of their antigen receptors express a membrane-bound form of OPG-L, a molecule of the TNF receptor superfamily (25–29). OPG-L is a key regulator of the development and activation of osteoclasts (26–30). Loss of OPG-L expression in gene-targeted mice results in severe osteopetrosis and a defect in tooth eruption due to a complete loss of osteoclast development (27). Further, we have recently shown that activated T cells can regulate systemic and local bone loss and joint destruction in a rat model of adjuvant-induced arthritis via OPG-L expression (31). These results prompted us to investigate the role of OPG-L in alveolar bone destruction during periodontal infections using our animal model.

To test whether CD4+ T cells activated by challenge with an oral microorganism expressed OPG-L, CD4+ T cells from *Aa*-HuPBL-NOD/SCID mice were collected and subjected to in vitro restimulation with *A. actinomycetemcomitans* sonicate antigens for

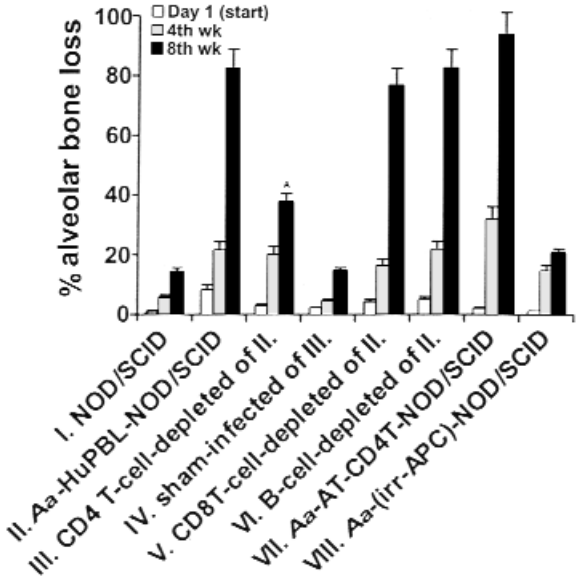

Figure 2
Regulation of alveolar bone destruction by *A. actinomycetemcomitans*–reactive CD4+ T cells. Groups of mice as indicated were either left untreated or inoculated with *A. actinomycetemcomitans*. CD4+ T, CD8+ T, or B cells were depleted from mice using specific Ab's and human complement as described in Methods. Data shown are the relative amounts (± SD) of alveolar bone loss accumulated by the end of 8 weeks, expressed as a percentage of the bone loss in the positive control, *Aa*-infected BALB/c mice (100%). Group I, sham-infected, nondepleted NOD/SCID mice ($n = 10$); group II, *Aa*-infected, nondepleted HuPBL-NOD/SCID mice ($n = 32$); group III, *Aa*-infected, CD4+ T cell–depleted NOD/SCID mice ($n = 20$); group IV, sham-infected, CD4+ T cell–depleted NOD/SCID mice ($n = 9$); group V, *Aa*-infected, CD8+ T cell–depleted NOD/SCID mice ($n = 12$); group VI, *Aa*-infected, B cell–depleted NOD/SCID mice ($n = 16$); group VII, *Aa*-infected NOD/SCID mice bearing adoptively transferred *Aa*-reactive CD4+ T cells (AT-CD4T) plus irradiated autologous monocytes/macrophages as APCs ($n = 10$); group VIII, *Aa*-infected NOD/SCID mice bearing adoptively transferred irradiated autologous monocytes/macrophages as APCs (irr-APC; $n = 6$). [A]Statistically significant difference in bone loss between group III and groups II, IV, V, and VI ($P < 0.005$).

2 days, followed by FACS analysis. Our data indicated that microbial stimulation did induce OPG-L expression on the surface of $CD4^+$ T cells (Figure 4, a–d). Interestingly, $CD4^+$ T cells from *Aa*-HuPBL-NOD/SCID mice failed to produce OPG-L in response to restimulation in vitro with a third-party species, *P. gingivalis* sonicate antigens (see Figure 4 legend), indicating that OPG-L induction is antigen-specific. Moreover, using RT-PCR we observed OPG-L mRNA expression in $CD4^+$ T cells isolated from the periodontal tissues of *Aa*-HuPBL-NOD/SCID and human LJP patients (data not shown). To determine whether the elevated expression of OPG-L correlated with an increase in osteoclast numbers, we determined the numbers of osteoclasts in the alveolar crestal bone areas of molars using TRAP staining, an enzymatic staining that specifically identifies osteoclast lineage cells (31, 36). Whereas only a few $TRAP^+$ osteoclasts were detected in noninfected HuPBL-NOD/SCID mice (used as a negative control), osteoclast numbers were significantly increased in *Aa*-HuPBL-NOD/SCID mice (Table 1). Thus, microbial stimulation of human $CD4^+$ T cells triggers the production of the key osteoclast activation and differentiation factor OPG-L, and local microbial infections increase the numbers of osteoclasts in the periodontal tissues. These data provide the first direct evidence, to our knowledge, that environmental pathogens, i.e., the oral microorganism *A. actinomycetemcomitans*, can activate OPG-L production in T cells.

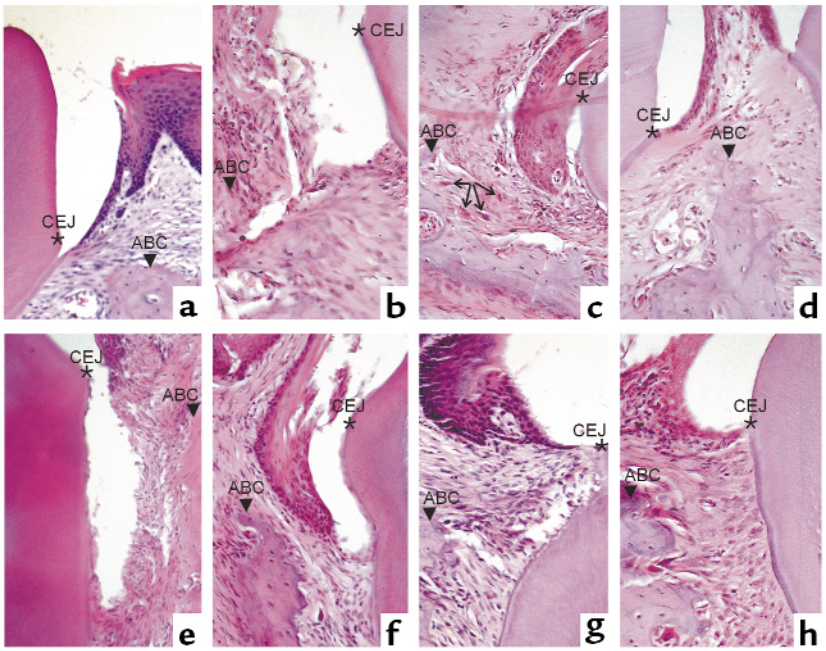

Figure 3
Histopathological lesions and bone loss in *Aa*-HuPBL-NOD/SCID mice. CEJ, cementoenamel junction, a landmark for tissue loss; ABC, alveolar bone crest. The distance between CEJ and ABC reflects the amount of tissue or alveolar bone loss measured as described in Methods. (**a**) A representative tissue section from sham-infected HuPBL-NOD/SCID mice demonstrating the normal periodontal and alveolar bone structures (as in Figure 1b, group II). (**b**, **c**) Typical histopathological lesions show significant inflammatory infiltration, connective tissue loss below CEJ (in **b**), and alveolar bone loss with apical growth of sulcular epithelium below CEJ and into alveolar bone area (in **c**) in *Aa*-HuPBL-NOD/SCID mice (as in Figure 2, group II) by the end of 8 weeks. Some multinucleated giant cells (arrows in **c**) can be observed along the surface of alveolar crestal bone. (**d**) Mild inflammatory infiltrates without obvious alveolar bone loss, as evidenced by sulcular epithelium located at CEJ, in the periodontal tissues of $CD4^+$ T cell–depleted *Aa*-HuPBL-NOD/SCID mice (as in Figure 2, group III) by the end of 8 weeks. (**e**, **f**) In vivo depletion of $CD8^+$ T cells (**e**) (as in Figure 2, group V) or B cells (**f**) (as in Figure 2, group VI) has no apparent effects on existing periodontal inflammation accompanied by tissue loss below CEJ (in **e**) or alveolar bone loss with apical growth of sulcular epithelium below CEJ and ABC (in **f**). These findings are consistent with those observed in nondepleted *Aa*-HuPBL-NOD/SCID mice in **b** and **c**. (**g**, **h**) In vivo inhibition of OPG-L via the decoy receptor OPG abrogates alveolar bone destruction, as evidenced by sulcular epithelium located at CEJ and above ABC (in both panels), in *Aa*-HuPBL-NOD/SCID mice by the end of 8 weeks (as in Figure 4e, group III). Note that OPG treatment does not affect periodontal inflammation observed in **g** and **h**. *Aa*-infected HuPBL-NOD/SCID mice were treated with an OPG-Fc fusion protein as described in Methods. Parts **g** and **h** are representative of eight mice studied in this group. Magnifications: **a**, **b**, **d**–**h**, ×200; **c**, ×100.

Inhibition of OPG-L in vivo blocks alveolar bone destruction in A. actinomycetemcomitans–inoculated "humanized" mice. To test whether OPG-L expression has biological relevance in alveolar bone destruction during periodontal infections, that is, whether the inhibition of OPG-L activity in vivo could reduce *A. actinomycetemcomitans*–induced alveolar bone loss, we took advantage of the existence of OPG, the natural decoy receptor of OPG-L (26–27, 31). Soluble recombinant OPG-Fc fusion protein was injected intraperitoneally into *Aa*-HuPBL-NOD/SCID mice every other day for 4 weeks. By the end of 8 weeks, the amount of alveolar bone loss detected in OPG-Fc–treated *Aa*-HuPBL-NOD/SCID mice was significantly reduced (Figure 3, g and h; Figure 4e, group III: mean = 38.2% of the positive control) compared with that of *Aa*-HuPBL-NOD/SCID mice that were not injected with OPG-Fc (Figure 4e: group II). This reduction of alveolar bone destruction (Figure 4e, group III) was significant (≈ 72.6% of that of *Aa*-HuPBL-NOD/SCID mice) after taking account of the background levels of spontaneous bone loss. Control injection of PBS, the vehicle for OPG-Fc, into *Aa*-HuPBL-NOD/SCID mice (Figure 4e, group IV) did not significantly alter alveolar bone loss when compared with group II. In a separate experiment, injecting ten times the amount of OPG-Fc in vivo ($n = 4$) did not result in any further reduction of alveolar bone destruction (data not shown), suggesting that (a) maximal dose inhibition was attained, and/or (b) another cell type or types may be

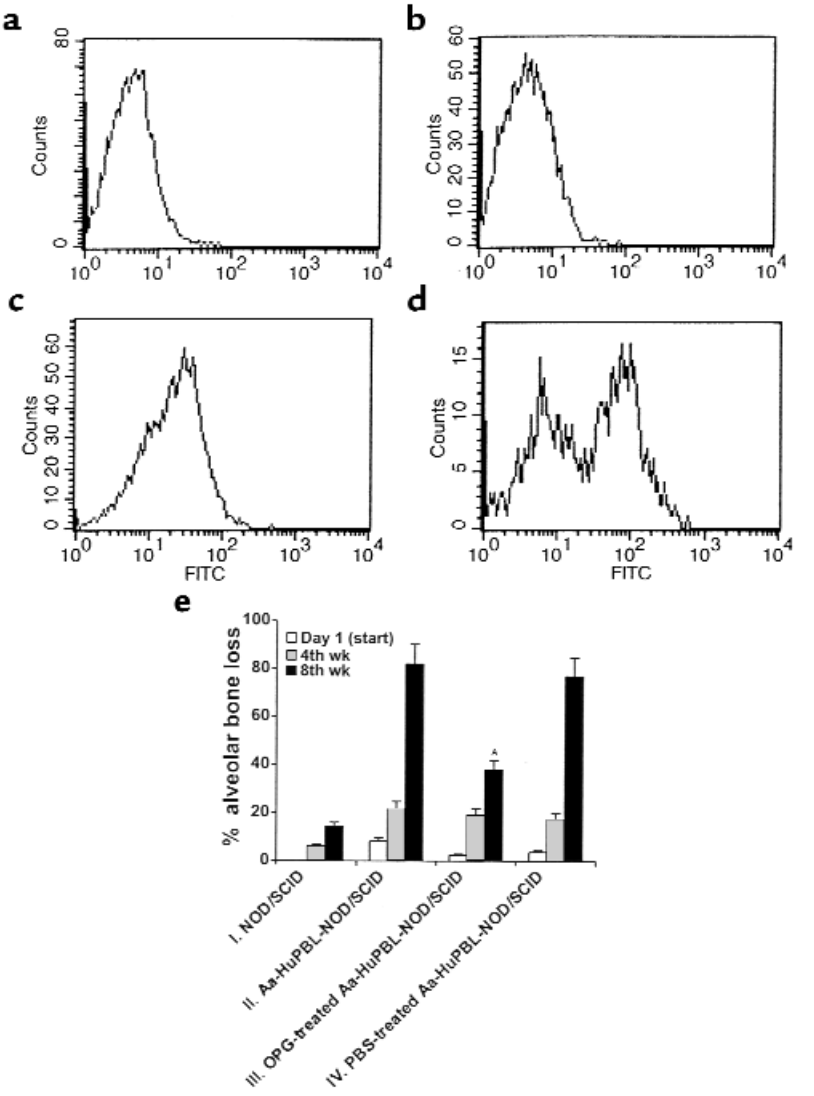

Figure 4
(**a**–**d**) *A. actinomycetemcomitans* stimulates OPG-L expression on periodontal CD4+ T cells. (**a**) Unstimulated HuPBL-derived CD4+ T cells stained with OPG-FITC; negative control. (**b**) HuPBL-derived CD4+ T cells restimulated with anti-TCR plus CD28 mAb's, followed by staining with isotypic control Ab; background control. (**c**) HuPBL-derived CD4+ T cells restimulated with anti-TCR plus CD28 mAb's, followed by staining with OPG-FITC; positive control. (**d**) Periodontal CD4+ T cells derived from *Aa*-HuPBL-NOD/SCID mice stimulated with *A. actinomycetemcomitans* sonicate antigens, followed by staining with OPG-FITC. Periodontal CD4+ T cells restimulated with *P. gingivalis* sonicate antigens did not induce significant membrane OPG-L expression over the background level (data not shown). OPG-L membrane expression was determined by FACS analyses 48 hours later. (**e**) Reduction of alveolar bone destruction in OPG-Fc treated *Aa*-HuPBL-NOD/SCID mice. Groups of mice as indicated were infected with *A. actinomycetemcomitans* followed by in vivo treatment with soluble human OPG-Fc fusion protein. Alveolar bone loss (mean values ± SD) was assessed at 8 weeks and normalized to the positive control, *Aa*-infected BALB/c mice (100%). Group I, sham-infected NOD/SCID mice (n = 10); group II, *Aa*-infected HuPBL-NOD/SCID mice not injected with OPG-Fc (n = 16); group III, *Aa*-infected HuPBL-NOD/SCID mice injected with OPG-Fc (n = 10); group IV, *Aa*-infected HuPBL-NOD/SCID mice injected with PBS (n = 8). [A]The differences in alveolar bone loss between group III and groups II and IV are statistically significant (P < 0.002).

involved in mediating alveolar bone loss (see below for discussion). Thus, inhibition of OPG-L via OPG significantly decreases alveolar bone destruction and local bone resorption in *Aa*-HuPBL-NOD/SCID mice. Moreover, whereas the number of osteoclasts was significantly increased in HuPBL-NOD/SCID mice in response to microbial infection, only a few TRAP+ osteoclasts could be detected in *Aa*-HuPBL-NOD/SCID mice treated with OPG (Table 1). These results indicate that the alveolar bone destruction observed in periodontitis is due at least in part to the action of osteoclasts activated by OPG-L. Interestingly, inhibition of OPG-L activity via OPG had no obvious effect on the severity of periodontal inflammation itself (Figure 3, g and h), consistent with our previous findings in the rat arthritis model (31) and in the collagen-induced arthritis model (40). This observation implies that tissue inflammation and alveolar bone destruction are relatively independent aspects of periodontal infections. In summary, our functional data show that *A. actinomycetemcomitans* activates CD4+ T cells in the periodontium and induces them to express OPG-L, and that OPG-L is a key mediator of alveolar bone destruction in microorganism-induced periodontal infection.

Discussion

Periodontitis, a prime cause of tooth loss in humans, results from the interplay between specific bacterial infections and host immune responses. Human periodontitis is characterized by prominent inflammatory infiltrates and is associated with irreversible loss of alveolar bone and/or connective tissue attachment in the periodontium. We generated HuPBL-NOD/SCID mice, using human leukocytes from LJP patients transplanted into NOD/SCID mice (24), to elucidate the mechanisms by which antimicrobial host immune responses contribute to alveolar bone destruction. Oral challenge of HuPBL-NOD/SCID mice with *A. actinomycetemcomitans* produced a microorganism-specific human periodontal infection in a "humanized" mouse model.

Perturbation of the T-cell immune repertoire is often observed in chronic inflammatory diseases such as rheumatoid arthritis (41–42). Recently, we have shown using a rat model of arthritis that activated T cells participate in regulating bone loss and joint

Table 1
The numbers of TRAP+ osteoclasts in the alveolar crestal bone area of molars

	No. ± SEM
Aa-HuPBL-NOD/SCID mice (n = 12)	14.1 ± 4.5
OPG-L–treated *Aa*-HuPBL-NOD/SCID mice (n = 10)	3.2 ± 2.2[A]
PBS-treated *Aa*-HuPBL-NOD/SCID mice (n = 8)	12.5 ± 3.6
Aa-infected control BALB/c mice (n = 10)	17.3 ± 4.2
Noninfected HuPBL-NOD/SCID mice (n = 5)	2.6 ± 1.7

The numbers of TRAP+ osteoclasts were identified at week 8 *of A. actinomycetemcomitans* infection (see Methods). [A]Significant differences (Student's t test; $P < 0.02$) were found between OPG-L–treated *Aa*-HuPBL-NOD/SCID mice and the following: *Aa*-HuPBL-NOD/SCID mice, PBS-treated *Aa*-HuPBL-NOD/SCID mice, and *A. actinomycetemcomitans*–infected control BALB/c mice.

destruction through their expression of OPG-L, a key mediator of osteoclastogenesis and osteoclast activation (31). While T cells are probably not required for normal bone homeostasis (27, 29–30), we proposed that local or systemic pathology within an osseous environment due to microbial or viral infections, autoimmune conditions (31), tumorigenesis or metastasis (43), or injuries could attract T cells capable of contributing to bone remodeling through the production of OPG-L.

Our results show that the oral microorganism *A. actinomycetemcomitans* can activate OPG-L production in CD4+ T cells, and they provide, albeit indirectly, the first evidence that environmental pathogens regulate expression of OPG-L on pathogen-specific T cells. Recently, OPG-L–independent triggering of osteoclast differentiation has been reported (44), a finding consistent with our observation that injection of soluble OPG (for 4 weeks) did not completely block alveolar bone destruction in vivo (approximately 75%; Figure 4e). It must be acknowledged that there are auxiliary factors of potential importance in the model we describe. Thus it is known that the expression of proinflammatory cytokines (e.g., IL-1 and TNF-α) can also regulate the relative balance of OPG-L and OPG (osteoclastogenesis inhibitory factor) in the bone microenvironment and/or mesenchymal tissues adjacent to bone (45–46), thus contributing to bone destruction independently of cell-mediated immunity. Additionally, the potential contribution of local mucosal cells, dental cells, and/or phagocytes (e.g., monocytes/macrophages, etc.; refs. 47, 48) in the early stages of infection or inflammation has yet to be defined, either in this model or in human periodontitis.

Thus while our data suggest that microorganism-activated CD4+ T cells are implicated as an important feature of alveolar bone loss in our model system, they do not prove unequivocally that OPG-L produced by those CD4+ T cells is responsible for the increased alveolar bone destruction seen. Selective depletion of such cells prior to adoptive transfer would address this question but has not yet proven to be technically feasible. Nevertheless, it is likely that CD4+ T cells of LJP patients differ fundamentally (in phenotype and/or genotype) from those of normal healthy subjects (49). This possibility is supported by our recent separate study, in which CD4+ T cells isolated from *Aa*-N-HuPBL-NOD/SCID mice (using HuPBL from normal healthy subjects; Figure 1b: group V) showed significantly less OPG-L expression by FACS analysis (data not shown). However, the underlying mechanism(s) for any differences awaits further investigation.

In summary, our results show that CD4+ T cell–mediated immunity is involved in the modulation of periodontal bone destruction in HuPBL-NOD/SCID mice after oral inoculation of *A. actinomycetemcomitans*. In vivo and in vitro *A. actinomycetemcomitans* stimulation results in the activation of CD4+ T cells and upregulation of OPG-L. Inhibition of OPG-L function via the decoy receptor OPG significantly reduces alveolar bone destruction in HuPBL-NOD/SCID mice in response to oral microbial infections. Moreover, OPG treatment significantly reduces the numbers of osteoclasts at the sites of local periodontal infection. Experiments are currently under way to test whether the same strategy can be used to prevent alveolar bone destruction before the full burst of periodontal inflammation and immune activation is established. Our results suggest a critical role for human CD4+ T cells reactive to oral microorganisms in periodontal disease. Microbial induction of OPG-L expression on T cells and OPG-L–mediated osteoclast activation and bone loss thus can provide a molecular explanation for the alveolar bone destruction observed in local periodontal infections. Inhibition of the function of OPG-L may thus have therapeutic value to prevent or to interrupt alveolar bone and/or tooth loss in human periodontal disease.

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1. Clark, W.B., and Loe, H. 1993. Mechanisms of initiation and progression of periodontal disease. *Periodontol. 2000.* **2**:72–82.
2. Brown, L.J., Oliver, R.C., and Loe, H. 1990. Evaluating periodontal status of US employed adults. *J. Am. Dent. Assoc.* **121**:226–232.
3. Eklund, S.A., and Burt, B.A. 1994. Risk factors for total tooth loss in the United States; longitudinal analysis of national data. *J. Public Health Dent.* **54**:5–14.
4. Genco, R. 1997. Research news. Healthy gums for a happy heart. *Science.* **276**:203.
5. Mattila, K.L., Valle, M.S., Nieminen, M.S., Valtonen, V.V., and Hietaniemi, K.L. 1993. Dental infections and coronary atherosclerosis. *Atherosclerosis.* **103**:205–211.
6. DeStefano, F., Anda, R.F., Kahn, S., Williamson, D.F., and Russell, C.M. 1993. Dental disease and risk of coronary heart disease and mortality. *Br. Med. J.* **306**:688–691.
7. Scannapieco, F.A., and Mylotte, J.M. 1996. Relationship between periodontal disease and bacterial pneumonia. *J. Periodontol.* **67**:1114–1122.
8. Danesh, J. 1999. Coronary heart disease, Helicobacter pylori, dental disease, Chlamydia pneumoniae, and cytomegalovirus: meta-analyses of prospective studies. *Am. Heart J.* **138**:S434–S437.

9. Becker, J., Garcia, R., Heiss, G., Vokonas, P.S., and Offenbacher, S. 1996. Periodontal disease and cardiovascular disease. *J. Periodontol.* **67**:1123–1137.
10. Zambon, J.Z. 1996. Periodontal diseases: microbial factors. *Ann. Periodontol.* **1**:879–925.
11. Brown, L.J., and Loe, H. 1991. Juvenile periodontitis in the United States of America. *J. Periodontol.* **62**:608–616.
12. Zambon, J.J. 1985. *Actinobacillus actinomycetemcomitans* in human periodontal disease. *J. Clin. Periodontol.* **12**:1–20.
13. Zambon, J.J., et al. 1988. *Actinobacillus actinomycetemcomitans* in the pathogenesis of human periodontal disease. *Adv. Dent. Res.* **2**:269–274.
14. Meyer, D.H., and Fives-Taylor, P.M. 1994. Characterization of adherence of *Actinobacillus actinomycetemcomitans* to epithelial cells. *Infect. Immun.* **62**:928–935.
15. Fives-Taylor, P., Meyer, D.H., and Mintz, K. 1996. Virulence factors of the periodontopathogen *Actinobacillus actinomycetemcomitans*. *J. Periodontol.* **67**(Suppl.):291–297.
16. Zambon, J.J., Haraszthy, V.I., Lally, E.T., and Denmuth, D.R. 1996. The microbiology of early-onset periodontitis: association of highly toxic *Actinobacillus actinomycetemcomitans* strains with localized juvenile periodontitis. *J. Periodontol.* **67**:282–290.
17. Lally, E.T., Kieba, I.R., Golub, E.E., Lear, J.D., and Tanaka, J.C. 1996. Structure/function aspects of *Actinobacillus actinomycetemcomitans* leukotoxin. *J. Periodontol.* **67**:298–308.
18. McArthur, W.P., and Clark, W.B. 1993. Specific antibodies and their potential role in periodontal diseases. *J. Periodontol.* **64**:807–818.
19. Ebersole, J.L., and Taubman, M.A. 1994. The protective nature of host responses in periodontal diseases. *Periodontol. 2000.* **5**:112–141.
20. Stoufi, E.D., Taubman, M.A., Ebersole, J.E., Smith, D.J., and Stashenko, P.P. 1987. Phenotypic analysis of mononuclear cells recovered from healthy and diseased human periodontal tissues. *J. Clin. Immunol.* **77**:235–245.
21. Suzuki, J., Park, S.K., and Falker, W.A., Jr. 1984. Immunological profiles of juvenile periodontitis. I. Lymphocyte blastogenesis and the autologous mixed lymphocyte response. *J. Periodontol.* **55**:453–459.
22. Yamashita, K., Eastcott, J.W., Taubman, M.A., Smith, D.J., and Cox, D.S. 1991. Effect of adoptive transfer of cloned *Actinobacillus actinomycetemcomitans*-specific T helper cells on periodontal disease. *Infect. Immun.* **59**:1529–1534.
23. Eastcott, J.W., Yamashita, K., Taubman, M.A., Harada, Y., and Smith, D.J. 1994. Adoptive transfer of cloned T helper cells ameliorates periodontal disease in nude rats. *Oral Microbiol. Immunol.* **9**:284–289.
24. Teng, Y.-T., et al. 1999. Periodontal immune responses of human lymphocytes in *Actinobacillus actinomycetemcomitans*-inoculated NOD/SCID mice engrafted with peripheral blood leukocytes of periodontitis patients. *J. Periodontol. Res.* **34**:54–61.
25. Simonet, W.S., et al. 1997. Osteoprotegerin: a novel secreted protein involved in the regulation of bone density. *Cell.* **89**:309–319.
26. Lacey, D.L., et al. 1998. Osteoprotegerin ligand is a cytokine that regulates osteoclast differentiation and activation. *Cell.* **93**:165–176.
27. Kong, Y.Y., et al. 1999. OPGL is a key regulator of osteoclastogenesis, lymphocyte development and lymph-node organogenesis. *Nature.* **397**:315–323.
28. Takahashi, N., Udagawa, N., and Suda, T. 1999. A new member of tumor necrosis factor ligand family, ODF/OPGL/TRANCE/RANKL, regulates osteoclast differentiation and function. *Biochem. Biophys. Res. Commun.* **256**:449–455.
29. Anderson, D.M., et al. 1997. A homologue of the TNF receptor and its ligand enhance T-cell growth and dendritic-cell function. *Nature.* **390**:175–179.
30. Hsu, H., et al. 1999. Tumor necrosis factor receptor family member RANK mediates osteoclast differentiation and activation induced by osteoprotegerin ligand. *Proc. Natl. Acad. Sci. USA.* **96**:3540–3545.
31. Kong, Y.Y., et al. 1999. Activated T cells regulate bone loss and joint destruction in arthritis via OGPL. *Nature.* **402**:304–309.
32. Sandhu, J., Shpitz, B., Gallinger, S., and Hozumi, N. 1994. Human primary immune response in SCID mice engrafted with human peripheral blood lymphocytes. *J. Immunol.* **152**:3808–3813.
33. Hozumi, N., Gorczynski, R.M., Peters, W., and Sandhu, J.S. 1994. A SCID mouse model for human immune response and disease. *Res. Immunol.* **145**:370–379.
34. Evans, R.T., et al. 1992. Periodontopathic potential of two strains of *Porphyromonas gingivalis* in gnotobiotic rats. *Arch. Oral Biol.* **37**:813–819.
35. Teng, Y.-T., Williams, D., Hozumi, N., and Gorczynski, R.M. 1996. Multiple levels of regulation for self tolerance in beef insulin transgenic mice. *Cell Immunol.* **173**:183–191.
36. Asotora, S., Gupta, A.K., Sodek, J., Aubin, J.E., and Heersche, J.N.M. 1994. Carbonic anhydrase 2 mRNA expression in individual osteoclasts under "resorbing" and "nonresorbing" conditions. *J. Bone Miner. Res.* **9**:1115–1122.
37. Baker, P.J., Evans, R.T., and Roopenian, D.C. 1994. Oral infection with *Prophyromonas gingivalis* and induced alveolar bone loss in immunocompetent and severe combined immunodeficient mice. *Arch. Oral. Biol.* **39**:1035–1040.
38. Page, R.C. 1982. *Periodontitis in man and other animals: a comparative review.* Blackwell Publisher. Toronto, Canada. 60–71.
39. Baker, P.J., Dixon, M., Evans, R.T., Dufour, L., and Roopenian, D.C. 1999. CD4 (+) T cells and the proinflammatory cytokines gamma interferon and interleukin-6 contribute to alveolar bone loss in mice. *Infect. Immun.* **67**:2804–2809.
40. Lubberts, E., et al. 2000. IL-4 gene therapy for collagen arthritis suppresses synovial IL-17 and osteoprotegerin ligand and prevents bone erosion. *J. Clin. Invest.* **105**:1697–1710.
41. Wagner, U.G., Koetz, K., Weyand, C.M., and Goronzy, J.J. 1998. Perturbation of the T cell repertoire in rheumatoid arthritis. *Proc. Natl. Acad. Sci. USA.* **95**:14447–14452.
42. Goronzy, J.J., Zettl, A., and Weyand, C.M. 1999. T cell receptor repertoire in rheumatoid arthritis. *Int. Rev. Immunol.* **17**:339–363.
43. Honore, P., et al. 2000. Osteoprotegerin blocks bone cancer-induced skeletal destruction, skeletal pain and pain-related neurochemical reorganization of the spinal cord. *Nat. Med.* **6**:521–528.
44. Kobayashi, K., et al. 2000. Tumor necrosis factor alpha stimulates osteoclast differentiation by a mechanism independent of the ODF/RANKL-RANK interaction. *J. Exp. Med.* **191**:275–286.
45. Hofbauer, L.C., et al. 1999. Interleukin-1beta and tumor necrosis factor-alpha, but not interleukin-6, stimulate osteoprotegerin ligand gene expression in human osteoblastic cells. *Bone.* **25**:255–259.
46. Hofbauer, L.C., et al. 2000. The roles of osteoprotegerin and osteoprotegerin ligand in the paracrine regulation of bone resorption. *J. Bone Miner. Res.* **15**:2–12.
47. Rani, C.S., and MacDougall, M. 2000. Dental cells express factors that regulate bone resorption. *Mol. Cell Biol. Res. Commun.* **3**:145–152.
48. Kawashima, N., and Stashenko, P. 1999. Expression of bone-resorptive and regulatory cytokines in murine periapical inflammation. *Arch. Oral Biol.* **44**:55–66.
49. Gainet, J., et al. 1999. Neutrophil dysfunction, IL-8, and soluble L-selectin plasma levels in rapidly progressive versus adult and localized juvenile periodontitis: variations according to disease severity and microbial flora. *J. Immunol.* **163**:5013–5019.