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*J Immunol* 2000; 164:2102-2109; doi: 10.4049/jimmunol.164.4.2102

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Requirement of B7 Costimulation for Th1-Mediated Inflammatory Bone Resorption in Experimental Periodontal Disease¹

Toshihisa Kawai,* Ronit Eisen-Lev,* Makoto Seki, † Jean W. Eastcott,* Mark E. Wilson, ‡ and Martin A. Taubman²**

The CD28 costimulation at TCR signaling plays a pivotal role in the regulation of the T cell response. To elucidate the role of T cells in periodontal disease, a system of cell transfer with TCR/CD28-dependent Th1 or Th2 clones was developed in rats. Gingival injection of specific Ag, Actinobacillus actinomycetemcomitans 29-kDa outer membrane protein, and LPS could induce local bone resorption 10 days after the transfer of Ag-specific Th1 clone cells, but not after transfer of Th2 clone cells. Interestingly, the presence of LPS was required not only for the induction of bone resorption but also for Ag-specific IgG2a production. LPS injection elicited the induction of expression of both B7-1 and B7-2 expression on gingival macrophages, which otherwise expressed only MHC class II when animals were injected with Ag alone. The expression of B7 molecules was observed for up to 3 days, which corresponded to the duration of retention of T clone cells in gingival tissues. Either local or systemic administration of CTLA4Ig, a functional antagonist of CD28 binding to B7, could abrogate the bone resorption induced by Th1 clone cells combined with gingival challenge with both Ag and LPS. These results suggest that local Ag-specific activation of Th1-type T cells by B7 costimulation appeared to trigger inflammatory bone resorption, whereas inhibition of B7 expression by CTLA4Ig might be a therapeutic approach for intervention with inflammatory bone resorption. *The Journal of Immunology, 2000, 164: 2102–2109.

Periodontal disease (PD)¹ (1) and rheumatoid arthritis (RA) (2) are diseases that bring about local bone resorption in conjunction with inflammation. T cell immune reactions to bacteria seem to play a key role in connective tissue destruction and bone resorption in PD (3). T lymphocytes are abundant in diseased periodontal tissue (4). Elevated levels of IgG Ab response to bacterial protein Ags in patients compared with healthy subjects (5) suggests a T cell association with host immune reaction to these bacteria. IgG production cannot be induced to T-dependent Ags without T cell help (6). However, the regulatory role of T cells in PD is unclear. The study of T cell involvement in RA has been facilitated by an animal model of collagen-induced arthritis (CIA) in which collagen-specific Th1-type cells cause joint inflammation (7). Therefore, development of an animal model of PD is required to gain insight into the immunopathological mechanisms of PD, especially the role of T cells in the context of connective tissue destruction and inflammatory bone resorption.

The effects of a variety of cytokines have been investigated in vitro models of osteoclast induction from bone marrow precursor cells. Proinflammatory cytokines such as IL-1αβ and TNF-α induce bone resorption by promoting differentiation of osteoclast precursor cells and by activating osteoclast cells (8). In contrast, Th2-type cytokines IL-4 and IL-13 abrogate the bone resorption induced by IL-1α (9). Given the evidence of production of both Th1- and Th2-type cytokines in the gingival tissue of PD patients (10), a subtle imbalance of cytokine profile may affect the induction of bone resorption in PD. For example, the Th1 cytokine IFN-γ induces the production of IL-1 by macrophages as the result of a second signal provided by LPS (11). The Th2 cytokines IL-4 or IL-10 inhibit secretion of IL-1 and TNF-α by macrophages treated with LPS (12, 13). Although there are many in vitro studies of the effects of Th1- or Th2-type cytokines on osteoclast differentiation and macrophage stimulation, the physiological role of Th1- or Th2-type T cells on bone resorption in vivo is unknown.

Complete T cell activation requires two signals, one from the TCR and the other from costimulatory molecules (14). B7/CD28 seem to provide the major costimulatory signals, which regulate T cell proliferation and production of IL-2 (15). CTLA4Ig, a fusion protein of human CTLA4 and the Fc fragment of human IgG has been demonstrated to be a dramatic inhibitor of Ag-specific T cell response (16). Blocking of B7/CD28 costimulatory signaling with human CTLA4Ig, which cross reacts with many other species including mice and rats, has inhibited the progression of autoimmune diseases in vivo (17) and rejection of allo- or xenografts (18, 19). Because B7/CD28 signaling itself is not functional until MHC/TCR signaling is provided to the T cells (20), the inhibition of an immune response with CTLA4Ig can indicate the involvement of Ag-specific T cells in that response. In the present studies of a rodent model, we found that 1) local stimulation of Th1-type T cells, but not of Th2-type cells, by MHC class II⁺/B7⁺ APC could induce bone resorption in rat periodontal tissue; 2) LPS was
Materials and Methods

Animals

Rowett strain (Forsyth Institute inbred over 20 generations) female rats, rnu+/− (heterozygous normal) with restricted oral flora, were bred in plastic isolators and maintained under pathogen-free conditions in laminar flow cabinets (21). All T cell clones used in this study were derived from these Rowett strain rats.

Bacteria, bacterial Ags, and LPS

Actinobacillus actinomycetemcomitans, American Type Culture Collection no. 43718 (strain Y4; Manassas, VA) was grown in pleuro-pneumonia-like organisms broth (Difco Laboratories, Detroit, MI) with glucose (3 g/L) and sodium bicarbonate (1 g/L) for 72 h at 37°C under increased CO2 (candle jar). The cultured bacteria in the log-growth phase were killed with formalin (5%) and served as a T cell Ag. The A. actinomycetemcomitans 29-kDa outer membrane protein (Omp29) (5, 22) and A. actinomycetemcomitans LPS (23) were prepared as previously described.

Monoclonal Abs and fusion proteins

Several mouse mAb to rat surface markers were used in this study, including anti-MHC class II (OX6; Serotec, Bicester, U.K.), anti-B7-1 and anti-B7-2 (CD80, 3H5; and CD86, 24F; Ref. 24; gifts of Dr. K. Okumura), mAb ED1 (specific for rat monocytes, macrophages, and dendritic cells; Serotec), and anti-rat CD62L and anti-rat CD44 mAb (Serotec). CTLA4lg, a fusion protein of human CTLA4 and Fc fragment of human IgG, and control fusion protein L6 were gifts of Dr. P. S. Linsley.

T cell clones

Th1-type clone (G23) and Th2-type clone (F13) specific for A. actinomycetemcomitans Omp29 (21) were activated by incubation with sterile formalin-killed A. actinomycetemcomitans and irradiated (3300 rad) syngeneic rat spleen cells. Rat recombinant IL-2 (1 U/ml; Serotec) or conditioned medium from Con A-stimulated spleen culture was added to the Th2-type cell culture. Both G23 and F13 are CD28+ (CD28R−) and proliferate in the presence of IL-2, IL-4, CD3, CD4, CD25, LFA-1, VLA-4, ICAM-1, and CCR5 by G23, and production of IL-4, expression of CD3, CD4, CD25, LFA-1, VLA-4, and ICAM-1 by F13, have been described previously (21, 25). We considered these clones as memory cells based on the following criteria: CCR5+ (G23), CD25+, VLA-4+/high, CD45RC+, CD44high, CD62L−.

Microinjection of Ag into gingivae and T cell i.v. transfer

Rowett strain female rats (2–3 mo old) received three palatal gingival injec-
tions (1 μl/site) on the mesial of the first molar and in the papillae between first and second and third molars on the right and left sides (total of three sites on each side) of the maxilla. To accomplish microinjection into rat gingivae, which is composed of thin epithelium and connective tissue, the tip of a 28.5-gauge MicroFine needle (Becton Dickinson, Moun-
tain View, CA) was cut into a double bevel and used to inject 1 μl/site into gingivae. Injection consisted of Omp29 alone, Omp29 plus A. actinomycetemcomitans LPS, LPS alone (left, experimental side), and PBS as a control (right, control side) on day 0. T cell clones, G23 or F13, stimulated with APC and killed A. actinomycetemcomitans in culture plates 3 days in advance, were isolated by gradient centrifugation on day 0. Each of the T cell clones was transferred i.v.

Tissue preparation

T cells infiltrating into the gingival tissues, cervical lymph nodes (CLN) and spleen were isolated as previously described (21). Briefly gingivae were removed by dissection, then washed thoroughly to eliminate any blood and diced into 1-mm3 pieces. The segments were incubated at 37°C for 1 h in collagenase (160 U/ml, Worthington type IV) in 1 ml RPMI 1640 containing 1000 U/ml heparin and 10% FBS. The tissues were gently com-
pressed over a 60-gauge stainless steel screen and single cells were passed through a nylon wool and glass wool column to enrich T cells (≥90% CD3+ cells). To obtain T cells from CLN or spleen, single-cell sus-
pensions also were passed through a nylon wool and glass wool column.

For tartrate-resistant acid phosphatase (TRAP) staining, the whole max-
illa was dissected and fixed in 5% formaldehyde-saline solution overnight at 4°C. Tissues were decalcified in 10% EDTA/0.1 M Tris, pH 6.9, solution for 1 mo at 4°C. Decalcified samples were embedded in paraffin and 6-μm sections were cut. Tissues for immunohistochemistry were embedded in OCT compound (Miles, Elkhart, IN) and frozen at −70°C immediately after dissection. Cryostat sections were cut at 6 μm. For detection of B7-1 mRNA, each animal’s gingivae was injected with LPS (0.5 μg/site) or Omp29 (0.5 μg/site) at several time periods. Animals were sacrificed and the entire gingivae were dissected and removed. Total RNA was extracted immediately from the tissue by homogenizing in a glass tissue grinder on ice using RNAzolB described in the protocol of the manufacturer (Tel-
Test, Friendswood, TX).

TRAP staining

TRAP staining, as modified from the methods of Barka et al. (26) was used to identify osteoclasts in alveolar bone. After deparaffinization, sections were incubated in acetate buffer, pH 5.5, in the presence of 150 nM sodium tartrate (J. T. Baker Chemicals, Phillipsburg, NJ) for 90 min at room temperature. Samples were then incubated in acid phosphatase substrate to develop red color. Methyl green was used to counterstain cell nuclei. Osteoclasts were identified as multinucleated red cells.

Immunohistochemistry

Frozen sections of each tissue were fixed with 2% paraformaldehyde in PBS at 4°C for 10 min. After blocking with 1.5% horse serum in PBS, each section was incubated with mouse mAb (OX6, ED1, 3H5, and 24F) in PBS for 30 min at room temperature. Then, biotin-labeled horse anti-mouse IgG (rat-absorbed; Vector Laboratories, Burlingame, CA) in the presence of 1.5% horse serum and 1.5% rat serum was applied for 30 min at room temperature. Endogenous peroxidase activity was neutralized by incubation of the sections with 3% H2O2 for 10 min. The sections were reacted with preformed ABC (Elite ABC; Vector Laboratories) for 30 min at room temperature, followed by extensive washing with PBS. Each Ag was visualized by cellular localization of color development after incubation in diaminobenzidine H2O2 solution for 3 min (OX6, ED1) or 10 min (3H5, 24F) and counterstaining with methyl green.

Quantitation of B7-1-specific mRNA expression by competitive RT-PCR

Total RNA extraction and RT-PCR have been previously described (25). Primers for rat B7-1 were designed from the cDNA sequence from GenBank (P02039) as follows: 5′ primer, TAAAGGCGATTGCATGACATCT-GTGCACG (sense, bases 12–36) and 3′ primer, CAGCTGGACTCTCATACTCAATGGA (anti-sense, bases 683–708). The specificity of this primer set was searched and confirmed by basic local alignment search tool. To construct a competitive template DNA of rat B7-1, cDNA of rat B7-1 was amplified by RT-PCR from the total RNA of Con A-activated spleen cells and subcloned into a pCR3 vector (Invitrogen, Portland, OR). After digestion at AccI and Clal sites, both cohesive ends were filled in by T4 DNA polymerase (Invitrogen) and ligated by T4 DNA ligase (Invitrogen). To verify the accuracy of the competitive template, its DNA sequence was analyzed (Automatic Sequencing and Genotyping Facility of Brigham and Women’s Hospital, Boston, MA). The final size of the competitive template DNA was 330 bp when compared with the intact B7-1 DNA product size (697 bp) as amplified by RT-PCR using the primers mentioned above. The competitive template was serially diluted from 10−15 to 10−21 mol/50 μl in the PCR solution in the presence of 1 μg/ml of yeast tRNA. The total RNA isolated from gingivae (1 μg) was transcribed to cDNA and amplified by the B7-1-specific primers designed above in the presence of the serially diluted competitive template (35 cycles at 94°C for 30 s, 60°C for 1 min, 72°C for 1 min, and a final elongation time of 10 min at 72°C). Rat β-actin was used as an internal control (data not shown), and the primer design has been previously described elsewhere (27).

Ag-specific T cell responses in vitro

The T cell fraction recovered from gingivae at various time periods after T cell clone transfer was cultured in 96-well plates (5 × 103/well) supplemented with rat recombinant IL-2 (1 U/ml; Life Technologies, Gaithers-
burg, MD) in the presence of irradiated splenic APC (3300 rad) alone or also with killed A. actinomycetemcomitans (105/well). T cells recovered from CLN or spleen (105/well) were also cultured in 96-well plates in the presence of splenic APC with or without killed A. actinomycetemcomitans

H₂SO₄ and measured at 490 nm. Hyperimmune serum to Omp29 was prepared in Rowett rats (3 mo old) by immunization with purified Omp29 (10 μg, was determined by liquid scintillation spectrometry.

Previously, we found Ag-specific retention of T clone cells in ginspleen

Results

Measurement of bone resorption

At various times (3–20 days) after T clone transfer with gingival challenge with Omp29 and/or LPS or saline, animals were sacrificed, the jaws were defleshed, and periodontal bone resorption was measured on the palatal surface of the maxillary molars (28). The distances from cemento-enamel junction (CEJ) to the alveolar ledge (AL) of injected sites (upper left palatal side) and saline injected control sites (upper right palatal side) were measured using a reticule eyepiece at 25× magnification as previously described (28). A total of five measurements were evaluated, including one point corresponding to the root axis of the second and third roots of the first molar, both roots of the second molar, and the first root of the third molar.

Influence of transferred Th1 clone cells on humoral response

To verify the immunological influence of transferred Th1 clone cells in recipient animals, the serum IgG2a Ab levels to Omp29 were determined by ELISA (Fig. 2). The sera from animals receiving G23 or receiving no T cells were collected on day 0 and day 10 after Omp29 challenge into gingivae with or without LPS. When both Omp29 and LPS were used for gingival challenge,
which only received gingival challenge with Ag and LPS, showed no significant bone resorption up to 20 days (Fig. 3B).

**Th2 clone transfer has no effect on periodontal bone resorption**

The effect of Th2 clone transfer on periodontal bone resorption was also examined (Fig. 4A). The Th2 clone, F13, which is also Omp29 specific and whose Th2-type characteristics have been confirmed (21, 25, 29), was used. Although from 10^5 to 10^7 G23 cells per animal could induce bone resorption after 10 days of transfer into Ag- and LPS-injected gingivae, Th2 clone cells did not induce bone resorption at the highest number (10^7 per animal) of cells (Fig. 2A). It is noteworthy that like G23 this Th2 clone is also retained in the gingival tissue after Omp29 and LPS challenge (21).

**Requirement of LPS for the Th1-associated bone resorption**

We have previously reported a requirement of LPS for T clone cell retention in gingivae (21). In the absence of LPS, after challenge with specific Ag, T cell retention was not observed in gingivae (21), and also Ag-specific IgG2a reaction was not induced (Fig. 2). To evaluate whether LPS is required for bone resorption, G23 clone cells were transferred into animals that received gingival challenge with Ag alone. No bone resorption was induced in the absence of LPS even when Th1 clone-transferred animals received gingival challenge with Ag alone. It is noteworthy that LPS alone (0.5 μg/site) in the absence of Ag also did not result in bone loss, although the animals received G23 clone transfer.

**TRAP-positive cells on the AL of periodontal bone**

To further investigate periodontal bone resorption in this model, and to determine the localization of osteoclast cells, these were evaluated by TRAP staining (Fig. 5). Rats were killed 10 days after transfer of G23 Th1 clone cells and gingival challenge with or without Ag and/or LPS. The entire maxilla was decalcified, and histomorphology of the bone was analyzed. TRAP-positive cells were only observed in the periodontal bone of G23-transferred rats that were challenged with Ag...
and LPS (Fig. 5, A and B). Rats whose gingivae were challenged with LPS and Ag without T cells did not show any TRAP-positive cells on the AL (Fig. 5, C and D). Also, no TRAP-positive cells were observed in animals receiving gingival challenge with saline alone or saline plus G23 transfer (data not shown).

B7-1, B7-2, and MHC class II expression on local macrophages

Because local Th1 retention in gingivae seemed to be responsible for alveolar bone resorption, the presence of APC that are capable of stimulating T cells (MHC class II+/B7+) was examined by means of immunohistochemical analysis (Fig. 6). In the LPS-challenged gingivae, expression of B7-1 (Fig. 6B) and B7-2 (Fig. 6D) was seen at 48 h after challenge (stained large cells), but little or no staining was observed in Omp29-challenged gingivae (Fig. 6, A and C, no large stained cells detected) or in saline-injected control (not shown) at the same time period. The expression of B7-1 and B7-2 peaked at between 24 and 48 h and diminished at 72 h after LPS challenge (B7-1 at 24 h, 5.7 ± 1.5; at 72 h, 1.3 ± 1.2; B7-2 at 24 h, 11.7 ± 2.5; at 72 h, 3.0 ± 1.0 mean positive cells/400 microscopic field ± SD). Macrophages were observed in the saline-treated gingivae (Fig. 6E) and also in the gingival challenge with LPS or Omp29 (not shown). MHC class II expression was also observed in macrophages together with endothelial cells in LPS-challenged samples (Fig. 6F) to approximately the same degree as Omp29-challenged gingivae, but less than in saline-injected gingivae (not shown).

B7-1 mRNA expression by gingivae

When gingivae were challenged with LPS, both B7-1 and B7-2 expression was observed along with MHC class II expression on gingival macrophages. To further determine if B7 expression was induced by LPS stimulation, competitive RT-PCR was used to investigate B7-1 mRNA expression (Fig. 7). B7-1 mRNA expres-
significantly different from saline control, by Student’s *t* test.

Systemic (i.v.) administration

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Local administration

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**FIGURE 8.** Inhibition of Th1 retention and abrogation of bone resorption by CTLA4-Ig. CTLA4-Ig inhibits in vitro Ag-specific proliferation (A). G23 cells (10^4/well) were stimulated with Ag and irradiated spleen APC for 3 days in the presence or absence of anti-MHC class II, anti-B7-1, anti-B7-2 mAb, or CTLA4-Ig or control L6 fusion protein (10 μg/ml each). Proliferation was detected by [3H]thymidine incorporation and expressed as mean cpm ± SD. One representative result of three experiments is shown. *, *p < 0.01; **, *p < 0.001; significantly different from T cells stimulated with Ag (Omp29) and APC in medium by Student’s *t* test. B, Animals receiving gingival challenge of saline, Omp29 (0.5 μg/site), or Omp29 plus LPS (0.5 μg/site) were sacrificed 1 day after G23 transfer. Some Omp29- and LPS-challenged animals were treated with CTLA4-Ig by local gingival injection (1 μg/site) or systemic administration (100 μg/rat) 1 day before T cell transfer. Gingival T cells were isolated and stimulated in vitro in culture with APC with or without Ag in the presence of IL-2 (1 U/ml). Each of paired column result of three experiments is shown. *, *p < 0.01; **, *p < 0.001; significantly different from saline control, by Student’s *t* test. ***, *p < 0.01; significantly different T cell response to APC plus Ag from Omp29 plus LPS group by Student’s *t* test. C, Spleen T cells from the same animals in B were isolated, and Ag-specific T cell reaction was tested in vitro. CTLA4-Ig was administrated systemically (i.v.). *, *p < 0.01; significantly different from T cell response to APC plus Ag in saline-challenged control group, by Student’s *t* test. One representative result of three is shown. D, Abrogation of bone resorption induced by Th1 transfer. Animals receiving G23 cells (10^7/animal) and gingival challenge with Omp29 plus LPS (0.5 μg each/site) were sacrificed 10 days after G23 transfer. Omp29- and LPS-challenged animals were treated with CTLA4-Ig or L6 by local gingival injection (1 μg/site) or systemic administration (100 μg/rat) 1 day before and 1 day after G23 cell transfer. *, *p < 0.01; significantly different from L6 controls by Student’s *t* test.

**Abrogation of bone resorption by local and systemic administration of CTLA4-Ig**

Given the evidence that B7 expression on local APC is related to gingival challenge with LPS, we tested whether this B7 expression is functionally related to the bone resorption induced by G23 transfer with gingival challenge of Ag and LPS. The human fusion protein CTLA4-Ig functionally inhibited in vitro proliferation of G23 Th1 clone cells in the presence of APC and Ag (Fig. 8A). Th1 clone G23 proliferation in the presence of APC and Ag was significantly inhibited by anti-MHC class II, anti-B7-1, and/or anti-B7-2 mAbs. The inhibitory effect of CTLA4-Ig was comparable to inhibition by both anti-B7-1 and anti-B7-2 mAbs. The control human fusion protein L6 did not effect G23 proliferation. To analyze the influence of systemic administration of CTLA4-Ig, reactivity of G23 cells isolated from gingivae (Fig. 8B) and spleen (Fig. 8C) of G23-transferred animals was tested in vitro. Again, LPS was required in addition to Ag for G23 cell retention in gingivae (Fig. 8B). After G23 transfer, the Ag-specific response of isolated T cells from gingivae challenged with Ag and LPS was diminished significantly by systemic or local administration of CTLA4-Ig, but not with control fusion protein L6, suggesting a role for B7 in Ag-specific T cell retention in gingivae. The Ag-specific response of T cells isolated from spleens of animals receiving G23 cell transfer was greatly diminished by systemic administration of CTLA4-Ig (Fig. 8C). Also, systemic administration of CTLA4-Ig abrogated the IgG2a response to Omp29, which was induced in the animals transferred with G23 and receiving gingival challenge with Omp29 and LPS as indicated in Fig. 2 (data not shown). The influence of systemic or local administration of CTLA4-Ig on the bone resorption induced by transferred G23 was examined (Fig. 8D). Bone resorption was measured 10 days after transfer of G23 into rats with Ag and LPS. CTLA4-Ig or control L6 was administered systemically (100 μg/rat) or locally (1 μg/site) 1 day before and 1 day after transfer of G23 cells. Both systemic and local administration of CTLA4-Ig showed significant inhibition of Ag-specific Th1 cell retention in gingivae (Fig. 8B) and bone resorption related to G23 cells (Fig. 8D).

**Discussion**

Our data indicated that local Ag presentation to Ag-specific Th1 cells, but not Th2 cells, could induce periodontal bone resorption in the context of MHC class II and B7 costimulation. To the best of our knowledge, this is the first finding that shows Ag-specific memory T cell involvement in inflammatory bone resorption. Direct T cell involvement in bone resorption was reported by Horwood et al. (30), who demonstrated that GM-CSF produced by T
cells inhibits in vitro osteoclast formation. Naive (non-Ag stimulated) T cells were used in their study, as compared with the memory type T cells that were used in the present study. Therefore, we assume that a different level of T cell activation, such as naive T cells vs memory T cells, effects bone resorption by regulating the differentiation/activation of osteoclast cells. Ag-experienced memory-type T cells can migrate to local inflammatory tissue more than naive T cells (31) did. Thus, our model delineates the memory T cell involvement in local inflammatory bone resorption, but not necessarily in systemic bone resorption like osteoporosis.

Previous studies of Th1 or Th2 cytokine mRNA expression by CD4+ T cells in PD suggested prominent production of IFN-γ, IL-6, IL-10, and IL-13 (10). IFN-γ appeared to be the predominant cytokine produced by gingival T cells. In addition, CD4+ and CD8- subsets in PD have shown a similar pattern of cytokine production with predominant production of IFN-γ (56). Both Th1-type and Th2-type cells appear to be retained in rat gingivae challenged with Ag and LPS (21). In the present study, we demonstrated that Th1-type, but not Th2-type, cells could trigger periodontal bone resorption. The sum of these observations seems to indicate that a subtle imbalance toward Th1 polarization can play a key role in the progression of PD. This hypothesis is supported by a report of Lee et al. (32) that IL-2 levels in gingival crevicular fluid are significantly higher in active periodontal pockets, which showed bone loss in 3 mo, than in inactive periodontal pockets, which did not show bone loss in the same time period.

Gram-negative bacteria are more frequently isolated from the microbial flora of active/progressing periodontal pockets than from dental plaque microflora of healthy subjects or healthy sites of the gingival sulcus in the same patient (33). LPS, which is exclusively produced by Gram-negative bacteria, has long been suggested to be an important factor in the pathogenesis of PD (34). Induction of bone resorption in vitro osteoclastogenesis by LPS is mediated by TNF-α from bone marrow macrophages (35). However, in animal models in vivo, an injection of a high dose of LPS (500 μg/site) (36) or long-term multiple injections of LPS (5 μg/site up to 10 consecutive injections) (37) is required to induce bone resorption. Interestingly, in the latter experiment (37), bone resorption by long-term LPS injection was associated with the presence of T cells. In the present study, a much lower dose of LPS (0.5 μg/site) administered with Ag induced bone resorption by Th1-type cells. It is noteworthy that in the current experiments a single injection of a lower dose (0.5 μg/site) of LPS alone did not induce significant bone resorption unless Th1 cells and Ag were also administered.

Although macrophages in the gingivae expressed MHC class II by either challenge with LPS or Omp29, B7 expression on macrophages in this system was induced by LPS challenge. LPS also can induce rapid production of the Th1 differentiation cytokine, IL-12, by dendritic cells (38). Therefore, we suggest that infusion of LPS into the gingival tissue triggers differentiation and activation of Th1-type T cells under physiological conditions. In general, isolated human monocytes do not express B7-1 or B7-2 costimulatory molecules, unless LPS is added (39). Recent studies of the human Toll-like receptor (TLR) family have demonstrated the importance of LPS to induce B7 costimulatory molecules where innate immunity elicits adaptive immunity (40). TLR-2 is a signaling component of the cellular receptor (CD14) for LPS (41). Defective response to bacterial LPS by the C3H/HeJ mouse strain was linked to a mutation in a TLR family gene (42). This evidence supports our finding that LPS can induce B7 in gingival macrophages. Interestingly, B7-1 and B7-2 are expressed on lymphocytes in PD tissue (43), and there is an absence of B7 expression in the healthy gingival tissue of PD patients (our unpublished observations), suggesting a relationship between B7 expression and progression of PD.

Acknowledgments

We thank Dr. Peter S. Linsley (Bristol-Myers Squibb Pharmaceutical Research Institute, Seattle, WA) for CTLA4Ig and L6 and Dr. Ko Okumura (Juntendo University, Tokyo, Japan) for mAb 3H5 and 24F. We also thank Justine Dobek for technical assistance with immunohistochemistry and Jan Schafer for secretarial assistance.

References


Th1-ASSOCIATED INFLAMMATORY BONE RESORPTION

1. The present study, a much lower dose of LPS (0.5 μg/site) is required to induce bone resorption. Inter-


American Society for Microbiology, Washington, DC, p. 147.


