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Human cytomegalovirus, Epstein-Barr virus and bone resorption-inducing cytokines in periapical lesions of deciduous teeth

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Background: A connection of herpesvirus periapical infection with symptomatic and large-size periapical lesions has been recognized in adult patients, but no data exist about a possible involvement of herpesviruses in severe periapical pathosis in children. Herpesviruses have the potential to elicit potent bone resorption-inducing cytokines in mammalian cells.
Aim: This study aimed to determine the occurrence of human cytomegalovirus and Epstein-Barr virus DNA, and mRNA transcripts of receptor activator of nuclear kappa B ligand (RANKL), osteoprotegerin, core binding factor alpha-1, colony stimulating factor-1, transforming growth factor-β, and monocyte chemoattractant protein-1 in periapical symptomatic pathosis of deciduous teeth.
Material and methods: Twelve deciduous molar teeth from patients aged 2–8 years were extracted due to severe periapical infection, and granulomatous tissue adherent to the root tip of the extracted teeth was collected using a surgical knife. Non-diseased pulpal tissue, obtained from 12 teeth extracted for orthodontic reasons, served as negative control. Polymerase chain reaction assays were employed to identify herpesvirus DNA and cytokine gene expression, using established polymerase chain reaction primers and procedures.
Results: Seven (58%) of the periapical lesions yielded human cytomegalovirus and eight (67%) Epstein-Barr virus. Only one (8%) periapical lesion showed neither human cytomegalovirus nor Epstein-Barr virus. In healthy pulpal tissue, one (8%) specimen demonstrated human cytomegalovirus and another (8%) specimen revealed Epstein-Barr virus. Of the cytokines examined, RANKL expression showed significantly higher occurrence in periapical pathosis than in healthy pulpal tissue ($P < 0.040$). No relationship was identified between the type of herpesvirus and cytokine expression in the periapical lesions studied.
Conclusions: The present findings provide evidence of a putative role of human cytomegalovirus and Epstein-Barr virus in the pathogenesis of symptomatic periapical pathosis in deciduous teeth. Increased RANKL expression in periapical lesions may be of pathogenetic significance.

Key words: colony stimulating factor-1; core binding factor alpha-1; cytokines; Epstein-Barr virus; human cytomegalovirus; monocyte chemoattractant protein-1; osteoprotegerin; periapical lesions; receptor activator of nuclear kappa B ligand; transforming growth factor-β

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A positive relationship exists between periapical herpesvirus infection and symptomatic and large-size periapical lesions in adult patients (18). Sabeti et al. (14) found human cytomegalovirus active infection in 92% and Epstein-Barr virus active infection in 62% of symptomatic periapical lesions. They also showed that human cytomegalovirus and Epstein-Barr virus dual infection occurred at a significantly higher frequency in periapical lesions showing radiographic bone destruction of 5×7 mm or larger than in smaller size lesions (14). In another study, Sabeti et al. (13) detected human cytomegalovirus and Epstein-Barr virus active infections in, respectively, 100% and 86% of symptomatic periapical lesions, but only identified each virus in 14% of asymptomatic periapical lesions with a similar radiographic size as the symptomatic lesions. Slots et al. (18) hypothesized that human cytomegalovirus and Epstein-Barr virus infections may cause periapical pathosis by inducing cytokine and chemokine release from inflammatory or connective tissue cells, or by impairing local host defenses, resulting in heightened virulence of resident bacterial pathogens. It may be that the pathophysiologic mechanisms of severe periapical pathosis and the processes of periapical release of herpesvirus-associated cytokines are intimately related.

Human cytomegalovirus and Epstein-Barr virus active infection leads to activation of mammalian cells and production of numerous cytokines and chemokines (chemotactic cytokines), which in itself can induce further cell activation and cytokine production in a complex system of regulation and cross-regulation (16). Cytokines have been implicated in the development, maintenance and healing of periapical lesions (19). In the context of periapical pathosis, cytokines capable of inducing bone resorption are of immediate interest. However, it should be noted that, due to redundancy in cytokine/chemokine systems, the contribution of individual cytokines to the pathogenesis of periapical pathosis is difficult to evaluate.

A cytokine network of key importance in the regulation of bone cell biology and in the maintenance of bone mass is receptor activator of nuclear factor-κB ligand (RANKL), its cellular receptor, receptor activator of nuclear factor-κB (RANK), and the decoy receptor osteoprotegerin (5). RANKL and RANK are cytokines belonging to the tumor necrosis factor superfamily. RANKL produced by activated T lymphocytes and osteoblastic lineage cells activates its specific receptor RANK located on osteoclasts and dendritic cells, thereby inducing osteoclast formation, fusion, activation, and survival. Human cytomegalovirus and other herpesviruses may induce the activation of RANKL-associated T lymphocytes. In binding to RANKL, osteoprotegerin blocks the differentiation and activation of osteoclasts. RANKL and osteoprotegerin are regulated by various hormones (glucocorticoids, estrogen), vitamin D, cytokines (tumor necrosis factor-α, interleukins 1, 4, 6, 11, and 17), and mesenchymal transcription factors, such as the core binding factor α-1 (5). Dysregulation of the RANKL/osteoprotegerin system has been implicated in the pathogenesis of periodontal disease, postmenopausal osteoporosis, rheumatoid arthritis, Paget's disease, benign and malignant bone tumors, bone metastases, and hypercalcemia of malignancy (5).

The membrane-bound colony stimulating factor-1 is a critical early modulator of osteoclast differentiation. Colony stimulating factor-1 enhances the survival of monocytic stem cells, thereby permitting them to respond to direct inducers of differentiation such as RANKL (4, 20). Transforming growth factor-β at low levels acts directly upon osteoclasts to enhance RANKL-mediated differentiation and formation, whereas at high levels it may repress osteoclast differentiation (7). Transforming growth factor-β can also cause the suppression of $CD8^+$ effector T lymphocytes (22), which are important in controlling herpesviruses, and thereby can give rise to Epstein-Barr virus activation in infected B lymphocytes (3). Transforming growth factor-β seems to play an important role in infection-stimulated bone resorption and repair processes in periapical lesions (2). Monocyte chemoattractant protein-1 is a chemokine that is produced by and acts on monocytes and macrophages (1). Monocyte chemoattractant protein-1 is present in periapical lesions and has been implicated in the progression of periapical granulomas to cysts (15).

As information is still sparse on herpesviruses in periapical symptomatic lesions, and because the cytokine production in such lesions needs to be further elucidated, this study examined the presence of human cytomegalovirus and Epstein-Barr virus DNA, and the expression of mRNA transcripts of RANKL, osteoprotegerin, core binding factor α-1, colony stimulating factor-1, transforming growth factor-β and monocyte chemoattractant protein-1 in periapical granulomatous lesions of extracted deciduous molar teeth. The present study also investigated a possible relationship of periapical human cytomegalovirus and Epstein-Barr virus with the cytokines studied. Polymerase chain reaction (PCR) assays were used to identify the study molecules.

Material and methods

The study included eight boys and four girls, aged 2–8 years, with deciduous teeth that showed large radiolucent lesions around pulpally infected roots. All study lesions were symptomatic (i.e. exhibited swelling, discomfort on biting, sensitivity by percussion or palpation). Granulomatous tissue adherent to extracted tooth was scraped off with a surgical knife. Non-diseased pulpal tissue, obtained from 12 permanent premolar teeth extracted for orthodontic reasons from individuals 8–12 years of age, served as negative control. After disinfectng the crown of a tooth with povidone-iodine, a sterile bur was used to gain access to the pulp chamber. Immediately before entering the pulp chamber, the cavity was rinsed with 2% chlorhexidine and sterile deionized water. The dental pulp was retrieved by means of sterile endodontic files. Since periapical lesions encompass reactive tissue composed mainly of granulomatous inflammatory tissue replacing normal bone, no true normal tissue equivalent exists to serve as negative control. The patients had not taken antibiotics or anti-inflammatory medications during the preceding 2 months. The study was approved by the ethics committee of the Selcuk University, Faculty of Dentistry.

Samples for PCR assays, which were obtained immediately after tooth extraction, were placed in 500 μl denaturing solution of the EZ-RNA total RNA isolation kit (Biological Industries, Kibbutz Beith Haemek, Israel). The tissue samples were then homogenized with a low-rotary device and divided into two aliquots. One aliquot was used for the extraction of viral DNA and one aliquot for total RNA isolation.

The sample preparation and PCR assay conditions for herpesvirus identification have been described previously (23). In brief, the samples were suspended in 250 μl of K-buffer containing DNA extracted using an alkaline phenol-chloroform-isoamyl alcohol (25 : 24: 1) procedure. Extracted DNA was re-suspended in 100 μl of distilled water, and 5 μl of the DNA solution was used in the PCR assay.

Table 1 lists the primers used in the study. The primers were designed and

Table 1. Polymerase chain reaction primers used in the study

Target molecule*		PCR primer	Size of amplicon
HCMV	Forward primer	5 ′GGA TCC GCA TGG CAT TCA CGT ATG T 3′	240 bp
	Reverse primer	5′ GAA TTC AGT GGA TAA CCT GCG GCG A 3′	
EBV	Forward primer	5′ CTC CCG CAC CCT CAA CAA GCT A 3′	494 bp
	Reverse primer	5′ GAA CCA GAA GHA CCC AAA AGC A 3′	
RANKL	Forward primer	5′ CTC AGC CTT TTG CTC ATC TCA CTA T 3′	115 bp
	Reverse primer	5′GTC ATG TTG GAG ATC TTG GCC CAA 3′	
OPG	Forward primer	5′ GTACAGCAAAGTGGAAGACCGTGTG 3′	361 bp
	Reverse primer	5′ GTTAGCAGGAGACCAAAGACACTGC 3′	
Cbf α-1	Forward primer	5′ TCC TTC CAG AAT GCT TCC GCC AT 3′	128 bp
	Reverse primer	5′ TCA AAA CAG TTG GGG AAC TGC TG 3′	
CSF-1	Forward primer	5′ AGC TGC TTC ACC AAG GAT TAT G 3′	132 bp
	Reverse primer	5′ CCA GTC CTT GTC AAG GAG ATT C 3′	
TGF-β	Forward primer	5′ TTT CCG TGG GAT ACT GAG ACA C 3′	104 bp
	Reverse primer	5′ CAA AAG GTA GGA GGG CCT CGA G 3′	
MCP-1	Forward primer	5′ CGT CTT CTA TCT CAC CTT GAG CCT G 3′	164 bp
	Reverse primer	5′ GCT ACT CTG CAA TCC CTT TCC TGT C 3′	
GAPDH	Forward primer	5′ GTT GCC ATC AAT GAC CCC TTC ATT G 3′	700 bp
	Reverse primer	5′ GCT TCA CCA CCT TCT TGA TGT CAT C 3′	

*HCMV, human cytomegalovirus; PCR, polmerase chain reaction; EBV, Epstein-Barr virus; RANKL, receptor activator of nuclear κB ligand; OPG, osteoprotegerin; Cbfα-1, core binding factor alpha-1; CSF-1, colony stimulating factor-1; TGF-β, transforming growth factor-β; MCP-1, monocyte chemoattractant protein-1; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

analyzed using OLIGOWARE 2.0 (8) and synthesized by MWG Biotech (Ebersberg bei Munich, Germany). PCR assays were performed to detect herpesviruses using a final volume of a 50-μl mixture containing 30 pmol of each primer (MWG Biotech), 2 U *Taq* DNA polymerase (Bioron, Ludwigshafen, Germany), 1.5 mM $MgCl_2$, 0.1 mM dNTP mix, 5 μl of 10× Reaction Buffer (Bioron), and 5 μl of extracted DNA sample. PCR procedures included a 40-round amplification process and were performed in three steps covering a denaturation at 94°C for 30 s, an annealing at 55°C for 30 s, and an extension at 72°C for 40 s. Positive and negative controls for human cytomegalovirus and Epstein-Barr virus included infected and noninfected leukocytes from human peripheral blood.

Total RNA for detecting cytokine gene transcripts was prepared from the homogenized tissue samples using the EZ-RNA total RNA isolation kit according to the package insert instructions (Biological Industries). Extracted RNA was dissolved in 100 μl of DNase- and RNase-free distilled water.

Reverse transcriptase-PCR reaction was performed in 50 μl of reverse transcription mix [10 U reverse transcriptase (Fermentase, Vilnius, Lithuania), 0.15 mM dNTP mix, and 10 μl of 5× reverse transcriptase buffer (Fermentase)]. After incubation for 2 h at 42°C, PCR procedures including a 40-round amplification process were performed in three steps covering a denaturation at 94°C for 30 s, an annealing at 57 (59)°C for 30 s, and an extension at 72°C for 40 s. Each set of PCR analysis included a negative control (water blank) and a positive control (cloned plasmids). The housekeeping gene, glyceraldehyde-3-phosphate dehydrogenase, also served as an internal control.

The specificity of the PCR procedures was confirmed by examining the size of the amplicons and the restriction endonuclease digest of the amplicons. The PCR detection limit, determined by using serially diluted (10^1–10^4) cloned plasmid DNA for herpesviruses and cytokines, was found to be 100 cloned plasmids/ml.

Detection of PCR amplification products was performed electrophoretically in a 2% agarose gel containing 0.5 μg/ml ethidium bromide. Each test run included a size marker (100 bp PCR marker; Bioron). The intensity of the amplification bands was compared by QUANTITY ONE software (Bio-Rad Laboratories, Hercules, CA).

The Mann–Whitney *U*-test was used to compare the PCR results from periapical tissue samples and healthy control dental pulps. The Pearson correlation test was used to assess the relationship between herpesvirus presence and cytokine expression in the periapical tissue samples.

Results

Figure 1 illustrates PCR electrophoresis of the herpesviruses and cytokines studied. In each positive sample, the amplification yielded a single electrophoretic band of expected size with no nonspecific amplification products.

Tables 2 and 3 display the detection of human cytomegalovirus and Epstein-Barr virus in, respectively, the periapical lesions and the healthy pulpal tissues studied. Of the 12 periapical lesions, 58% showed human cytomegalovirus, 67% Epstein-Barr virus, and 33% both human cytomegalovirus and Epstein-Barr virus; only 8% displayed no test herpesvirus. Of the 12 healthy pulpal tissue samples, only one (8%) demonstrated human cytomegalovirus, and one (8%) revealed Epstein-Barr virus. Periapical lesions showed a significantly higher occurrence of human cytomegalovirus ($P < 0.040$) and Epstein-Barr virus ($P < 0.040$) than healthy pulpal tissue.

Table 2 demonstrates the cytokine expression in the periapical lesions studied. The occurrence of positive cytokine samples was as follows: RANKL, 8/12 (67%); osteoprotegerin, 4/12 (33%); core binding factor α-1, 12/12 (100%); colony stimulating factor-1, 9/12(75%); transforming growth factor-β, 9/12 (75%); and monocyte chemoattractant protein-1, 9/12 (75%). In the healthy pulpal tissue group, the occurrence of positive cytokine samples was as follows: RANKL, 2/12 (17%); osteoprotegerin, 6/12 (50%); core binding factor α-1, 12/12 (100%); colony stimulating factor-1, 8/12 (67%); transforming growth factor-β, 12/12 (100%); and monocyte chemoattractant protein-1, 8/12 (67%) (Table 3). Only the RANKL expression revealed a statistically significant difference between the periapical pathosis and the healthy pulpal tissue group ($P < 0.040$). No correlation was identified between herpesvirus presence and cytokine expression in the periapical lesions studied.

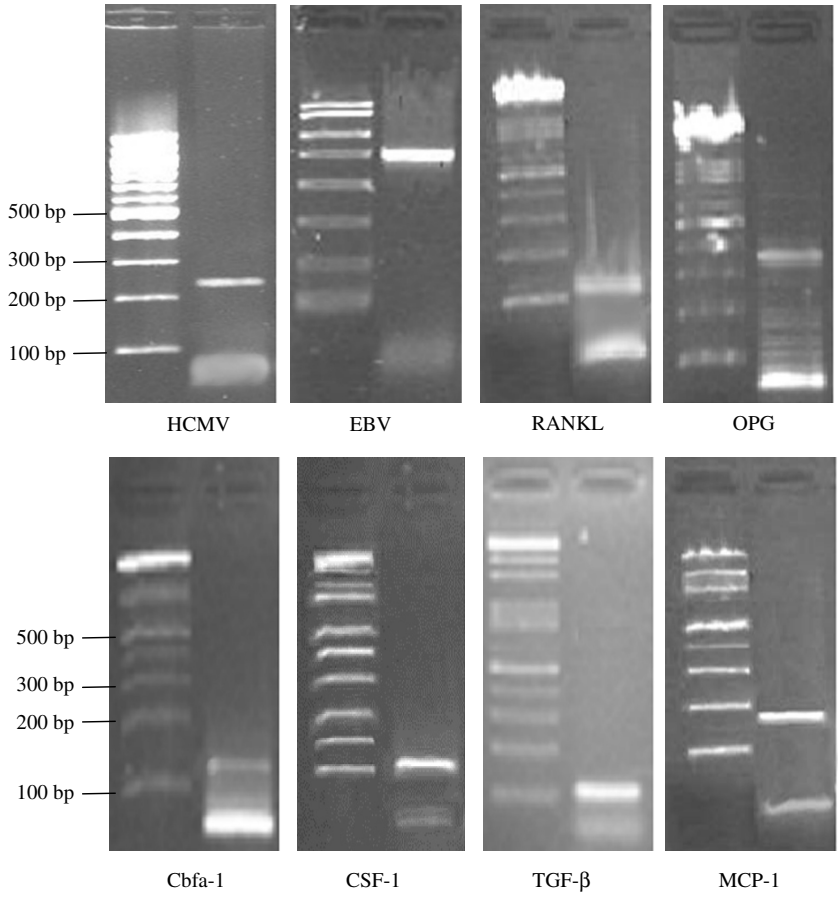

Fig. 1. Amplicons of human cytomegalovirus DNA, Epstein-Barr virus DNA and cytokine expression. For abbreviations see footnote to Table 1.

Table 2. Human cytomegalovirus DNA, Epstein-Barr virus DNA and cytokine expression in symptomatic periapical lesions of deciduous teeth*

Subject No.	Age in years	Sex	HCMV	EBV	RANKL	OPG	Cbfα-1	CSF-1	TGF-β	MCP-1
1	8	♂	+	–	+	–	+	–	–	–
2	6	♂	+	+	–	–	+	+	–	+
3	6	♂	+	+	+	+	+	+	+	+
4	2	♀	–	+	+	+	+	+	+	+
5	7	♀	–	+	+	+	+	+	+	+
6	8	♂	+	+	–	–	+	–	+	–
7	5	♂	–	+	+	+	+	+	+	+
8	6	♂	+	–	+	–	+	+	+	+
9	6	♀	+	+	–	–	+	+	+	+
10	7	♂	–	+	+	–	+	+	+	+
11	5	♂	+	–	+	–	+	+	+	+
12	7	♀	–	–	–	–	+	–	–	–

*For abbreviations see footnote to Table 1.

Discussion

The present study detected human cytomegalovirus DNA in 58% and Epstein-Barr virus DNA in 67% of periapical symptomatic lesions of deciduous teeth, whereas each of the two viruses was only demonstrated in 8% of control samples from noninflamed pulpal tissue. Only one periapical study lesion showed neither human cytomegalovirus nor Epstein-Barr virus DNA. Sabeti et al. (14), studying permanent teeth, detected human cytomegalovirus active infection in 92% and Epstein-Barr in 62% of periapical symptomatic lesions. Despite experimental differences, this study and the study by Sabeti et al. (14) demonstrated a high occurrence of human cytomegalovirus and Epstein-Barr virus in symptomatic periapical lesions, attesting to the robustness of the relationship between the two herpesviruses and acute periapical infection.

Herpesvirus infections give rise to a wide range of cytokine release from mammalian cells (10). It has been speculated that a herpesvirus-induced sudden 'storm' of proinflammatory cytokines, including interleukin-1β, tumor necrosis factor-α, interleukin-6, prostaglandins and interferons, can cause periapical symptoms (17). The present study focused on the periapical presence of cytokines implicated in bone loss. Osteoclasts are primarily regulated by the RANKL/RANK/osteoprotegerin axis of mediators (21). RANKL stimulates osteoclast differentiation, activates mature osteoclasts, and inhibits osteoclast apoptosis, and works together with core binding factor α-1, colony stimulating factor-1 and transforming growth factor-β in stimulating bone resorption. The process of bone resorption debuts following the binding of RANKL to the RANK receptor. This study demonstrated a significantly elevated occurrence of RANKL expression in periapical lesions compared to healthy pulpal tissue. Sabeti et al. (12) has identified RANKL in periapical lesions as well. The expression and number of periapical RANKL-immunopositive cells has also been associated with the level of bone resorption in experimental periapical pathosis of rats (24). In addition, RANKL immunoreactivity has been detected in rat periodontal ligament osteoclasts during excessive occlusal loading associated with increased remodeling of the alveolar bone (6). RANKL immunoreactivity has also been demonstrated in pulpal tissue cells of developing mouse teeth (11) and of deciduous teeth undergoing root resorption (9), which agrees with our finding of RANKL expression in some healthy pulps.

Although no relationship was found between osteoprotegerin, core binding factor α-1, colony stimulating factor-1, transforming growth factor-β or monocyte chemoattractant protein-1 and symptomatic periapical pathosis in deciduous teeth, this does not rule out the importance of these cytokines in the development of periapical lesions. These cytokines are part of a complex, semiredundant cytokine system, and some cytokine responses may predominate in normal physiological bone turn-over and some in pathological bone resorption. Also, the lack of a correlation of human cytomegalovirus or Epstein-Barr virus with the cytokines examined here does not exclude the existence of important pathogenetic interactions. The qualitative

Table 3. Human cytomegalovirus DNA, Epstein-Barr virus DNA and cytokine expression in healthy pulpal tissue*

Subject No.	Age in years	Sex	HCMV	EBV	RANKL	OPG	Cbfα-1	CSF-1	TGF-β	MCP-1
1	9	♂	–	–	–	–	+	+	+	–
2	10	♂	–	–	–	+	+	–	+	+
3	9	♀	–	–	–	+	+	–	+	–
4	9	♂	+	–	–	–	+	+	+	+
5	8	♂	–	+	–	–	+	+	+	+
6	8	♂	–	–	+	+	+	+	+	+
7	10	♂	–	–	–	–	+	+	+	+
8	11	♂	–	–	–	+	+	–	+	–
9	12	♀	–	–	–	+	+	–	+	–
10	10	♂	–	–	–	–	+	+	+	+
11	9	♂	–	–	–	–	+	+	+	+
12	9	♀	–	–	+	+	+	+	+	+

*For abbreviations see footnote to Table 1.

PCR procedure used in this study was unable to detect possible significant associations between herpesvirus loads and cytokine levels. The employment of mRNA expression and real-time PCR techniques to quantify periapical active herpesvirus infections and the level of transcription of bone-resorption-inducing cytokines may help clarify the role of the studied infectious agents and mediators in periapical pathosis.

In summary, the present study supports the notion that human cytomegalovirus and Epstein-Barr virus are important infectious agents in periapical symptomatic pathosis. This study also points to the RANKL/RANK system as a likely mediator of periapical bone resorption.

References

1. Daly C, Rollins BJ. Monocyte chemoattractant protein-1 (CCL2) in inflammatory disease and adaptive immunity: therapeutic opportunities and controversies. Microcirculation 2003: **10**: 247–257.
2. Danin J, Linder LE, Lundqvist G, Andersson L. Tumor necrosis factor-alpha and transforming growth factor-beta1 in chronic periapical lesions. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2000: **90**: 514–517.
3. di Renzo L, Altiok A, Klein G, Klein E. Endogenous TGF-beta contributes to the induction of the EBV lytic cycle in two Burkitt lymphoma cell lines. Int J Cancer 1994: **57**: 914–919.
4. Gyda M, Corisdeo S, Zaidi M, Troen BR. Macrophage colony-stimulating factor suppresses osteoblast formation. Biochem Biophys Res Commun 2001: **285**: 328–334.
5. Hofbauer LC, Heufelder AE. Role of receptor activator of nuclear factor-kappaB ligand and osteoprotegerin in bone cell biology. J Mol Med 2001: **79**: 243–253.
6. Kaku M, Uoshima K, Yamashita Y, Miura H. Investigation of periodontal ligament reaction upon excessive occlusal load – osteopontin induction among periodontal ligament cells. J Periodontal Res 2005: **40**: 59–66.
7. Karst M, Gorny G, Galvin RJ, Oursler MJ. Roles of stromal cell RANKL, OPG, and M-CSF expression in biphasic TGF-beta regulation of osteoclast differentiation. J Cell Physiol 2004: **200**: 99–106.
8. Kubar A, Yapar M, Besirbellioglu B, Avci IY, Guney C. Rapid and quantitative detection of mumps virus RNA by one-step real-time RT-PCR. Diagn Microbiol Infect Dis 2004: **49**: 83–88.
9. Lossdorfer S, Gotz W, Jager A. Immunohistochemical localization of receptor activator of nuclear factor kappaB (RANK) and its ligand (RANKL) in human deciduous teeth. Calcif Tissue Int 2002: **71**: 45–52.
10. Mogensen TH, Paludan SR. Molecular pathways in virus-induced cytokine production. Microbiol Mol Biol Rev 2001: **65**: 131–150.
11. Rani CS, MacDougall M. Dental cells express factors that regulate bone resorption. Mol Cell Biol Res Commun 2000: **3**: 145–152.
12. Sabeti M, Simon J, Kermani V, Valles Y, Rostein I. Detection of receptor activator of NF-kappa beta ligand in apical periodontitis. J Endod 2005: **31**: 17–18.
13. Sabeti M, Simon JH, Slots J. Cytomegalovirus and Epstein-Barr virus are associated with symptomatic periapical pathosis. Oral Microbiol Immunol 2003: **18**: 327–328.
14. Sabeti M, Valles Y, Nowzari H, Simon JH, Kermani-Arab V, Slots J. Cytomegalovirus and Epstein-Barr virus DNA transcription in endodontic symptomatic lesions. Oral Microbiol Immunol 2003: **18**: 104–108. (Erratum in Oral Microbiol Immunol 2005: 20: 322).
15. Silva TA, Garlet GP, Lara VS, Martins W Jr, Silva JS, Cunha FQ. Differential expression of chemokines and chemokine receptors in inflammatory periapical diseases. Oral Microbiol Immunol 2005: **20**: 310–316.
16. Slots J. Herpesviruses in periodontal diseases. Periodontol 2000 2005: **38**: 33–62.
17. Slots J, Nowzari H, Sabeti M. Cytomegalovirus infection in symptomatic periapical pathosis. Int Endod J 2004: **37**: 519–524.
18. Slots J, Sabeti M, Simon JH. Herpesviruses in periapical pathosis: an etiopathogenic relationship? Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2003: **96**: 327–331.
19. Stashenko P, Teles R, D'Souza R. Periapical inflammatory responses and their modulation. Crit Rev Oral Biol Med 1998: **9**: 498–521.
20. Troen BR. Molecular mechanisms underlying osteoclast formation and activation. Exp Gerontol 2003: **38**: 605–614.
21. Ueland T. GH/IGF-I and bone resorption *in vivo* and *in vitro*. Eur J Endocrinol 2005: **152**: 327–332.
22. von Boehmer H. Mechanisms of suppression by suppressor T cells. Nat Immunol 2005: **6**: 338–344.
23. Yapar M, Saygun I, Ozdemir A, Kubar A, Sahin S. Prevalence of human herpesviruses in patients with aggressive periodontitis. J Periodontol 2003: **74**: 1634–1640.
24. Zhang X, Peng B. Immunolocalization of receptor activator of NF kappa B ligand in rat periapical lesions. J Endod 2005: **31**: 574–577.