J Clin Periodontol 2011; 38: 122–130 doi: 10.1111/j.1600-051X.2010.01645.x

Journal of Clinical Periodontology

Mechanism of alveolar bone loss in a collagen-induced arthritis model in mice

Jung-Chul Park[1]*, Chuanxin Su[2]*, Im-Hee Jung[1], Seong-Ho Choi[1], Kyoo-Sung Cho[1], Chong-Kwan Kim[1], Yong-Beom Park[2], Soo-Kon Lee[2] and Chang-Sung Kim[1]

[1]Department of Periodontology, Research Institute for Periodontal Regeneration, College of Dentistry, Yonsei University, Seoul, Korea; [2]Department of Internal Medicine, Division of Rheumatology, College of Medicine, Yonsei University, Seoul, Korea

Park J-C, Su C, Jung I-H, Choi S-H, Cho K-S, Kim C-K, Park Y-B, Lee S-K, Kim C-S: Mechanism of alveolar bone loss in a collagen-induced arthritis model in mice. J Clin Periodontol 2011; 38: 122–130. doi: 10.1111/j.1600-051X.2010.01645.x

Abstract

Objective: The aim of this study was to understand the cellular/molecular mechanisms of periodontal breakdown in a collagen-induced arthritis (CIA) model in mice to enhance the understanding of rheumatoid arthritis (RA)-associated alveolar bone loss in humans.

Materials and Methods: All analyses were performed on paired samples from CIA and control group mice. Mandibles were retrieved for micro-computed tomography (micro-CT), histomorphometric analysis, and isolation of alveolar bone cells (ABCs). In vitro osteoclastogenic/osteogenic/adipogenic potentials of ABCs were evaluated and the mRNA expression of downstream effector genes was assessed. Bone formation of ABCs was assessed using an ectopic transplantation model.

Results: Histomorphometric and micro-CT data showed that alveolar bone loss was significantly increased in the CIA group ($p < 0.05$). Osteoclastogenesis was significantly increased in the CIA group in vivo ($p < 0.05$), with upregulated mRNA expressions of osteoclastogenesis-associated genes. Osteoblasts appeared to undergo increased apoptosis, and the bone-forming activity of ABCs concomitantly decreased with in vitro osteogenic differentiation and in vivo ectopic transplantation ($p < 0.05$). Also, adipogenesis-associated mRNA expression was highly expressed in the CIA group, resulting in significantly enhanced adipocyte differentiation in vitro ($p < 0.05$).

Conclusions: These data demonstrate that increased osteoclastic activity, decreased bone-forming activity and enhanced adipogenesis promote alveolar bone loss in a CIA model in mice, and they suggest that these mechanisms could account for the same outcome in human RA.

Key words: bone resportion; experimental arthritis; mice; periodontitis; rheumatoid arthritis

Accepted for publication 9 October 2010

Periodontitis (PD) is one of the most common chronic inflammatory diseases (Williams, 1990, Bartold et al. 2005), and it is initiated by a pathogenic biofilm leading to loss of attachment, including progressive destruction of the alveolar bone and periodontal ligament, and apical migration of the junctional epithelium. Although pathogenic biofilms are the primary aetiologic agents for PD, the severity and progression of tissue destruction depends on the complex cellular and immunological interactions within the host. In light of this association between periodontal infection and host defence mechanisms, recent studies have proposed a potential correlation between periodontal disease and rheumatoid arthritis (RA), and explained it in part by induction of autoimmunity or excessive production of pro-inflammatory cytokines (Mercado et al. 2003).

RA is also a chronic inflammatory disease that is characterized by the accumulation and persistence of inflammatory infiltrates in the synovial membrane, which often results in synovitis, structural damage, deformity, and loss of function. RA and PD have been reported to share numerous common pathogenic characteristics, and a number of studies have found a potential

*Contibuted equally.

Conflict of interest and source of funding statement

All authors declare no conflict of interest. This study was supported by a grant of the Korea Healthcare technology R&D Project, Ministry for Health, Welfare & Family Affairs, Republic of Korea (A084447).

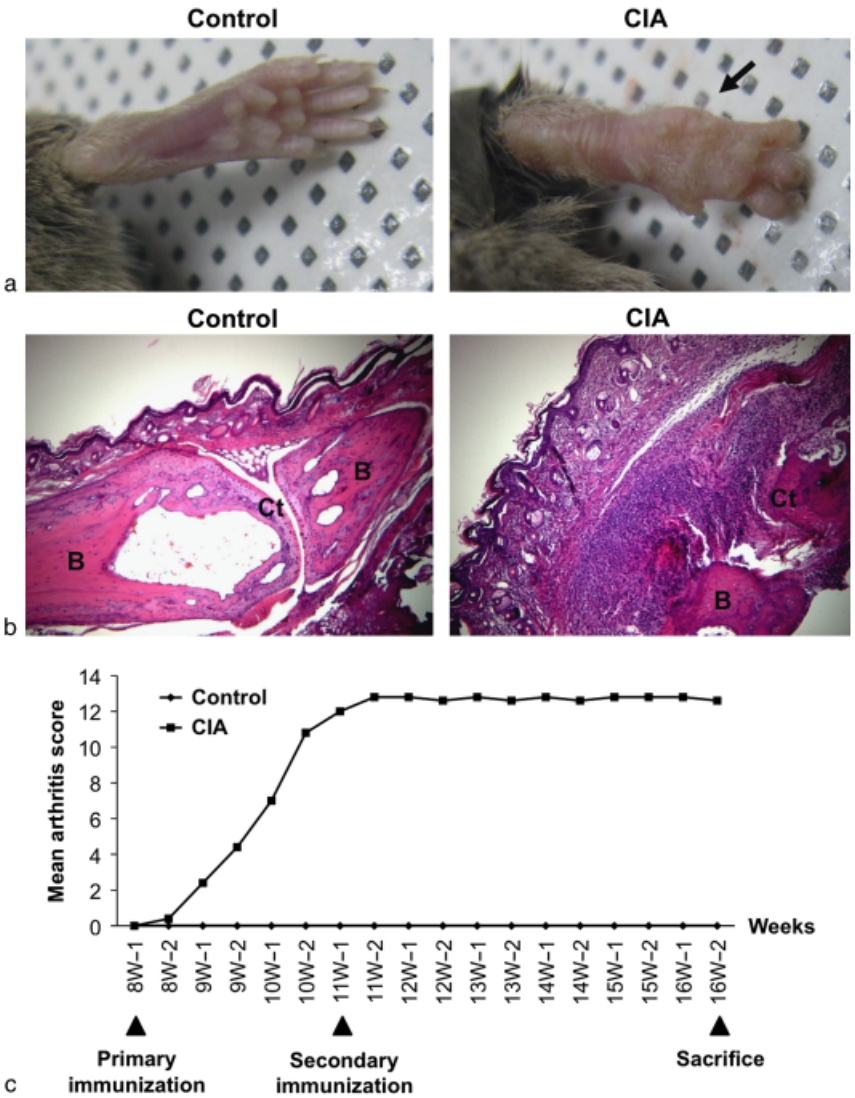


Fig. 1. Clinical and histological assessment of collagen-induced arthritis (CIA) development in DBA/1 mouse 8 weeks after CIA induction. (a) Appearance of rear paws in mice from the control (CTL) and the CIA group. Note joint swelling and severe deformity in the CIA group (arrow). (b) Histolopathological findings of the joint area from the toes of the hind feet. The CTL group shows normal histology of mouse joint. Severe bone erosion and resorption of cartilage is observed in the CIA group, with high infiltration of inflammatory cells. Tissue sections were stained with haematoxylin & eosin. Original magnification $\times$ 20. (B, bone, Ct, cartilage) (c) Severity of arthritis was measured twice a week until sacrifice using the clinical arthritis score.

association between RA and PD (Mercado et al. 2000, Mercado et al. 2001, de Pablo et al. 2008, Pischon et al. 2008). Interestingly, common genetic variants are associated with both diseases (Ogrendik et al. 2005), and other studies have shown a remarkably similar involvement of mediators, including prostaglandins, cytokines, matrix metalloproteinases, and cell types (Bozkurt et al. 2000, Mercado et al. 2003, Petropoulou et al. 2003), leading to the stimulation of local osteoclastogenesis (Cochran, 2008).

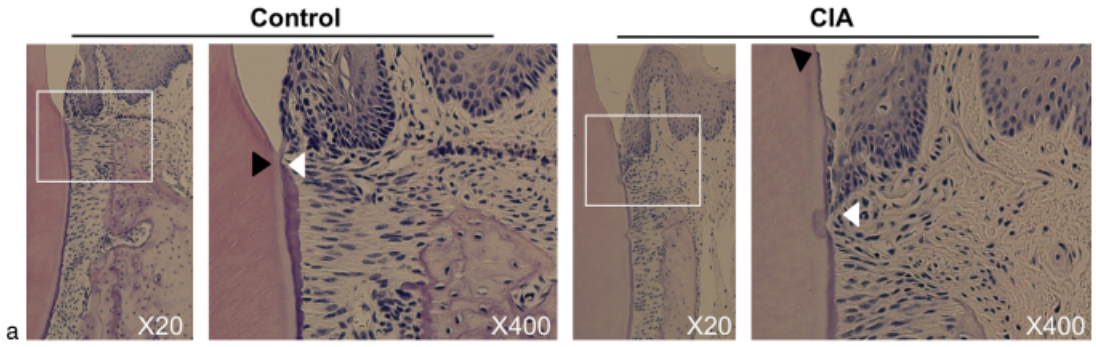
Normal bone is a dynamic tissue that is continuously remodelled by bone-resorbing osteoclasts and bone-forming osteoblasts, a process that is referred to as bone coupling (Parfitt, 1982). However, the imbalance of bone coupling and sequential bone loss can also be induced by systemic diseases (Salvi et al. 1997, Liu et al. 2006, Graves, 2008). For instance, Graves (2008) demonstrated that type 2 diabetes may aggravate periodontal diseases by both increasing bone loss and limiting reparative bone formation, resulting in a defect in bone coupling. An imbalance in osteoclast/osteoblast activity in RA may also cause impaired bone formation and together may lead to a coupling defect. However, the results of recent studies question the additional mechanism underlying the coupling defect (Pei and Tontonoz, 2004), and it is now suspected that the reciprocal relationship between adipogenesis and osteogenesis is also related to skeletal homeostasis, especially in terms of peroxisome proliferator-activated receptor γ (PPARγ), a key transcription factor implicated in adipogenesis(Akune et al. 2004).

The objectives of this study were to evaluate the association between the development of RA and PD in a mouse model of collagen-induced arthritis (CIA) and to elucidate the mechanism underlying periodontal breakdown, focusing on alveolar bone resorption.

Materials and Methods

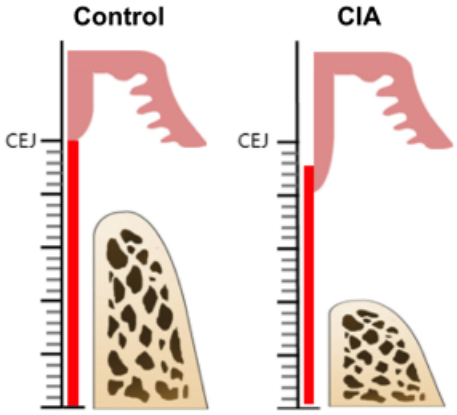
Animals

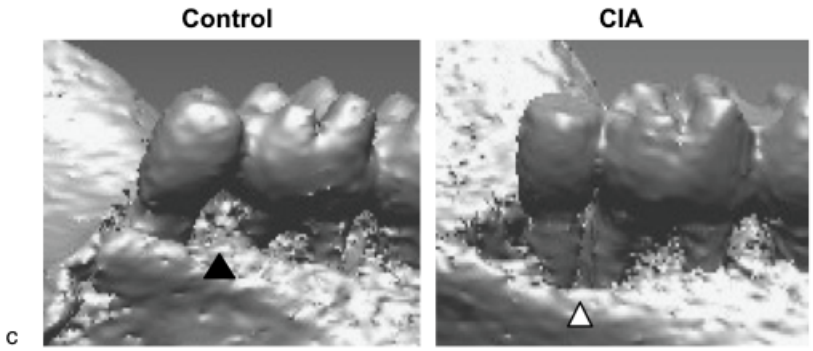
Ten DBA/1 mice at 8 weeks of age (SLC, Shizoka, Japan) were divided into two groups. The CIA group ($n = 5$), in which mice were injected with type II collagen (bovine type II collagen, Sigma-Aldrich, St. Louis, MO, USA), was compared with an age-matched control (CTL) group ($n = 5$). CIA was induced by an intradermal injection of 100 μg of collagen type II emulsified in complete Freund's adjuvant into the tail base (Lee et al. 2009). After 3 weeks, mice received a booster injection. The animals were observed twice a week for 8 weeks after primary immunization, then anaesthetized and killed at 8 weeks after induction of CIA. CIA development was confirmed by clinical and histological inspection (Fig. 1). The severity of arthritis was evaluated by visual inspection. All four

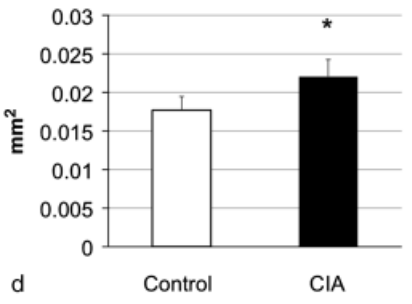


	Distance from CEJ to Cementum (μm)	Distance from CEJ to Junctional epithelium (μm)	Distance from CEJ to bone crest (μm)
Control	0	0	65.07 ±37.96
CIA	28.59 ±22.76*	183.95 ±89.45*	144.28 ±99.85*

b







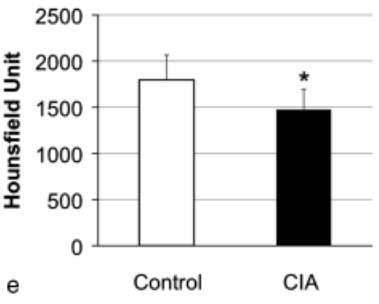


Table 1. Primers for RT-PCR

Gene	Forward	Reverse	GeneBank no.	Annealing temperature (°C)	Product size (bp)
RANKL	AGCCGAGACTACGGCAAGTA	GATGGTGAGGTGTGCAAATG	NM011613	50	495
TRAP	AAATCACTCTTCAAGACCAG	TTATTGAACAGCAGTGACAG	NM001102405	51.3	312
PPARγ2	CATGGTTGACACAGAGATGCC	TCACAAGCATGAACTCCATAG	U09138	50	380
aP2	CCTCGA AGGTTTACAAAATGTGTGA	AAACTCTTGTGGAAGTCACGCCTTT	NM024406	60	401
β-actin	AGCCATGTACGTAGCCATCC	CTCTCAGCTGTGGTGGTGAA	X03672	50	228

RANKL; receptor activator of nuclear factor κ-light-chain-enhancer of activated B cells' ligand; TRAP; tartrate-resistant acid phosphatase; PPARγ2; peroxisome proliferators-activated receptor γ; aP2; adipocyte fatty acid-binding protein; RT-PCR, reverse transcription-polymerase chain reaction.

legs of the mice were evaluated and scored from 0 to 4 according to the following scale: 0, no signs of arthritis; 1, swelling and/or redness of the paw or one digit; 2, two joints involved; 3, more than two joints involved; and 4, severe arthritis of the entire paw and all digits (Cho et al. 2006). Arthritis scoring was performed by two independent observers in a blinded fashion. All animal experiments were performed under an animal study protocol approved by the Institutional Animal Care and Use Committee, Yonsei University, Seoul, Korea.

Alveolar bone destruction analyses by micro-computed tomography (micro-CT) and histomorphometric analysis

Left mandibles were analysed by micro-CT (SkyScan, SCANCO USA, Southeastern, PA, USA), with a minor modification of a previously published protocol (Park et al. 2007). Using three-dimensional image reconstructions, the cementoenamel junction (CEJ) and marginal bone crest were drawn manually. Alveolar bone resorption was measured as the exposed root surface area between the CEJ and the alveolar bone crest. Bone density was measured using two-dimensional cross-sectional images and determined as the average of Hounsfield unit measurement at the apical area of the molar tips.

For histology, samples were fixed in 4% paraformaldehyde and then decalcified with 10% EDTA (pH 8.0) for paraffin embedding. Paraffin sections were deparaffinized, hydrated, and step-serial sectioned in a bucco-lingual vertical plane (5 μm thick). The sections were stained with haematoxylin and eosin (H&E). The most central sections of two molars selected based on the width of the root canal were used for the histological and histometric analysis. Two experienced examiners performed the histopathologic evaluations of the tissue specimens in a blinded fashion using light microscopy (Olympus multiview microscope BH2, Tokyo, Japan) and a PC-based image-analysis system (Image-Pro Plus, Media Cybernetic, Silver Spring, MD, USA). The following parameters modified from the previous report (Kim et al. 2005) were analysed: (A) CEJ junction to cementum: distance from the CEJ to the top of the remaining cementum on the root surface. (B) CEJ junction to junctional epithelium: distance from the CEJ to the apical extension of the junctional epithelium. (C) CEJ junction to bone crest: distance from the CEJ to the top of the alveolar bone crest.

Measurements were made in micrometres, and schematic diagrams were drawn to compare the patterns of periodontal breakdown of the CIA group with that of the CTL group (Fig. 2). In addition to H&E staining, the deoxynucleotidyl transferase-mediated dUTP-biotin nick-end labelling (TUNEL) method (in situ apoptosis detection kit; Trevigen, Gaithersburg, MD, USA) was performed in accordance with the manufacturer's recommendations for the detection of apoptotic cells, and osteoclasts were visualized by tartrate-resistant acid phosphatase (TRAP) staining using an appropriate detection kit (Sigma-Aldrich) according to the manufacturer's instructions.

Mouse alveolar bone cell (ABC) isolation

ABCs were isolated and cultured from the right mandibular jaws of each group as reported previously (Shi et al. 2002). ABCs formed adherent colonies after approximately 3–7 days of culture. Primary cultures were passed to disperse the colony-forming cells – passage P1. P3–P5 ABCs were used for the experiments.

Fig. 2. Periodontal breakdown including alveolar bone loss in the mouse model of collagen-induced arthritis (CIA). (a) Intact periodontal tissue in the control (CTL) group is shown on the left panel (haematoxylin & eosin). The periodontal attachment is well maintained at the level of the cementoenamel (CEJ) junction (black arrowhead), and the top of the alveolar bone crest is close to the CEJ. In the CIA group, diseased periodontium with reduced alveolar bone height and several root resorption pits along the root surface are observed (right panel). The cementum is resorbed down the root surface (white arrowhead) and the junctional epithelium has migrated apically along the denuded root surface. (b) Histometric measurements revealed the degree of destruction of the periodontium. The following parameters were analysed in the most central section of molars [CEJ to cementum, CEJ to junctional epithelium, and CEJ to bone crest (vertical alveolar bone loss)]. Schematic diagrams of the state of the periodontium in CTL and CIA groups are shown on the right panels. (c) Significant alveolar bone resorption was observed in three-dimensional reconstructions of micro-computed tomographic images. Alveolar bone is covering the interproximal area (black arrowhead) in the CTL group, while active alveolar bone resorption is observed in the corresponding area in the CIA group (white arrowhead). (d) Assessment of exposed root surface area. There was a statistically significant increase in the CIA group. (e) Assessment of alveolar bone density. Bone density was determined as the average of Hounsfield unit measurement at the apical area of the molars, and there was a significant decrease in the CIA group compared with the CTL condition. *Statistically significant difference from the CTL group ($p < 0.05$).

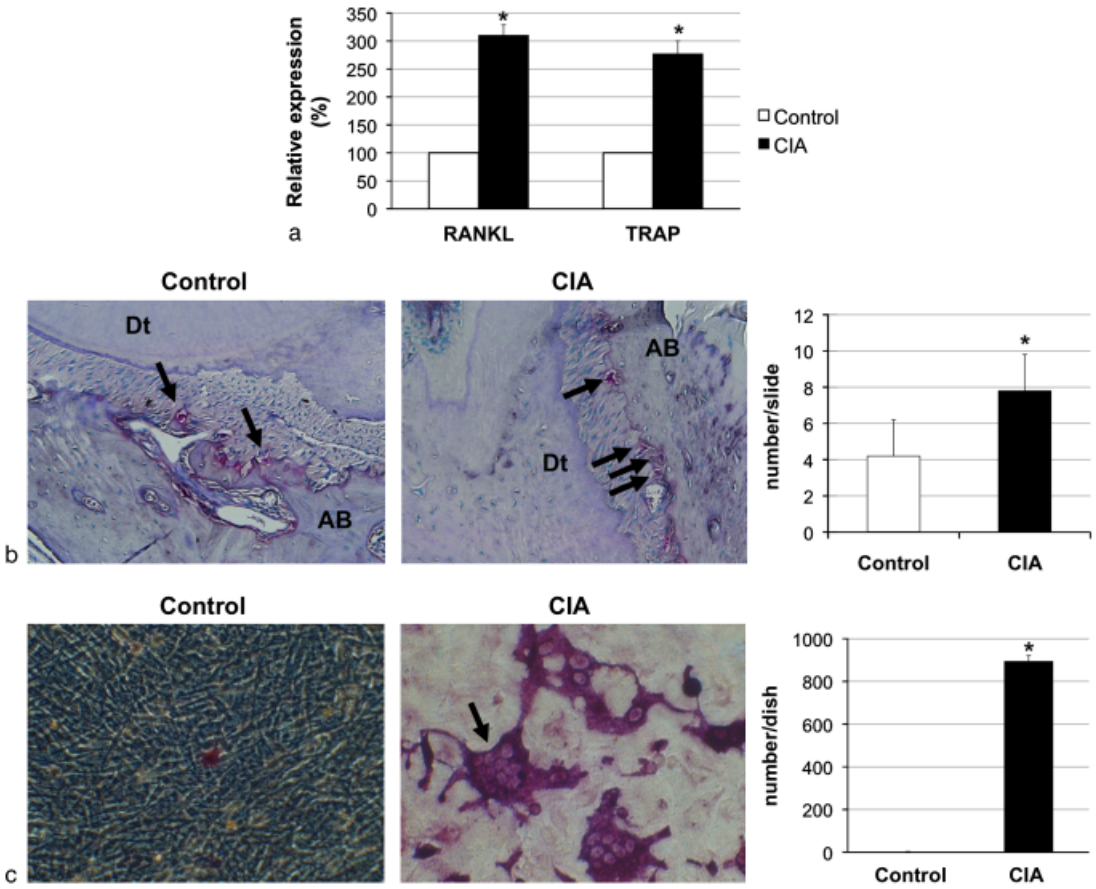


Fig. 3. Increased osteoclastic activity in alveolar bone cells (ABCs) from collagen-induced arthritis (CIA) mice. (a) Reverse transcription-polymerase chain reaction analysis was performed to determine the mRNA expression associated with osteoclastogenic differentiation. ABCs were harvested and cultured in an osteoclastogenic medium. mRNA expressions for the receptor activator of nuclear factor κ-light-chain-enhancer of activated B cells ligand (RANKL) and tartrate-resistant acid phosphatase (TRAP) were considerably increased in the CIA group. (b) Increased number of osteoclasts in the CIA group in vivo. TRAP-positive multinucleated cell numbers (MNCs, arrows) were significantly increased in the CIA group ($p < 0.05$). Original magnification × 100. (Dt, dentin, AB, alveolar bone) (c) Increased number of osteoclasts in the CIA group in vitro. ABCs harvested from alveolar bone were cocultured with bone marrow cells to induce the production of osteoclasts. Cells with more than three nuclei were considered as osteoclasts (arrow); MNCs were not detected in the control (CTL) group, whereas a large number of MNCs were observed in the CIA group. Original magnification × 100. *Statistically significant difference from the control group ($p < 0.05$).

Induction of osteogenic and adipogenic differentiation

ABCs from each group were cultured and induced for osteogenic and adipogenic differentiation. Cells (P5) were seeded into 35-mm culture dishes at 1×10^5 cells per well and cultured until they reached a subconfluent stage. The culture medium for osteogenic differentiation comprised α-MEM (Gibco BRL, Grand Island, NY, USA) containing 20% FBS (Gibco BRL), 2 mM L-glutamine (Gibco BRL), 100 μM L-ascorbic acid 2-phosphate, 10 nM dexamethasone (Sigma-Aldrich), 2 mM β-glycerophosphate (Sigma-Aldrich), 55 μM 2-mercaptoethanol (Amersco), 100 U/ml penicillin (Gibco BRL), and 100 μg/ml streptomycin (Gibco BRL), and was changed at 3-day intervals. The culture medium for adipogenic differentiation comprised α-MEM containing 15% FBS, 2 mM L-glutamine, 100 μM L-ascorbic acid 2-phosphate, 0.5 mM isobutyl-methylxanthine (Sigma-Aldrich), 60 μM indomethacin (Sigma-Aldrich), 0.5 μM hydrocortisone (Sigma-Aldrich), 10 μM insulin (Sigma-Aldrich), 100 U/ml penicillin, and 100 μg/ml streptomycin. After 2 weeks (adipogenic differentiation) or 4 weeks (osteogenic differentiation) of induction, the cells were stained with Oil Red O stain and alizarin red, respectively. To evaluate the induction of each differentiation type, areas of adipocyte formation and newly formed nodules were measured using an automated image-analysis system (Image-Pro Plus).

Induction of osteoclasts by coculture with bone marrow cells

In vitro osteoclast differentiation was conducted as described previously (Yun et al. 2007). Briefly, bone marrow cells were collected from the tibiae of mice, washed with α-MEM, and added to the ABCs with 1 α,25-dihydroxyvitamin D_3 (10^{-8} M, Enzo Life Sciences, Farmingdale, NY, USA). After 7 days, the cells were fixed and stained for TRAP, which is an osteoclastogenesis marker, using an acid phosphatase kit (Sigma Chemical, St. Louis, MO, USA). TRAP-positive multinucleated cells with

more than three nuclei were considered as osteoclasts.

Total RNA extraction and reverse transcription-polymerase chain reaction (RT-PCR)

RT-PCR was performed using samples from adipogenic and osteoclastogenic differentiations. Total RNA was isolated using TRIzol (Invitrogen, Carlsberg, CA, USA). RT-PCR was performed with 1 μg of RNA using a commercial kit (Cat. No. 25081, Maxime RT PreMix, Oligo dT primer, iNtRon Biotechnology, Daejon, Korea). The cDNA synthesized was amplified by PCR using the gene-specific primers (Table 1). PCR products were visualized by electrophoresis on agarose gels stained with ethidium bromide and analysed using a gel-documentation system (Gel Doc XR, BIO-RAD, Hercules, CA, USA).

Transplantation of ABCs into immunocompromised mice and histological examination

ABCs (6.0×10^6) were mixed with 80 mg of hydroxyapatite/tricalcium phosphate (HA/TCP) ceramic powder (Biomatlante, Vigneux, France) and then transplanted into the dorsal surface of 8-week-old immunocompromised mice as described previously (Kuznetsov et al. 1997). Transplants were harvested after 8 weeks, fixed with 4% formalin, decalcified with buffered 5% EDTA (pH 7.2–7.4), and then embedded in paraffin. Sections (5 μm) were deparaffinized and stained with H&E. Histometric analysis of new bone regeneration in vivo was performed using Image-Pro software, as described previously (Shi et al. 2002).

Statistical analysis

Student's *t*-test was used to analyse the statistical significance of differences between CIA and CTL groups. The level of statistical significance was set at $p < 0.05$.

Results

Alveolar bone destruction in CIA mice

Systemic development of arthritis was confirmed by clinical and histologic findings (Fig. 1). CIA-induced periodontal tissue breakdown was determined by histologic and radiographic analyses. The histological appearance of alveolar bone is shown in Fig. 2. A normal periodontium was observed in the CTL group, as evidenced by undamaged periodontal attachments, including complete coverage of the root surface by the cementum, connective tissue insertion into the cementum, and maintenance of the junctional epithelium and the crest of alveolar bone at the level of the CEJ. In comparison, the CIA group exhibited loss of attachments, including severe bone resorption, apical migration of junctional epithelium, and cementum demineralization. Quantitative histomorphometric analysis revealed a significant decrease in bone height in the CIA group ($p < 0.05$). Specifically, the junctional epithelium migrated twice the distance away from the CEJ in the apical direction in the CIA group compared with the CTL group ($p < 0.05$), and the connective tissue attachment with collagen fibre insertion into the cementum was partially destroyed. Several resorption pits were also observed on the cementum surface. Three-dimensional reconstruction from micro-CT images revealed the alveolar bone loss, and the amount of exposed root surface was significantly higher in the CIA group ($p < 0.05$). Also, the alveolar bone density measured at the apical area of molars by Hounsfield unit demonstrated relevant change in bone density. Finally, the alveolar bone of the CIA group appeared to be comparatively porous and had a lower bone density ($p < 0.05$).

Increased osteoclastic activity in ABCs from the CIA group

We examined changes in osteoclast activities in ABCs from the CIA group in several different ways (Fig. 3). First, the RT-PCR results showed that mRNA expressions for receptor activator of nuclear factor κ-light-chain-enhancer of activated B cells ligand (RANKL) and TRAP, among others, were considerably increased in the CIA group. Also, we were able to observe actively resorbing osteoclasts along the alveolar bone surface in the CIA group. The number of osteoclasts was also significantly increased in the CIA group after the induction of osteoclast differentiation using coculture of ABCs with bone marrow cells ($p < 0.05$). This specific increase in osteoclast activity appeared to be contributing considerably to the resorption of alveolar bone and the reduction in alveolar bone density.

Reduced osteoblastic activity in CIA mice

In addition to increased osteoclastic activity, we also considered a defect in osteoblastic bone formation as a potential mechanism of alveolar bone loss. When tested, TUNEL staining revealed a large number of apoptotic cells inside the alveolar bones of CIA group mice, which were assumed to be osteoblasts (Fig. 4). Consequently, the reduced bone formation could be, at least in part, accounted for by increased cell death. The bone-forming activity of ABCs soaked in HA/TCP particles in the ectopic model was studied by transplanting ABCs into a subcutaneous pocket on the dorsal surface of immunocompromised mice. Histologic results from harvested implants after 8 weeks showed that ABCs had induced new bone formation around the residual biomaterials in the CTL group, while ABCs of the CIA group failed to do so. Immunohistochemical analysis also revealed increased expression of the osteogenic markers in the CTL group with decreased expression in the CIA group (data not shown). The results from the in vitro cell-culture analysis were consistent with those of the in vivo data. ABCs were cultured in osteogenic medium and bone-nodule formation, stained by alizarin red, was examined. There was a drastic reduction in bone-nodule formation in the CIA group, suggesting inferior bone-forming ABC activity in that group. In addition, the RT-PCR results showed that the expression of osteogenic markers such as osteocalcin was down-regulated in the CIA group (data now shown).

Increased adipocyte production in the CIA group

Bone remodelling is largely explained by a crosstalk mechanism between osteoblasts and osteoclasts. However, adipocytes are also heavily involved in bone-cell regulation by acting on osteoblasts, as is PPARγ2, which is known for its inhibitory action against osteogenesis. To evaluate the changes in adipocytes or adipogenesis, we cultured ABCs in an adipogenic medium. ABCs from the CIA group up-regulated the expression of adipogenic markers such as PPARγ2 and aP2 as determined by RT-PCR (Fig. 5). In addition, the CIA

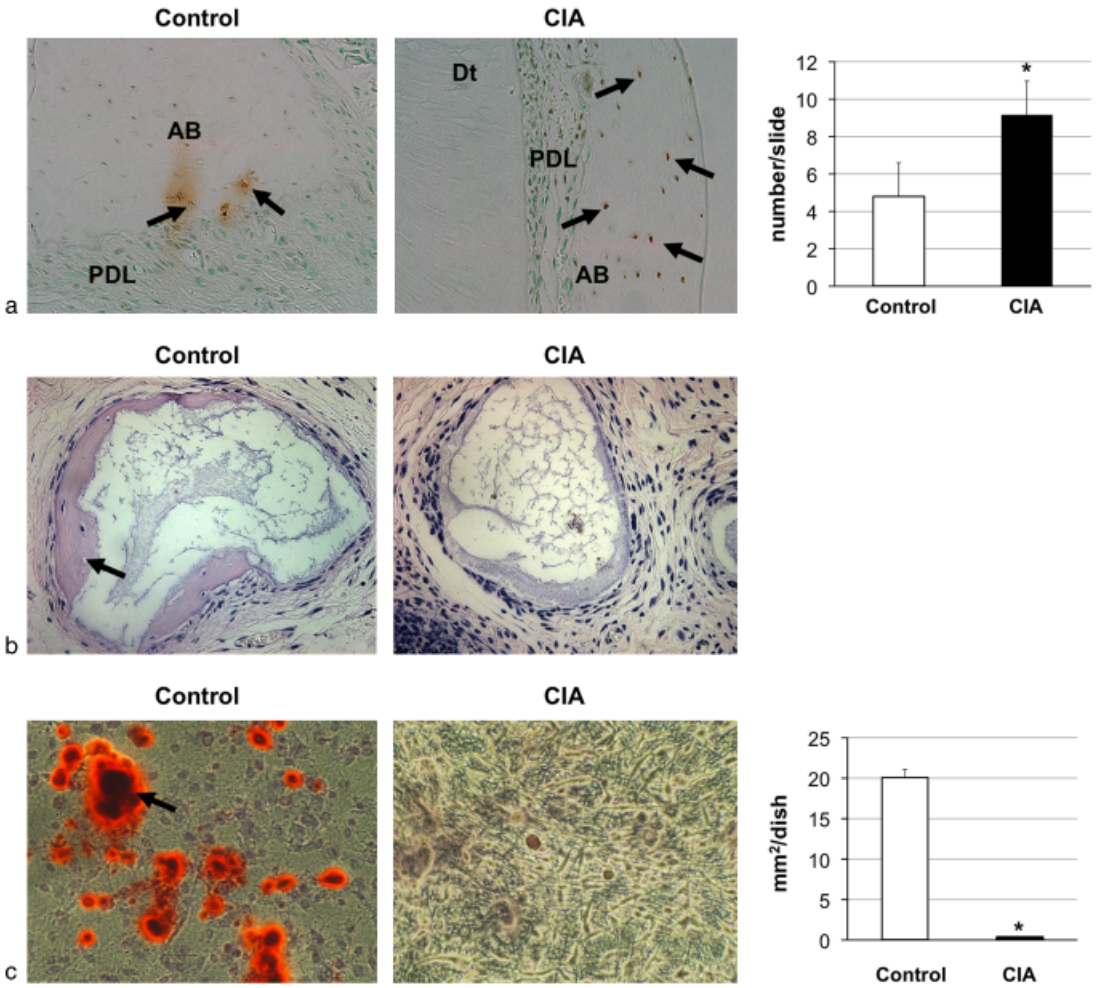


Fig. 4. Reduced osteoblastic activity in collagen-induced arthritis (CIA) model. (a) Deoxynucleotidyl transferase-mediated dUTP-biotin nick-end labelling staining revealed a few apoptotic cells in the normal periodontium while increased numbers of apoptotic cells were detected inside the alveolar bone in the CIA group (arrows). Osteoblasts were assumed to undergo increased apoptosis in the CIA group. (Dt, dentin; PDL, periodontal ligament; AB, alveolar bone). (b) ABCs were soaked in hydroxyapatite/tricalcium phosphate particles and then transplanted subcutaneously into the dorsal surface of a mouse. New bone formation was observed in the control (CTL) group (arrow) after 8 weeks. In contrast, no bone formation was observed and a large number of leucocytes were noted in the CIA group. (c) Osteogenic induction of ABCs. Normal bone-nodule formation (arrow) was observed in the CTL group (alizarin red staining). Little, if any, bone-nodule tissue was observed in the CIA group. Measurement of the area of bone-nodule tissue revealed a significant decrease in this parameter in the CIA group. *Statistically significant difference from control group ($p < 0.05$).

group exhibited an increased number of adipocytes (as revealed by Oil Red O staining), with an increased tendency towards adipogenic differentiation ($p < 0.05$).

Discussion

Here, we report that experimentally induced adjuvant arthritis causes severe periodontal breakdown with substantial resorption of alveolar bone and loss of the periodontal attachments in DBA/1 mice. While adjuvant arthritis is a well-established RA model in rats, there are contradicting reports on the association of alveolar bone loss with RA development (Ramamurthy et al. 2005, Bartold et al. 2010), making rats an unreliable animal model for arthritis-associated alveolar bone loss. In this study, we used DBA/1 mice with arthritis induced by an injection of type II collagen as reported previously (Lee et al. 2009), and we successfully observed significant alveolar bone loss and periodontal tissue destruction during the course of arthritis development. Although it does not fully duplicate the condition of human RA, we consider this model as a powerful tool to study a potential link between RA and PD.

First, to assess the possibility that CIA mice with increased alveolar bone loss are impaired in their ability to repair, we determined changes in the factors associated with bone remodelling or bone coupling (Parfitt, 1982). Additionally, we also assessed changes in adipogenesis as it has been reported recently to be involved in maintaining bone homeostasis. Recent reports further point towards up-regulation of the pro-inflammatory cytokines tumour necrosis factor-α (TNF-α) and interleukin (IL)-1β playing key roles in chronic inflammatory lesions (Okada and Murakami, 1998). These cytokines are known to induce bone resorption by

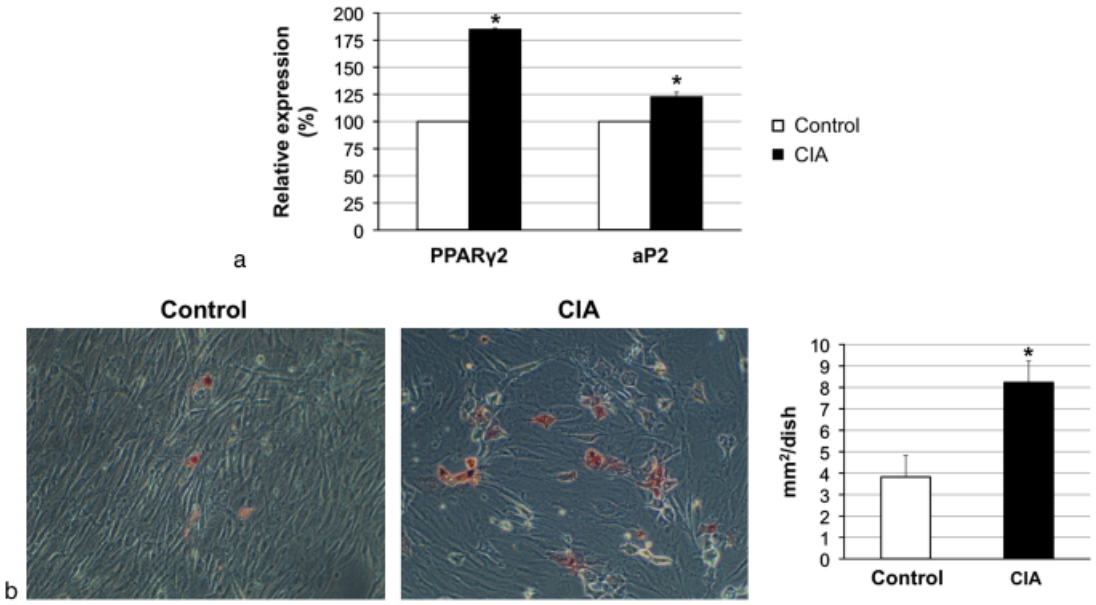


Fig. 5. Increased adipogenic differentiation in the collagen-induced arthritis (CIA) model. (A) Expression of adipogenesis related mRNA was analysed by reverse transcription-polymerase chain reaction (RT-PCR). RT-PCR shows that peroxisome proliferators-activated receptor γ (PPARγ2) and adipocyte fatty acid binding protein (aP2) were highly expressed in the CIA group. (B) ABCs were induced in an adipogenic medium for 14 days. A small number of lipid droplets were detected within the stimulated cells by Oil Red O staining at 2 weeks after stimulation in the control group. Increased accumulation of lipid droplets was observed in the CIA group. *Statistically significant difference from the control group ($p < 0.05$).

promoting the differentiation of osteoclast precursors and subsequently by activating osteoclasts (Pfeilschifter et al. 1989). Previously, we have shown that the systemic levels of cytokines such as TNF-α, IL-1β, IL-6, MMP-3, iNOS, and COX-2 increased during the course of the development of CIA (Lee et al. 2009). We assumed that both periodontal and arthritic bone losses were potentially mediated by increased levels of these cytokines, inducing osteoclastic activity. In this line, such up-regulation results in active resorption of bone tissue and destroys the balance of bone coupling. Our results show that osteoclasts were induced in CIA and that osteoclastogenesis was readily derived in ABCs from the CIA group. We conclude that this increased number of osteoclasts largely accounts for alveolar bone loss.

However, our results also documented increased apoptosis of osteoblasts and reduced expression of related mRNA in CIA mice, leading to diminished bone formation, which might result from a reduced production of osseous matrix or simply from a decreased number of cells with active matrix production. The bone formation observed in the transplantation model shows that reduced bone formation is more affected by decreased bone-forming activity of osteoblasts than by a diminished number of osteoblasts. Therefore, these data suggest that the inappropriate biologic activity of osteoblasts accounts for, at least in part, the imbalance in bone remodelling while elevated osteoclast activity could be a dominant factor.

CIA is a systemic disorder that is not limited to articular structures, and several mechanisms have been suggested to induce periodontal destruction. We have focused on the reciprocal relationship between the development of bone and adipose tissue and suspected that this relationship could be a potential mechanism underlying the alveolar bone loss in CIA-induced PD. It is well established that mesenchymal stem cells can differentiate to osteogenic and adipogenic lineages. Our hypothesis was that cell differentiation in CIA tends to be redirected into the adipogenic lineage, and that the accumulation of adipocytes reduces bone density and as such ultimately increases the susceptibility to bone resorption. Our RT-PCR results show that aP2 and PPARγ2 mRNA expressions were increased in the CIA group compared with the CTL group, and that ABCs from the CIA group were enhanced in their capacity to differentiate into the adipogenic pathway. aP2 is a key mediator of the intracellular transport and metabolism of fatty acids, and PPARγ2 is a well-known key regulator of osteoblast and adipocyte differentiation, its activation playing a pivotal role in the selection of adipogenesis over osteoblastogenesis (Lecka-Czernik et al. 1999). The up-regulated expression of PPARγ2 implies an enhanced differentiation of adipocytes in the alveolar bone and that it may be partially responsible for defective mineralization, decreased bone density, and ultimately, for alveolar bone loss. Several reports demonstrated that decreased PPARγ2 activity causes increased bone density and results in increased number of osteoblasts (Akune et al. 2004). On the other hand, increased PPARγ2 activity by PPARγ2 agonists results in a significant decrease in bone density along with decreased numbers of osteoblasts and increased numbers of adipocytes (Soroceanu et al. 2004). While we demonstrated a redirection of ABCs' differentiation into adipocytes, the molecular mechanism behind this process can be the subject of further studies.

In conclusion, the present study showed that increased osteoclastic activity, decreased bone-forming activity of

osteoblasts, and enhanced adipogenesis account for the alveolar bone loss in CIA. These findings provide a new perspective towards understanding the mechanism of alveolar bone loss in RA patients and improvement of existing treatments for RA patients.

Acknowledgments

This study was supported by a grant of the Korea Healthcare technology R&D Project, Ministry for Health, Welfare & Family Affairs, Republic of Korea (A084447).

References

Akune, T., Ohba, S., Kamekura, S., Yamaguchi, M., Chung, U. I., Kubota, N., Terauchi, Y., Harada, Y., Azuma, Y., Nakamura, K., Kadowaki, T. & Kawaguchi, H. (2004) PPARgamma insufficiency enhances osteogenesis through osteoblast formation from bone marrow progenitors. *Journal of Clinical Investigation* **113**, 846–855.

Bartold, M., Marino, V., Cantley, M. D. & Haynes, D. R. (2010) Effect of Porphyromonas gingivalis-induced inflammation on the development of rheumatoid arthritis. *Journal of Clinical Periodontology* **37**, 405–411.

Bartold, P. M., Marshall, R. I. & Haynes, D. R. (2005) Periodontitis and rheumatoid arthritis: a review. *Journal of Periodontology* **76**, 2066–2074.

Bozkurt, F. Y., Berker, E., Akkus, S. & Bulut, S. (2000) Relationship between interleukin-6 levels in gingival crevicular fluid and periodontal status in patients with rheumatoid arthritis and adult periodontitis. *Journal of Periodontology* **71**, 1756–1760.

Cho, M. L., Kang, J. W., Moon, Y. M., Nam, H. J., Jhun, J. Y., Heo, S. B., Jin, H. T., Min, S. Y., Ju, J. H., Park, K. S., Cho, Y. G., Yoon, C. H., Park, S. H., Sung, Y. C. & Kim, H. Y. (2006) STAT3 and NF-kappaB signal pathway is required for IL-23-mediated IL-17 production in spontaneous arthritis animal model IL-1 receptor antagonist-deficient mice. *Journal of Immunology* **176**, 5652–5661.

Cochran, D. L. (2008) Inflammation and bone loss in periodontal disease. *Journal of Periodontology* **79**, 1569–1576.

de Pablo, P., Dietrich, T. & McAlindon, T. E. (2008) Association of periodontal disease and tooth loss with rheumatoid arthritis in the US population. *Journal of Rheumatology* **35**, 70–76.

Graves, D. (2008) Cytokines that promote periodontal tissue destruction. *Journal of Periodontology* **79**, 1585–1591.

Kim, C. S., Choi, S. H., Cho, K. S., Chai, J. K., Wikesjo, U. M. & Kim, C. K. (2005) Periodontal healing in one-wall intra-bony defects in dogs following implantation of autogenous bone or a coral-derived biomaterial. *Journal of Clinical Periodontology* **32**, 583–589.

Kuznetsov, S. A., Krebsbach, P. H., Satomura, K., Kerr, J., Riminucci, M., Benayahu, D. & Robey, P. G. (1997) Single-colony derived strains of human marrow stromal fibroblasts form bone after transplantation in vivo. *Journal of Bone and Mineral Research* **12**, 1335–1347.

Lecka-Czernik, B., Gubrij, I., Moerman, E. J., Kajkenova, O., Lipschitz, D. A., Manolagas, S. C. & Jilka, R. L. (1999) Inhibition of Osf2/Cbfa1 expression and terminal osteoblast differentiation by PPARgamma2. *Journal of Cellular Biochemistry* **74**, 357–371.

Lee, S. W., Kim, J. H., Park, Y. B. & Lee, S. K. (2009) Bortezomib attenuates murine collagen-induced arthritis. *Annals of the Rheumatic Diseases* **68**, 1761–1767.

Liu, R., Bal, H. S., Desta, T., Krothapalli, N., Alyassi, M., Luan, Q. & Graves, D. T. (2006) Diabetes enhances periodontal bone loss through enhanced resorption and diminished bone formation. *Journal of Dental Research* **85**, 510–514.

Mercado, F. B., Marshall, R. I. & Bartold, P. M. (2003) Inter-relationships between rheumatoid arthritis and periodontal disease. A review.. *Journal of Clinical Periodontology* **30**, 761–772.

Mercado, F. B., Marshall, R. I., Klestov, A. C. & Bartold, P. M. (2001) Relationship between rheumatoid arthritis and periodontitis. *Journal of Periodontology* **72**, 779–787.

Mercado, F., Marshall, R. I., Klestov, A. C. & Bartold, P. M. (2000) Is there a relationship between rheumatoid arthritis and periodontal disease? *Journal of Clinical Periodontology* **27**, 267–272.

Ogrendik, M., Kokino, S., Ozdemir, F., Bird, P. S. & Hamlet, S. (2005) Serum antibodies to oral anaerobic bacteria in patients with rheumatoid arthritis. *Medscape General Medicine* **7**, 2.

Okada, H. & Murakami, S. (1998) Cytokine expression in periodontal health and disease. *Critical Reviews in Oral Biology & Medicine* **9**, 248–266.

Parfitt, A. M. (1982) The coupling of bone formation to bone resorption: a critical analysis of the concept and of its relevance to the pathogenesis of osteoporosis. *Metabolic Bone Disease and Related Research* **4**, 1–6.

Park, C. H., Abramson, Z. R., Taba, M. Jr., Jin, Q., Chang, J., Kreider, J. M., Goldstein, S. A. & Giannobile, W. V. (2007) Three-dimensional micro-computed tomographic imaging of alveolar bone in experimental bone loss or repair. *Journal of Periodontology* **78**, 273–281.

Pei, L. & Tontonoz, P. (2004) Fat's loss is bone's gain. *Journal of Clinical Investigation* **113**, 805–806.

Petropoulou, P., Zhang, Z., Curtis, M. A., Johnson, N. W., Hughes, F. J. & Winyard, P. G. (2003) Measurement of both native and inactivated forms of alpha1 proteinase inhibitor in human inflammatory extracellular fluids. *Journal of Clinical Periodontology* **30**, 795–801.

Pfeilschifter, J., Chenu, C., Bird, A., Mundy, G. R. & Roodman, G. D. (1989) Interleukin-1 and tumor necrosis factor stimulate the formation of human osteoclastlike cells in vitro. *Journal of Bone and Mineral Research* **4**, 113–118.

Pischon, N., Pischon, T., Kroger, J., Gulmez, E., Kleber, B. M., Bernimoulin, J. P., Landau, H., Brinkmann, P. G., Schlattmann, P., Zernicke, J., Buttgereit, F. & Detert, J. (2008) Association among rheumatoid arthritis, oral hygiene, and periodontitis. *Journal of Periodontology* **79**, 979–986.

Ramamurthy, N. S., Greenwald, R. A., Celiker, M. Y. & Shi, E. Y. (2005) Experimental arthritis in rats induces biomarkers of periodontitis which are ameliorated by gene therapy with tissue inhibitor of matrix metalloproteinases. *Journal of Periodontology* **76**, 229–233.

Salvi, G. E., Collins, J. G., Yalda, B., Arnold, R. R., Lang, N. P. & Offenbacher, S. (1997) Monocytic TNF alpha secretion patterns in IDDM patients with periodontal diseases. *Journal of Clinical Periodontology* **24**, 8–16.

Shi, S., Gronthos, S., Chen, S., Reddi, A., Counter, C. M., Robey, P. G. & Wang, C. Y. (2002) Bone formation by human postnatal bone marrow stromal stem cells is enhanced by telomerase expression. *Nature Biotechnology* **20**, 587–591.

Soroceanu, M. A., Miao, D., Bai, X. Y., Su, H., Goltzman, D. & Karaplis, A. C. (2004) Rosiglitazone impacts negatively on bone by promoting osteoblast/osteocyte apoptosis. *Journal of Endocrinology* **183**, 203–216.

Williams, R. C. (1990) Periodontal disease. *New England Journal of Medicine* **322**, 373–382.

Yun, J. H., Kim, C. S., Cho, K. S., Chai, J. K., Kim, C. K. & Choi, S. H. (2007) (-)-Epigallocatechin gallate induces apoptosis, via caspase activation, in osteoclasts differentiated from RAW 264.7 cells. *Journal of Periodontal Research* **42**, 212–218.

Address:
Chang-Sung Kim
Department of Periodontology
Research Institute for Periodontal Regeneration
College of Dentistry
Yonsei University
250 Seongsanno
Seodaemun-gu
Seoul 120-752
Korea
E-mail: dentall@yuhs.ac

Clinical Relevance

Scientific rationale for the study: PD is prevalent and more severe in patients with RA. The objective of this study was to investigate its underlying mechanism by assessing the process of periodontal breakdown in collagen-induced arthritis of mice, focusing on alveolar bone resorption.

Principal findings: In mice with CIA, significant alveolar bone loss was observed. The underlining mechanism involved increased osteoclastic activity, decreased bone-forming activity of osteoblasts, and upregulated adipogenesis.

Practical implications: Understanding the mechanism of alveolar bone loss associated with induced arthritis in an experimental mouse model will provide new avenues for interventions that can improve the course of periodontal breakdown in RA patients.