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TNF-α Upregulates Expression of BMP-2 and BMP-3 Genes in the Rat Dental Follicle – Implications for Tooth Eruption

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Abstract

The dental follicle appears to regulate both the alveolar bone resorption and bone formation needed for tooth eruption. Tumor necrosis factor-alpha (TNF-α) gene expression is maximally upregulated at postnatal day 9 in the rat dental follicle of the 1st mandibular molar, a time that correlates with rapid bone growth at the base of the tooth crypt, as well as a minor burst of osteoclastogenesis. TNF-α expression is correlated with the expression of bone morphogenetic protein-2 (BMP-2), a molecule expressed in the dental follicle that can promote bone formation. Because BMP-2 signaling may be augmented by bone morphogenetic protein-3 (BMP-3), it was the objective of this study to determine 1) if the dental follicle expresses BMP-3 and 2) if TNF-α stimulates the dental follicle cells to express BMP-2 and BMP-3. Dental follicles were collected from different postnatal ages of rat pups. Dental follicle cells were incubated with TNF-α to study its dosage and time-course effects on gene expression of BMP-2 and BMP-3, as determined by real-time RT-PCR. Next, immunostaining was conducted to confirm if the protein was synthesized and ELISA of the conditioned medium was conducted to determine if BMP-2 was secreted. We found that BMP-3 expression is correlated with the expression of TNF-α in the dental follicle and TNF-α significantly increased BMP-2 and BMP-3 expression in vitro. Immunostaining and ELISA showed that BMP-2 and BMP-3 were synthesized and secreted. This study suggests that TNF-α can upregulate the expression of bone formation genes that may be needed for tooth eruption.

Keywords

TNF-α; BMP-2; BMP-3; tooth eruption; dental follicle

Introduction

It is well documented that tooth eruption requires bone resorption to create an eruption pathway in the coronal part of the bony crypt such that the tooth can erupt (1–3). Such bone resorption is mainly regulated by the dental follicle (DF), a loose connective tissue sac that surrounds the unerupted tooth. The DF exerts its function by producing molecular signals to regulate osteoclastogenesis at a precise time during eruption (1, 4). In the rat first mandibular molar, a major burst in osteoclast numbers is seen at day 3 postnatally (5, 6), the time the DF maximally expresses osteoclastogeneic genes such as colony stimulating factor-1 (CSF -1) and monocyte chemoattractant protein-1 (MCP-l) (7, 8) while suppressing the expression of negative regulators of osteoclastogeneis such as osteoprotegerin (OPG) (9,

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4). In addition to this major period of osteoclastogenesis, there is a minor secondary burst of osteoclastogenesis at postnatal day 10 (6). We have proposed that the DF regulates this secondary burst of osteoclastogenesis by increasing its expression of vascular endothelial growth factor (VEGF) and TNF-α (10–13), as well as by increasing its expression of receptor activator for nuclear factor κ B ligand (RANKL) (14). VEGF, which is upregulated by TNF-α (11), can substitute for CSF-1 to upregulate osteoclastogenesis in the RANK-RANKL interaction pathway by enhancing receptor activator for nuclear factor κ B (RANK) gene expression in osteoclast precursors (13). In addition, TNF-α may directly promote osteoclastogenesis via a RANK-RANKL independent pathway (15).

Early studies of the dog fourth premolar indicated that the tooth crypt also undergoes bone formation in its basal part during tooth eruption (16). When the basal one-half of the DF was removed, no bone formation in the basal portion of the crypt occurred and the tooth did not erupt, suggesting that the DF plays a critical role in regulating alveolar bone formation (16). Recently, we have observed that this bone formation might be a motive force to push the tooth out of its bony crypt during eruption (17). To coordinate the opposite biological processes (osteogenesis vs osteoclastogenesis) during eruption, the DF may differentially express genes related to bone formation and resorption; i.e., the coronal portion of the follicle expresses a higher level of bone resorption molecules such as RANKL and the basal portion expresses a higher level of bone formation molecules such as bone morphogenetic protein-2 (BMP-2) (18).

Bone morphogenetic proteins (BMPs) belong to the transforming growth factor–β super-family and play an important role in postnatal bone formation (19). Given that there are reports that TNF-α upregulates the expression of BMP-2 to promote bone formation (20, 21) and given that bone growth is occurring at the base of the alveolar crypt at the time TNF-α is maximally expressed in the DF (17), perhaps TNF-α also is involved in regulating bone formation for tooth eruption. In particular, TNF-α may promote the expression of BMP-2 in the basal one-half of the DF to enhance alveolar bone formation in the base of the bony crypt.

Another important member of the BMPs is BMP-3, the most abundant BMP in bone. In developing mouse teeth, BMP-3 transcripts first were observed during late bud stage in the dental mesenchyme and expression was later restricted to the DF (22). The expression pattern of BMP-3 and its regulation in the postnatal DF are not known. Because the DF appears to regulate bone formation and bone resorption for tooth eruption, the expression of BMP-3 in the DF may be significant in that it may have a dual role of both promoting bone formation and bone resorption.

Thus, the objectives of this study were to: 1) determine the in vivo gene expression profile of BMP-3 in the dental follicle; and 2) determine if TNF-α can enhance the gene expression of BMP-2 and BMP-3 in vitro in the cultured dental follicle cells.

Materials and Methods

Experimental animals and Dental follicle (DF)

Breeding pairs of Sprague-Dawley rats were housed in the School of Veterinary Medicine at Louisiana State University and maintained using a protocol approved by the Intuitional Animal Care and Use Committee. The mandibles were collected every other day from postnatal days 1 to 11 with 1 pup per age in a given litter; i.e., 6 pups per litter. The DFs were surgically isolated from the first mandibular molar under a dissection microscope (23), and immediately homogenized in TRI REAGENT (Molecular Research Center Inc., Cincinnati, OH, USA) with a tissue grinder. The samples then were stored at −80°C until the

time of RNA extraction. Four litters with a total of 24 pups were used in this experiment. The experiment was conducted with a completely randomized block design with each litter as a block. Next, the RNA was extracted from the collected DF according to the manufacturer's protocol. The RNA samples were treated with DNase I to remove any DNA contamination. The RNA concentration and purity were measured at OD 260 and OD 280. RNA purity was ensured by the ratio of OD260/OD280 being greater than 1.9.

Culture of dental follicle cells (DFCs) and in vitro incubation experiments

The DFs were isolated from day 5 or day 6 postnatal rats and after trypsinization the cells were cultured in MEM medium with 10 % newborn calf serum, 1 mM sodium pyruvate and antibiotics as described by Wise et al., (23). At confluency, the cells were detached using 1% trypsin and passaged into new flasks. Cells of passage 6 to passage 9 were then used for experimentation. For incubation experiments, cells were grown in 25-cm^2 T -flasks until approximately 80 % confluent. On the day of treatment, the cells were placed in serum depletion medium containing 0.3 % bovine serum albumin (BSA) in Dulbecco's modified Eagle's medium (DMEM) for 5 hours. Next, for the dosage effect study, TNF-α was added to the cultures at concentrations of 0, 5, 10, 20 and 50 ng/ml and incubated for 1 hour (for BMP-2) or 4 hours (for BMP-3). These treatment durations were based on the time-course effects of TNF-α on expression of OPG and BMP-3 in the DFCs. The same experiment was repeated at least 3 times. For the time course study, cells were incubated in medium containing 10 ng/ml of TNF-α for 0.5, 1, 2, 4, 6 and 8 hours and cells incubated in medium without TNF-α were also collected at each time point as controls. The experiment was repeated 5 times. After the designated time of incubation, the cells were collected into TRI REAGENT for RNA extraction as described above.

RT -PCR

Equal amounts of total RNA (1 μg from DFs and 2 μg from DFCs) were reverse transcribed into cDNA with MLV reverse transcriptase (Invitrogen Corporation, Carlsbad, CA, USA) in 20 μl volume. 2 μl cDNA of each sample was used in Taqman real-time PCR to obtain the C_T value for a given gene in an ABI sequence detector (Applied Biosystems, Foster City, CA, USA). The real-time PCR for β-actin was run in parallel and served as the PCR control. Each sample was run in duplicate in PCR to determine gene expression of BMP-2 and BMP-3. The PCR primers and probe were as follows: for BMP-2, 5′-T AGTGACTTTTGGCC ACGACG-3′ (forward), 5′-GCTTCCGCTGTTTGTGTTTG-3′ (reverse) and probe 5′-FAM-AA GGACATCCACTCCA CAAACGAGAAAAGC-BHQ1-3′; for BMP-3, the primers and probe were 5 ′-AAGCGTGGTATCTAGCCTACAGA-3′(forward), 5′-CAGTCTGGGCACAGTTCCT- 3′ (reverse), and 5′-FAM-ACACCGCGATTTCACA-BHQ-3′ (probe). Relative gene expression (RGE) was calculated from the C_T values using the Delta C_T method.

ELISA and Immunostaining

DFCs, passages 7–9, were grown in 25 cm^2 flasks (T-25) in 5 ml minimum essential medium (MEM) supplemented with 10% newborn calf serum, 1 mM sodium pyruvate and antibiotics. Upon reaching 90% confluence, cells were incubated for 5 hours in DMEM without serum but supplemented with 0.3% BSA. After 5 hours of serum depletion, cells were incubated with 5 ml of DMEM containing 10 ng/ml TNF-α but no BSA for 2, 4, 6 and 8 hours. At the specified times, the conditioned medium was collected and 50 μl of protease inhibitor cocktail (Sigma-Aldrich, St. Louis, MO, USA) was added to the collected medium to prevent protein degradation. The total proteins in the medium were precipitated with 60% saturation of ammonium sulfate and pellets were collected after centrifugation for 1 hour at 14000 rpm/min. Supernatants were aspirated and the pellets dissolved in 80 μl of water. Protein concentration was determined using the Bradford protein assay kit (Pierce,

Rockford, IL, USA). BMP-2 ELISA was performed with 20 μg total protein of each sample using a Quantikine BMP-2 immunoassay kit (R&D Systems, Inc., Minneapolis, MN, USA) according to the manufacturer's instructions. The assay reaction was measured at OD 450 nm with the correction wavelength set at 570 nm using a Bio-Rad microplate reader model 550 (Bio-Rad, Hercules, CA, USA). The intensity of the yellow color measured was in proportion to the amount of BMP-2 bound. The experiment was repeated at least 4 times for each time point. The relative change in absorbance volume of a treatment was calculated by normalizing to the control set to 1.

For immunostaining, DFCs of passages 7–9 were plated on glass cover slips and cultured and treated under the same conditions described above except that treatments were conducted on cells at 50 % confluency. After 4 and 6 hours of TNF-α treatment, the cover slips with attached DFCs were washed 3 times with 3 ml of PBS and then fixed with cold (−20° C) methanol for 3 minutes. The cells were incubated with 0.1% Triton X-100 in PBS and 1% H_2O_2 for 5 minutes, followed by 3 washes of PBS. Samples were then incubated in 5% goat serum in PBS for 45 minutes, washed 3 times with PBS and incubated overnight with rabbit polyclonal anti-BMP-2 or anti-BMP-3 primary antibodies (human origin cross react with rat from Abcam, Inc., Cambridge, MA, USA) at 1: 100 dilution (i.e., 4 μg/ml) in 2% goat serum at 4° C overnight. The next day, the cells were incubated with secondary biotinylated anti-rabbit antibody (Vector Laboratories. Inc., Burlingame, CA, USA) for 45 minutes at room temperature, followed by washing 3 times with PBS to remove the unbound antibody. Next, the staining of the cells was conducted using a Vector DAB peroxidase substrate kit (Vector Laboratories Inc.), and the cells were then photographed under a light microscope.

Statistical Analysis

Analysis of variance (ANOVA) combined with the least significant difference (LSD) test was used to assess the treatment effect if multiple samples (more than 2 samples) were compared. A Student T test was used if comparison was between two samples such as for comparison of TNF-α effect between treatment and untreated control in each time point in the time course study. The statistical significant level was $P \leq 0.05$ for all tests.

Results

This study revealed that the DF expressed BMP-3. Its expression gradually increased starting at day 3 and peaked at day 9 when expression was more than threefold higher than at day 1 (Fig. 1), which was statistically significant. The expression from days 5 through 9 were not statistically significant from each other (Fig. 1). Although its expression appeared to drop at day 11, the reduction in expression was also not statistically significant.

Previously, we have reported increased gene expression of BMP-2 in the DF of the rat first mandibular molar at postnatal days 3 and 9 (24). In this study, real-time RT -PCR revealed that TNF-α could significantly increase BMP-2 expression in vitro in the DFCs at all concentrations tested (Fig. 2A). The enhancement was more than threefold at a TNF-α concentration of 5 ng/ml as compared to the untreated control. The expression level continued to increase to the maximal TNF-α concentration tested (50 ng/ml), in which the expression was about fivefold higher than the control. Of the concentrations tested, TNF-α concentrations of 10 ng/ml resulted in no statistically significant difference on BMP-2 expression when compared to the higher concentration of TNF-α. In the time course study with 10 ng/ml TNF-α, a significant enhancement of BMP-2 expression was also seen at all time points tested (Fig. 2B). The enhancement was twofold at 0.5 hour incubation and increased to fourfold at 2 hours of incubation. The trend of enhancement was continued until

6 hours, at which time the enhancement was maximal with more than a sixfold increase as compared to the control (Fig. 2B).

TNF-α also stimulated BMP-3 expression in the DFCs, but to a lesser extent than BMP-2 in terms of duration and dosage. When DFCs were incubated with TNF-α for 4 hours, BMP-3 expression was significantly increased at all concentrations tested as compared to the untreated control with the maximal expression seen at a TNF-α concentration of 25 ng/ml (Fig. 3A). The TNF-α concentrations of 5 ng/ml to 50 ng/ml resulted in no statistically significant difference among the concentrations on affecting BMP-3 expression. In a time-course study, significant upregulation of BMP-3 was seen when cells were incubated for 1, 2, 4 and 6 hours with 10 ng/ml of TNF-α, with the maximal enhancement at 4 hours of incubation (Fig. 3B).

Immunostaining with rat anti-BMP-2 and BMP-3 showed a greater staining in the DFCs incubated with TNF-α than in the controls (Fig. 4). This was true for both 4 and 6 hours of treatment (Fig. 4) and corresponded to the mRNA levels as seen in Figure 2. When the conditioned medium was analyzed by ELISA for the presence of BMP-2, a significantly higher absorbance reading was obtained in TNF-α treatments than in the controls after either 4 hours or longer of incubation (Fig. 5), indicating that significantly more BMP-2 was secreted into the medium after TNF-α treatment.

Discussion

Studies by us and others have suggested that bone growth at the base of the tooth crypt is required for a tooth to erupt (16, 17, 18). In a study of dog premolars, removal of the basal DF resulted in no alveolar bone formation in the base of the tooth crypt and no eruption, suggesting that the DF plays an essential role in regulating alveolar bone formation (16). In a recent scanning electron microscope study, we observed that the initial alveolar bone formation was seen at day 3 postnatally and rapid bone growth occurred after day 9 in the base of the alveolar bony crypt of the first mandibular molar (17). After measuring the size of the molar, the depth of the tooth socket and the bone deposition, we proposed that the bone formation at the base of tooth crypt may serve as a motive force to guide the tooth out of the bony crypt during eruption (17).

BMPs have been well-documented to possess osteogenic activity (25). Previously, we have reported that in the DF, BMP-2 expression is higher in the basal region than in the coronal region at postnatal days 3, 5, 9 and 11 (18). In turn, this could promote the bone formation at the base of the crypt that is needed for tooth eruption. Paralleling this is increased expression of TNF-α in the DF at day 3 with the maximal expression at day 9 (11). This study showed that TNF-α can stimulate the expression of BMP-2 in the DFCs (Figs. 2A, 2B). Studies by others also indicate that TNF-α stimulates BMP-2 expression in certain cell types such as chondrocytes and endothelial cells (20, 21). Thus, the increased TNF-α seen in the DF may play a role in regulating the regional differences of BMP-2 expression. In addition to BMP-2, this study reveals that the expression of BMP-3 in the DF is increased at day 3 and is subsequently and incrementally increased until day 9 (Fig. 1), which correlates with the expression of TNF-α (11). In conjunction with the in vivo correlation, this study reveals that TNF-α could significantly enhance the expression of BMP-3 in vitro in the DFCs (Fig. 3A and 3B), suggesting that TNF-α may contribute to the increased expression of BMP-3 seen in vivo. Thus, it appears that TNF-α is a positive regulator of the expression of BMP-2 and BMP-3 in the DF.

Cultured DFCs have been shown to secrete BMP-2 (24) and we now show that this secretion of BMP-2 from the DFCs is enhanced by TNF-α (Fig. 5). In turn, this BMP-2 secreted by

the DF cells could promote osteogenesis. Because the DF is adjacent to the alveolar bone, the BMP-2 secreted by it could stimulate osteoprogenitor cells in the bone to differentiate into osteoblasts.

In addition, recent studies have shown that the DF contains stem cells or progenitor cells (26, 27). We also have discovered that stem cells are present in the rat DF and that such stem cells are pluripotent in that they can differentiate into multiple cell lineages, including osteoblasts (28). Thus, it is possible that the BMP-2 secreted by the DFCs exerts a paracrine effect on these stem cells to induce their differentiation into osteoblasts in the basal region of DF, where BMP-2 is relatively higher than in the coronal region.

BMP-3 was originally purified as a bone-inductive protein from bone matrix (29). It has been shown to play important roles in initiating bone formation (30). When purified human recombinant BMP-3 was implanted into mouse thigh muscle, osteogenesis was observed, indicating the bone-inducing activity of BMP-3 in vivo (31). BMP-3 also stimulates the differentiation of osteoblast precursors in cultured human bone marrow stromal cells (32). This study has determined that BMP-3 expression is gradually increased from day 3 to the peak at day 9 in the rat DF of first mandibular molar. This expression pattern of BMP-3 is significant because it correlates with the bone growth status seen at the base of the tooth crypt (17). Given the fact that BMP-3 can induce bone formation as discussed, it is possible that BMP-3 expressed in the DF also contributes to the bone growth needed for tooth eruption.

In an experiment to study osteo-induction of BMP-2 and BMP-3, a recombinant DNA technique was used to construct BMP-2 and BMP-3b (a closely related BMP-3 protein) expression vectors, and the vectors were introduced alone or together into rat calf muscle in vivo by adenovirus (33). The study demonstrated that BMP-3b alone did not cause osteo-induction; however, BMP-2 and BMP-3b together resulted in a greater osteo-inductive activity than BMP-2 alone. Furthermore, the degree of osteo-induction was increased as the BMP-3b vector unit increased (33), suggesting that BMP-3 may augment the bone formation induced by BMP-2. Although the relative gene expression (RGE) of BMP-2 is higher in the basal than in the coronal region of the DF at certain postnatal days, the RGEs of BMP-2 in the base are similar for these days (18). However, because BMP-3 is maximally expressed at day 9, this may be of significance in explaining the rapid bone growth see at the base of the crypt starting at day 9 (17). Thus, even if BMP-3 does not independently contribute to osteo-induction, it may serve as a booster of the BMP-2 signal to induce the alveolar bone formation.

Despite the observation of osteo-inductive activity of BMP-3 in many reports, there are several studies that suggest that BMP-3 may work as a negative regulator of bone formation. A recent study reported that BMP-3 inhibits BMP-2-mediated induction of Msx2 and blocks BMP-2 mediated differentiation of osteo-progenitor cells into osteoblasts (34). Perhaps, BMP-3 may have either a positive or a negative effect on osteo-induction depending on the developmental conditions, cellular environments or tissue types. Regarding tooth eruption, alveolar bone resorption and formation concurrently occur in the coronal and basal region of the tooth crypt, respectively. We have reported that the coronal and basal regions of the DF possess distinct features in differential expression of BMP-2 and RANKL genes (18). Thus, it is possible that increased BMP-3 inhibits the bone formation in the-coronal region to favor bone resorption whereas it promotes bone formation in the basal region. Future experiments to determine this might be to attempt a coronal or basal regional knockdown of BMP-3 in the DF using siRNA and then determine the effect on bone resorption coronally and bone formation basally.

TNF-α is primarily considered as a positive regulator of bone resorption. We have reported that TNF-α can promote the expression of bone resorption molecules such as VEGF and RANKL in the DF (11, 14). This study shows that TNF-α can also promote the expression of bone formation molecules of BMP-2 and BMP-3 (although BMP-3 may posses dual roles). Therefore, TNF-α may be a cytokine capable of regulating both osteogenesis and osteoclastogenesis, the opposite cellular events required for bone remodeling.

¶ Regarding tooth eruption, TNF-α might concurrently upregulate bone resorption and bone formation to promote eruption. Specifically, it might differentially regulate the expression of osteoclastogenic or osteogeneic genes in the DF; i.e., TNF-α may directly upregulate expression of osteoclastogenic genes in the coronal region of the DF whereas in the basal region it upregulates the expression of osteogenic genes. Alternately, this osteoclastogenesis and osteogenesis also could be achieved by regulating genes such as BMP-3 that have dual functions (see above discussion).

In conclusion, BMP-3 is expressed in the DF of the postnatal rat first mandibular molar. Its expression profile closely correlates with the previously reported expression pattern of TNF-α. In vitro, TNF-α upregulates the expression of BMP-2 and BMP-3, but has a stronger effect on BMP-2. Together with the previous findings of TNF-α in promoting bone resorption, we speculate that TNF-α may have dual roles in regulating bone formation and bone resorption for tooth eruption.

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References

1. Wise GE, King GJ. Mechanisms of tooth eruption and orthodontic tooth movement. J Dent Res. 2008; 87:414–434. [PubMed: 18434571]
2. Marks, SC., Jr; Cieliski, MJ.; Sundquist, K.; Wise, GE.; Gorski, JP. The role of bone resorption in tooth eruption. In: Davidovitch, Z., editor. The Biological Mechanisms of Tooth Eruption, Resorption and Replacement by Implants. Birmingham, AL: EBSCO Media; 1994. p. 482-488.
3. Grier RL, Wise GE. Inhibition of tooth eruption in the rat by a bisphosphonate. J Dent Res. 1998; 77:8–15. [PubMed: 9437395]
4. Wise GE, Yao S, Odgren PR, Pan F. CSF-l regulation of osteoclastogenesis for tooth eruption. J Dent Res. 2005; 84:837–841. [PubMed: 16109994]
5. Wise GE, Fan W. Changes in the tartrate-resistant acid phosphatase cell population in dental follicles and bony crypts of rat molars during tooth eruption. J Dent Res. 1989; 68:150–156. [PubMed: 2465331]
6. Cielinski, MJ.; Jolie, M.; Wise, GE.; Ando, DG.; Marks, SC, Jr. Colony-stimulating factor-l (CSF-l) is a potent stimulator of tooth eruption in the rat. In: Davidovitch, Z., editor. The Biological Mechanisms of Tooth Eruption, Resorption and Replacement by Implants. Birmingham, AL: EBSCO Media; 1994. p. 429-436.
7. Wise GE, Lin F, Zhao L. Transcription and translation of CSF-1 in the dental follicle. J Dent Res. 1995; 74:1551–1557. [PubMed: 7560415]
8. Que BG, Wise GE. Colony-stimulating factor-1 and monocyte chemotactic protein-l chemotaxis for monocytes in the rat dental follicle. Arch Oral Biol. 1997; 42:855–860. [PubMed: 9460539]
9. Wise GE, Lumpkin SJ, Huang H, Zhang Q. Osteoprotegerin and osteoclast differentiation factor in tooth eruption. J Dent Res. 2000; 79:1937–1942. [PubMed: 11201042]
10. Wise GE, Yao S. Expression of vascular endothelial growth factor in the dental follicle. Crit Rev Eukaryot Gene Expr. 2003; 13:173–180. [PubMed: 14696965]
11. Wise GE, Yao S. Expression of tumour necrosis factor-alpha in the rat dental follicle. Arch Oral Biol. 2003; 48:47–54. [PubMed: 12615141]

12. Yao S, Wise GE. In vivo expression of classic PKC isoforms in the rat dental follicle as related to tooth eruption. Connect Tissue Res. 2004; 45:216–221. [PubMed: 15763930]
13. Yao S, Liu D, Pan F, Wise GE. Effect of vascular endothelial growth factor on RANK gene expression in osteoclast precursors and on osteoclastogenesis. Arch Oral Biol. 2006; 51:596–602. [PubMed: 16443190]
14. Liu D, Yao S, Pan F, Wise GE. Chronology and regulation of gene expression of RANKL in the rat dental follicle. Eur J Oral Sci. 2005; 113:404–409. [PubMed: 16202028]
15. Kitaura H, Sands MS, Aya K, Zhou P, Hirayama T, Uthgenannt B, Wei S, Takeshita S, Novack DV, Silva MJ, Abu-Amer Y, Ross FP. Marrow stromal cells and osteoclast precursors differentially contribute to TNF-alpha-induced osteoclastogenesis in vivo. J Immunol. 2004; 173:4838–4846. [PubMed: 15470024]
16. Marks SC Jr, Cahill DR. Regional control by the dental follicle of alterations in alveolar bone metabolism during tooth eruption. J Oral Pathol. 1987; 16:164–169. [PubMed: 3114454]
17. Wise GE, Yao S, Henk WG. Bone formation as a potential motive force of tooth eruption in the rat molar. Clin Anat. 2007; 20:632–639. [PubMed: 17415742]
18. Wise GE, Yao S. Regional differences of expression of bone morphogenetic protein-2 and RANKL in the rat dental follicle. Eur J Oral Sci. 2006; 114:512–516. [PubMed: 17184234]
19. Chen D, Zhao M, Mundy GR. Bone morphogenetic proteins. Growth Factors. 2004; 22:233–241. [PubMed: 15621726]
20. Fukui N, Zhu Y, Maloney WJ, Clohisy YJ, Sandell LJ. Stimulation of BMP-2 expression by pro-inflammatory cytokines IL-l and TNF-alpha in normal and osteoarthritic chondrocytes. J Bone Joint Surg Am. 2003; 85:59–66. [PubMed: 12925611]
21. Csiszar A, Smith KE, Koller A, Kaley G, Edwards JG, Ungvari Z. Regulation of bone morphogenetic protein-2 expression in endothelial cells: role of nuclear factor-kappa B activation by tumor necrosis factor-alpha, H_2O_2, and high intravascular pressure. Circulation. 2005; 111:2364–2372. [PubMed: 15851600]
22. Aberg T, Wozney J, Thesleff I. Expression patterns of bone morphogenetic proteins (bmps) in the developing mouse tooth suggest poles in morphogenesis and cell differentiation. Dev Dyn. 1997; 210:383–396. [PubMed: 9415424]
23. Wise GE, Lin F, Fan W. Culture and characterization of dental follicle cells from rat molars. Cell Tissue Res. 1992; 267:483–492. [PubMed: 1571962]
24. Wise GE, Ding D, Yao S. Regulation of secretion of osteoprotegerin in rat dental follicle cells. Eur J Oral Sci. 2004; 112:439–444. [PubMed: 15458504]
25. Wozney JM, Rosen V, Celeste AJ, Mitsock LM, Whitters MJ, Kriz RW, Hewick RM, Wang EA. Novel regulators of bone formation: molecular clones and activities. Science. 1988; 242:1528–1534. [PubMed: 3201241]
26. Morsczeck C, Gotz W, Schierholz J, Zeilhofer F, Kühn U, Möhl C, Sippel C, Hoffmann KH. Isolation of precursor cells (PCs) from human dental follicles of wisdom teeth. Matrix Biol. 2005; 24:155–165. [PubMed: 15890265]
27. Luan X, Ito Y, Dangaria S, Diekwisch TG. Dental follicle progenitor cell heterogeneity in the developing mouse periodontium. Stem Cells Dev. 2006; 15:595–608. [PubMed: 16978062]
28. Yao S, Pan F, Prpic V, Wise GE. Differentiation of stem cells in the dental follicle. J Dent Res. 2008; 87:767–771. [PubMed: 18650550]
29. Sampath TK, Muthukumaran N, Reddi AH. Isolation of osteogenin, an extracellular matrix-associated, bone-inductive protein, by heparin affinity chromatography. Proc Natl Acad Sci. 1987; 84:7109–7113. [PubMed: 3478684]
30. Reddi AH, Cunningham NS. Recent progress in bone induction by osteogenin and bone morphogenetic proteins: challenges for biomechanical and tissue engineering. J Biomech Eng. 1991; 113:189–190. [PubMed: 1875692]
31. Zhu B, Pu Q, Chen N, Chen S. Expression, purification, and bone-inducing activity of recombinant human bone morphogenetic protein-3 mature peptide. Clin J Biotechnol. 1999; 15:153–158.
32. Amedee J, Bareille R, Rouais F, Cunningham N, Reddi H, Harmand MF. Osteogenin (bone morphogenic protein 3) inhibits proliferation and stimulates differentiation of osteoprogenitors in human bone marrow. Differentiation. 1994; 58:157–164. [PubMed: 7890141]

33. Kaihara S, Bessho K, Okubo Y, Kaihara S, Bessho K, Okubo Y, Sonobe J, Komatsu Y, Miura M, Miyatake S, Nakao K, Iizuka TI. Over expression of bone morphogenetic protein-3b (BMP-3b) using an adenoviral vector promote the osteoblastic differentiation in C2C12 cells and augment the bone formation induced by bone morphogenetic protein-2 (BMP-2) in rats. Life Sci. 2003; 72:1683–1693. [PubMed: 12559390]

34. Daluiski A, Engstrand T, Bahamonde ME, Gamer LW, Agius E, Stevenson SL, Cox K, Rosen V, Lyons KM. Bone morphogenetic protein-3 is a negative regulator of bone density. Nat Genet. 2001; 27:84–88. [PubMed: 11138004]

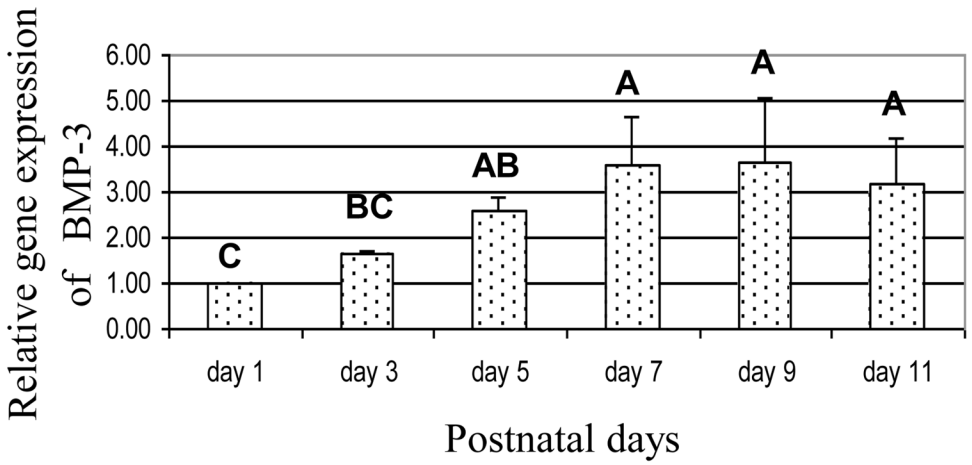
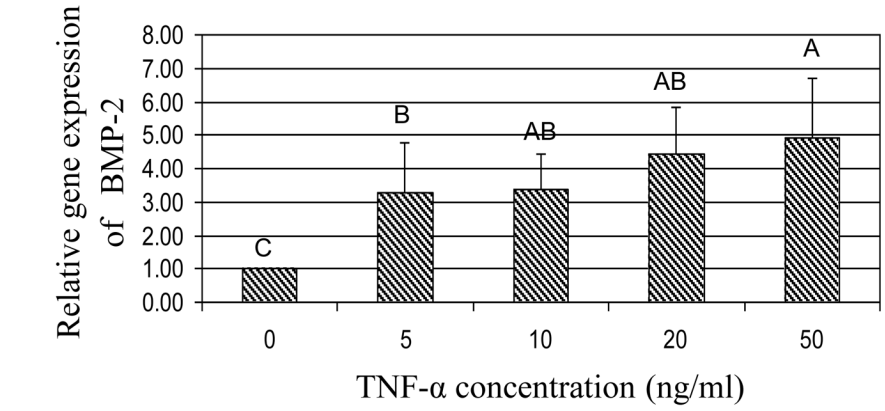


Figure 1.

Relative gene expression (RGE) of BMP-3 in the dental follicle of rat first mandibular molar as determined by RT-PCR. Note that BMP-3 begins to increase its expression at postnatal day 3 and reaches its peak at day 9. Bars labeled with the same letter indicate no significant difference at $P \leq 0.05$. For example, day 1 (labeled C) does not differ from day 3 (BC) but does differ from day 5 (AB) and subsequent days (labeled A).



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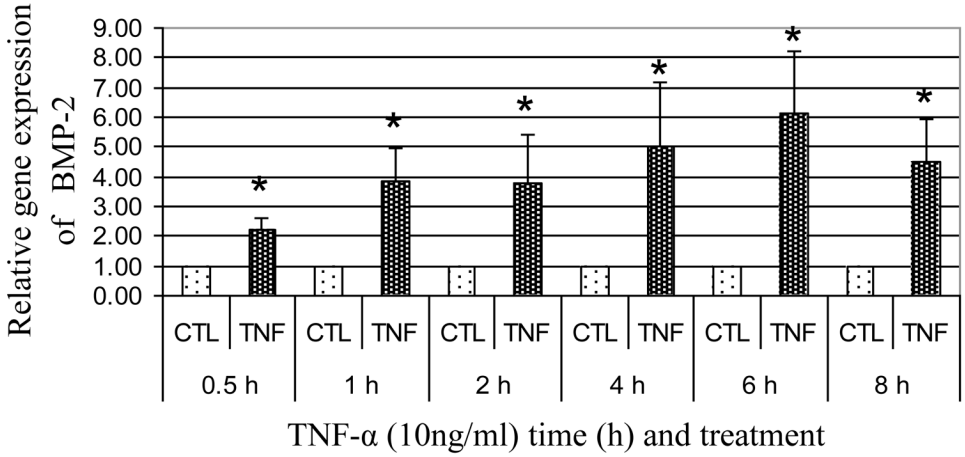

B

Figure 2.

Comparison of TNF-α effects on BMP-2 gene expression in cultured dental follicle cells. (A) Dosage effect of TNF-α on BMP-2 expression after 1 hr of incubation. RGE (relative gene expression). Bars with the same letter are not significantly different at $P \leq 0.05$. (B) Time-course effect of TNF-α on BMP-2 expression. Bar labeled with an asterisk (*) indicates a significant difference from its control ($P \leq 0.05$). Note that all time points tested were significant with the maximal effect at 6 hours of incubation.

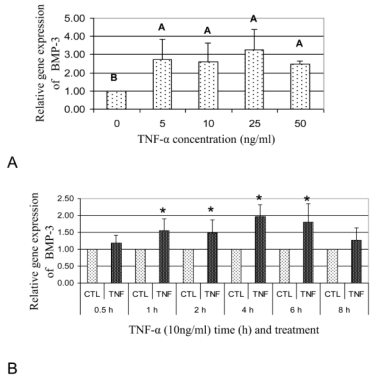

Figure 3.

Comparison of TNF-α effect on BMP-3 gene expression in cultured dental follicle cells. (A) Dosage effect of TNF-α on BMP-3 expression after 4 hrs of incubation. RGE (relative gene expression). Bars with the same letter are not significantly different at $P \leq 0.05$. (B) Time-course effect of TNF-α on BMP-3 expression. Bar labeled with an asterisk (*) indicates a significant difference from its control ($P \leq 0.05$). In brief, all concentrations used are significantly different from the control of 0ng/ml. Note that a significant effect was seen at 1, 2, 4 and 6 hours of treatment with the maximal effect at 4 hours of incubation.

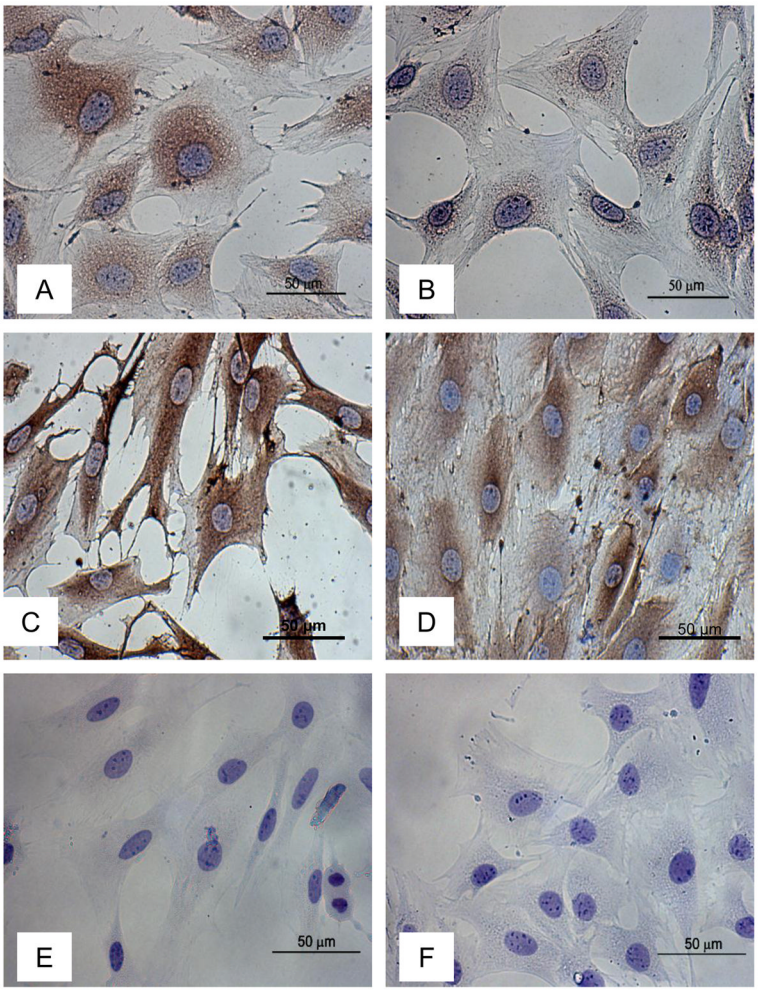

Figure 4.
Immunostaining for BMP-2 and BMP-3 in cultured DFCs with and without TNF-α treatment. The greater staining was seen in TNF-α treated cells (A: stained with BMP-2 antibody, C: stained with BMP-3 antibody) as compared to untreated cells (B: stained with BMP-2 antibody, D: stained with BMP-3 antibody) indicating that TNF-α enhanced BMP-2 and BMP-3 protein production. Omitting the primary antibody resulted in no staining in both treated and untreated cells (E and F, respectively), indicating that the immunostainings for BMP-2 and BMP-3 were specific.

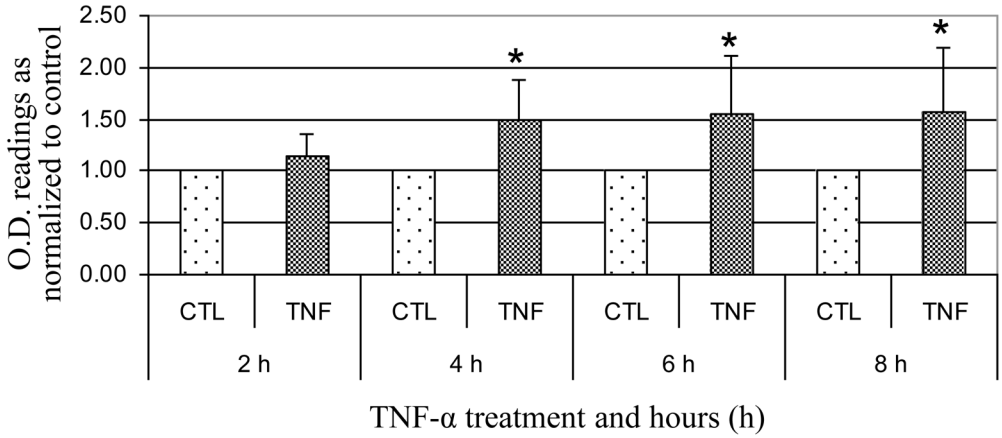

Figure 5.

TNF-α effect on BMP-2 secretion from DFCs as determined by ELISA. Note that a significantly higher OD reading was seen in the conditioned medium after 4 hours or longer of TNF-α incubation as compared to the untreated control (CTL) in each time point. Each bar labeled with an asterisk (*) indicates a significant difference from its control at $P \leq 0.05$.