Oral Microbiology Immunology 2008: 23: 510–514
Printed in Singapore. All rights reserved

ORAL MICROBIOLOGY
AND IMMUNOLOGY

Relationship between human cytomegalovirus transcription and symptomatic apical periodontitis in Iran

K. A. Yazdi[1], M. Sabeti[2], F. Jabalameli[3], M. Eman eini[3], S. A. Kolahdouzan[4], J. Slots[2]

[1]Department of Endodontics, School of Dentistry/Dental Research Center, Tehran University of Medical Sciences, Tehran, Iran, [2]University of Southern California School of Dentistry, Los Angeles, CA, USA, [3]Department of Microbiology, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran, [4]Private Endodontic Practice, Tehran, Iran

Yazdi KA, Sabeti M, Jabalameli F, Eman eini M, Kolahdouzan SA, Slots J. Relationship between human cytomegalovirus transcription and symptomatic apical periodontitis in Iran.
Oral Microbiol Immunol 2008: 23: 510–514. © 2008 The Authors. Journal compilation. © 2008 Blackwell Munksgaard.

Background/aims: Apical periodontitis of endodontic origin may develop as a result of cooperative interactions among herpesviruses, specific pathogenic bacteria and tissue-destructive inflammatory mediators. This study sought to identify the presence of Epstein–Barr virus (EBV) and human cytomegalovirus (HCMV) transcripts in symptomatic and asymptomatic periapical lesions of individuals living in Iran.

Material and methods: Fifty endodontic patients (28 with symptomatic periapical lesions and 22 with asymptomatic periapical lesions) were included in the study. In each study subject, a microbiological periapical sample was collected using a curette in conjunction with periapical surgery. A reverse transcription–polymerase chain reaction assay was used to identify transcripts of EBV and HCMV.

Results: Human cytomegalovirus transcript was detected in 15 of the 28 (53.6%) symptomatic and in six of the 22 (27.3%) asymptomatic periapical study lesions (significant difference between symptomatic and asymptomatic lesions; $P = 0.03$, chi-square test). Epstein–Barr virus transcript was identified in one symptomatic and in two asymptomatic periapical lesions.

Conclusion: This study establishes that HCMV transcription is common in apical periodontitis and is most frequent in symptomatic lesions. The high frequency of active herpesvirus infections in severe apical periodontitis changes the pathogenic paradigm of the disease and may also have preventive and therapeutic implications.

Key words: cytomegalovirus; Epstein–Barr virus; immunology; pathogenesis; periapical lesions

Jørgen Slots, University of Southern California School of Dentistry, MC-0641, Los Angeles, CA 90089-0641, USA
Tel.: + 1 213 740 1091;
e-mail: jslots@usc.edu
Accepted for publication June 18, 2008

Apical periodontitis of endodontic origin is usually described as a bacterial infection caused by specific pathogenic species (7, 10, 26, 28). Initial pulpitis lesions harbor mainly facultative gram-positive plaque bacteria, whereas periapical lesions are associated with anaerobic and proteolytic gram-negative species (6, 19). The population shift in the endodontic microbiota with disease progression is widely recognized but was never explained. Endodontic bacteria possess potent virulence factors (21) and induce vigorous host responses (20). However, it is not known if bacteria cause periapical lesions solely on their own, or if they play a part in the disease process as a microbiological consequence of some other pathophysiological events.

A major disturbance in the endodontic ecosystem must take place to cause the shift from a predominantly gram-positive facultative to a gram-negative anaerobic microbiota. Microbial factors, such as synergistic and antagonistic bacterial interactions (9, 35), host-specific factors, including innate and acquired immune functions (33), as well as specific

nutritional factors (7) determine the composition of the endodontic microbiota. However, it is not clear why fastidious anaerobic species would outcompete viridans streptococci and actinomyces species during the progression of endodontic disease. Endodontic gram-negative bacteria must overcome high levels of inhibitory serum antibodies (3), and bacteriocins from co-resident gram-positive species (37). Oral streptococci and actinomyces do not seem to face a similar degree of challenge from the host or from gram-negative species.

Various clinical features of apical periodontitis also require an explanation. Many periapical lesions are self-limiting with short duration morbidity, and do not proceed to major bone loss. On the other hand, some periapical lesions continue a progressive course of destruction to eventually involve a large part of the affected jaw. Also, after a prolonged period of disease stability, some periapical lesions become aggressive and symptomatic. Moreover, one or two roots of a molar may show periapical progressive disease, whereas other roots of the same tooth may show little or no periapical pathosis.

The critical pathogenic factors of periapical pathosis remain unclear, mainly because of difficulties in identifying the full spectrum of principal endodontopathogens and in distinguishing between destructive and protective features of the endodontic inflammatory response. It may be that different hypotheses of etiology fit separate parts of the endodontic disease process. There is little doubt that pulpitis lesions that communicate with the oral cavity are attributable to bacterial action. However, because of a lack of direct evidence for a pure bacterial etiology of periapical lesions, and the many puzzling clinical features of the disease, it is our contention that a simple bacterial cause of periapical disease may have been overemphasized. We suggest that recent studies showing the presence of herpesvirus infections in periapical lesions can provide new important insights into the causation of the disease (31). Most likely, herpesviral–bacterial concurrent infections are more pathogenic than single infections by either of the two types of infectious agents (30).

Published studies on mammalian viruses in endodontic lesions have virtually all originated from the USA. As the type and prevalence of herpesviruses differ among ethnic and geographic groups (27, 39), and microorganisms in endodontic infections also vary with geographic location (4, 17), it is important to determine the herpesvirus presence in endodontic lesions of several different populations. The present study was undertaken to determine the occurrence of transcripts of Epstein–Barr virus (EBV) and human cytomegalovirus (HCMV) in symptomatic and asymptomatic apical periodontitis lesions of individuals living in Iran.

Material and methods

Subjects

The study included 28 patients (aged 18–57 years) with symptomatic apical periodontitis and 22 patients (aged 15–55 years) with asymptomatic apical periodontitis. The 50 study participants were scheduled for endodontic examination at the Department of Endodontics, Tehran University of Medical Sciences. All patients were systemically healthy and had not received endodontic treatment or antibiotics for at least 3 months before the start of the study. The Institutional Internal Review and Ethics Board of the Tehran University of Medical Sciences approved the study. Written informed consent was obtained from each study subject after the nature of the procedures and possible discomforts and risks had been fully explained.

Symptomatic teeth exhibited swelling, pain, discomfort on biting, or sensitivity by percussion or palpation. Asymptomatic teeth revealed no signs or symptoms of acute periapical inflammation or pain at the time of the study. The apical area of each study tooth was examined in periapical radiographs obtained by a long cone paralleling technique. None of the study teeth demonstrated moderate or severe types of marginal periodontitis.

Virological sampling

Periapical samples were collected in conjunction with apicectomy. The surgery was performed because of radiographic evidence of incomplete periapical healing following conventional root canal treatment. Before administering local anesthetics, the teeth, gingiva and mucosa of the sample area were washed with 0.2% chlorhexidine and patients rinsed with 0.2% chlorhexidine mouthwash for 30 s. Using a sterile no. 15 blade, an intrasulcular incision was extended one or two teeth mesially and distally from the study tooth, followed by a vertical release incision mesially. A full-thickness mucoperiosteal flap was then reflected, exposing the periapical lesion area. Access through the cortical bony plate was obtained using a sterile explorer or No. 4 or 6 sterile high-speed surgical round burs with sterile water coolant in the area of osteotomy. Using a sterile curette, a periapical specimen for virological identification was placed in an empty plastic vial and immediately frozen at −70°C.

Virological examination

Epstein–Barr virus and HCMV transcripts were identified as previously described (24). Briefly, RNA was isolated using RNX-plus (CinnaGen Inc, Tehran, Iran). Complementary DNA (cDNA) was generated using a preamplification kit with dimer nucleotides and random hexamers (RevertAid™ First-Strand cDNA Synthesis Kit; Fermentas, Vilnius, Lithuania). Polymerase chain reaction (PCR) primers for EBV were TCC ACC ACA CCC AGG CAC and TGG AGA GGT CAG GTT ACT TAC, which amplified the genome encoding for the early antigen EBV nuclear antigen 2 (EBNA-2) (15). The size of the EBV amplification product is 205 base pairs (bp). Primers for HCMV were ACG CGC TGC CGC TCA AGA T and TGT AGT AGA CGT CGG GCT CTT T, which amplified the pp65 gene, transcribed late during the infectious cycle (22, 32). The size of the HCMV amplification product is 195 bp. The standardization, sensitivity, and validation of the cDNA methodology followed previously described procedures (36). The sensitivity of the PCR assay was 400 herpesvirus genomic copies, including the dilution factor of the samples. No cross-reactivity was observed with a variety of oral bacterial strains. Amplification products were identified in agarose electrophoretic gel, and visualized for size using a UV lamp at 320 nm wavelength.

Results

A total of 28 symptomatic and 22 asymptomatic periapical lesions were studied. The average radiographic size was 4.8×4.9 mm for symptomatic lesions and 4.3×4.7 mm for asymptomatic lesions.

Table 1 reveals that HCMV transcript was detected in 15 of the 28 (53.6%) symptomatic and in 6 of the 22 (27.3%) asymptomatic periapical study lesions. The difference in occurrence of HCMV transcript between symptomatic and asymptomatic lesions was statistically significant ($P = 0.03$, chi-square test). The EBV transcript was identified in one symptomatic and two asymptomatic periapical

Table 1. EBV and HCMV transcripts in symptomatic and asymptomatic periapical lesions

Periapical lesions	EBV alone, *n* (%)	HCMV alone, *n* (%)	EBV + HCMV, *n* (%)	No study viruses, *n* (%)
Symptomatic (*n* = 28)	1 (3.6)	15 (53.6)	0 (0)	12 (42.9)
Asymptomatic (*n* = 22)	1 (4.5)	5 (22.7)	1 (4.5)	15 (68.2)
Total (*n* = 50)	2 (4.0)	20 (40.0)	1 (2.0)	27 (54.0)

EBV, Epstein–Barr virus; HCMV, human cytomegalovirus.

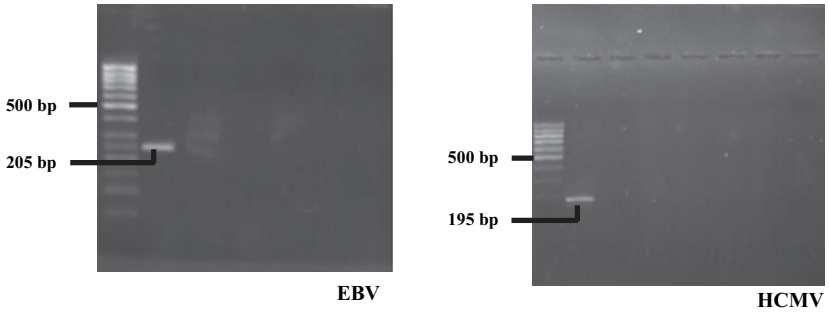

Fig. 1. Transcripts of Epstein–Barr virus (EBV; 205 bp) and human cytomegalovirus (HCMV; 195 bp) in periapical lesions.

lesions. Fig. 1 shows the identification of HCMV and EBV transcripts by agarose gel electrophoresis.

Discussion

This study determined EBV and HCMV genomic transcription with the aim of identifying pathogenetically important herpesvirus active infections. As the present PCR methodology would not recognize transcriptionally quiescent EBV and HCMV genomes, our study probably underestimated the total occurrence of these viruses in the periapical lesions studied.

The present HCMV data are in general agreement with previous findings, but the finding of a low rate of EBV transcription in symptomatic periapical lesions is at variance with earlier results (31). As this study employed the EBV PCR primer design used in previous studies (24), the difference in findings may be more related to the characteristics of the patients studied than to the PCR methodologies used.

Our data strengthen the hypothesis of a causal relationship between an active HCMV infection and symptomatic periapical pathosis. Saboia-Dantas et al. (25) identified EBV and HCMV serologically in enlarged periapical cells, which is suggestive of an active viral infection, and herpesvirus-infected cells were particularly prominent in HIV-infected patients. HCMV genome can be detected in periapical cysts as well, especially in those with a previous episode of acute infection (2).

Some symptomatic periapical lesions may develop in part because of reactivation of a latent HCMV infection. Herpesvirus reactivation takes place with a weakened host defense and has, by itself, the potential to impair important host defense systems. Obviously, the viral load needs to be of a sufficiently high magnitude to cause disease, and may have been relatively low in the periapical study lesions that showed herpesvirus transcription but remained asymptomatic.

Both innate and the adaptive immune systems play important roles in the host defense against herpesviruses (12, 29). Briefly, a herpesviral active infection initiates the activation of nuclear factor-kappa B and its translocation to the nucleus, promoting the expression of proinflammatory cytokines, chemokines, and adhesion molecules in virally infected cells. Proinflammatory cytokines recruit macrophages and natural killer cells to the site of infection and activate the cellular expression of various effector functions. Cells of the innate immune system lyze virally infected cells and are a rich source of T helper type 1 proinflammatory antiviral cytokines, including interleukin-1, interleukin-6, interleukin-12, interleukin-18, tumor necrosis factor-α and interferons. The concerted action of innate immune responses activates the adaptive immune system, especially $CD8^+$ cytotoxic T lymphocytes, in the defense against herpesviral infections. However, the pathophysiology of herpesviral infections also includes viral immunoevasins that exploit diverse cellular processes to interfere with host antiviral functions (29, 38). The inflammatory cells and cytokines associated with herpesvirus infections are present in large quantities in endodontic lesions (18).

Periapical lesions may develop as a result of complex immune responses against the herpesviral–bacterial combined infection. As T helper type 1 proinflammatory cytokines can undermine T helper type 2 cytokines, and vice versa (13, 29), periapical lesions may experience a changing dominance of either herpesviral or bacterial immune responses. The early phases of the development of apical periodontitis may involve immunosuppressive events for various reasons having the potential to activate a latent herpesvirus periapical infection. Herpesvirus active infections induce cytotoxic T-cell proliferation and proinflammatory cytokine release (5), which can adversely affect the production of antibacterial antibodies (16), and can subvert complement (16), neutrophil (1), and macrophage (8) functions. Exogenous-like pathogenic species, which are mainly controlled by antibody-mediated host responses (23), may particularly benefit from a reduction in antibacterial immunity and outgrow co-resident indigenous microorganisms, triggering a shift towards a more virulent flora. If a herpesvirus endodontic infection indeed induces high levels of proinflammatory cytokines and overgrowth of major endodontopathic bacteria, the (re)establishment of effective antiviral immunity may constitute an important step in achieving a long-term remission of apical periodontitis.

Even though proinflammatory cytokines have the potential to initiate alveolar bone resorption (14), the periapical cytokine response may actually be beneficial overall by preventing the activation and widespread dissemination of virulent viruses (11). If so, herpesvirus-associated apical periodontitis may develop as a result of the efforts of the host to control existing viruses and avoid viral dissemination and serious systemic diseases.

The concept of a pathogenic herpesvirus infection may help clarify at least some of the clinical features of periapical pathosis. In this context, herpesvirus-associated cytopathogenic effects, immune evasion, immunopathogenicity, latency, reactivation from latency and tissue tropism may constitute important aspects of periapical pathosis. A robust antiherpesvirus host

response may be responsible for a prolonged period of disease stability even in the presence of virulent bacteria; herpesvirus reactivation from the latent state may account for periapical disease exacerbation; and herpesviral propensity to tissue tropism may explain why signs and symptoms of endodontic pathosis can vary significantly between teeth/roots in the same patient.

The finding of herpesvirus active infections in severe apical periodontitis may have therapeutic implications. Antiviral chemotherapeutics are effective against viruses in the lytic phase, but not in the latent phase, which limits their use to acute/progressive dental infections. A patient with refractory marginal periodontitis and high EBV periodontal load was recently treated with the antiherpesvirus drug Valtrex® (GlaxoSmithKline, Brentford, Middlesex, UK) (valacyclovir HCl, 500 mg twice a day for 10 days), which led to suppression of the virus to undetectable levels and a 'dramatic' clinical improvement (34). Future management of apical and marginal periodontal diseases may also benefit from antiherpesviral immunotherapeutics: either prophylactic vaccines, which harness the immune system of healthy subjects to prevent infection by disease-causing viruses; or therapeutic vaccines, which stimulate the immune system into combating existing viruses and disease. The notion of herpesviral–bacterial pathogenetic synergism implies that effective vaccination against herpesviruses can help control endodontic bacteria as well.

In summary, the involvement of herpesviruses in periapical pathosis challenges several conventional concepts of endodontic immunology. We propose that unfavorable changes in the endodontic host defense suppress important antiviral immune responses, which then trigger herpesvirus reactivation and an increase in proinflammatory cytokines having the potential to induce bone resorption and overgrowth of major endodontopathic bacteria. Studies are warranted on methods to control or eliminate herpesviruses in endodontic sites.

Acknowledgments

This research was supported by the Tehran University of Medical Sciences, Health Services Grant #132/6466.

References

1. Abramson JS, Mills EL. Depression of neutrophil function induced by viruses and its role in secondary microbial infections. Rev Infect Dis 1988: **10**: 326–341.
2. Andric M, Milasin J, Jovanovic T, Todorovic L. Human cytomegalovirus is present in odontogenic cysts. Oral Microbiol Immunol 2007: **22**: 347–351.
3. Baumgartner JC, Falkler WA Jr, Bernie RS, Suzuki JB. Serum IgG reactive with oral anaerobic microorganisms associated with infections of endodontic origin. Oral Microbiol Immunol 1992: **7**: 106–110.
4. Baumgartner JC, Siqueira JF Jr, Xia T, Róças IN. Geographical differences in bacteria detected in endodontic infections using polymerase chain reaction. J Endod 2004: **30**: 141–144.
5. Botero JE, Contreras A, Parra B. Profiling of inflammatory cytokines produced by gingival fibroblasts after HCMV infection. Oral Microbiol Immunol 2008: **23**: 291–298.
6. Fabricius L, Dahlén G, Ohman AE, Möller AJ. Predominant indigenous oral bacteria isolated from infected root canals after varied times of closure. Scand J Dent Res 1982: **90**: 134–144.
7. Figdor D, Sundqvist G. A big role for the very small – understanding the endodontic microbial flora. Aust Dent J 2007: **52** (1 suppl): S38–S551.
8. Gafa V, Manches O, Pastor A et al. Human cytomegalovirus downregulates complement receptors (CR3, CR4) and decreases phagocytosis by macrophages. J Med Virol 2005: **76**: 361–366.
9. Gomes BP, Drucker DB, Lilley JD. Positive and negative associations between bacterial species in dental root canals. Microbios 1994: **80**: 231–243.
10. Gomes BP, Montagner F, Jacinto RC, Zaia AA, Ferraz CC, Souza-Filho FJ. Polymerase chain reaction of *Porphyromonas gingivalis*, *Treponema denticola*, and *Tannerella forsythia* in primary endodontic infections. J Endod 2007: **33**: 1049–1052.
11. Guidotti LG, Chisari FV. Cytokine-mediated control of viral infections. Virology 2000: **273**: 221–227.
12. Guidotti LG, Chisari FV. Noncytolytic control of viral infections by the innate and adaptive immune response. Annu Rev Immunol 2001: **19**: 65–91.
13. Haveman JW, Muller Kobold AC, Tervaert JW et al. The central role of monocytes in the pathogenesis of sepsis: consequences for immunomonitoring and treatment. Neth J Med 1999: **55**: 132–141.
14. Kawashima N, Stashenko P. Expression of bone-resorptive and regulatory cytokines in murine periapical inflammation. Arch Oral Biol 1999: **44**: 55–66.
15. Lin JC, Lin SC, De BK, Chan WC, Evatt BL, Chan WP [corrected to Chan WC]. Precision of genotyping of Epstein–Barr virus by polymerase chain reaction using three gene loci (EBNA-2, EBNA-3C, and EBER): predominance of type A virus associated with Hodgkin's disease. Blood 1993: **81**: 3372–3381 [Erratum in: Blood 1993: 82: 2268].
16. Loenen WA, Bruggeman CA, Wiertz EJ. Immune evasion by human cytomegalovirus: lessons in immunology and cell biology. Semin Immunol 2001: **13**: 41–49.
17. Machado de Oliveira JC, Siqueira JF Jr, Rôças IN et al. Bacterial community profiles of endodontic abscesses from Brazilian and USA subjects as compared by denaturing gradient gel electrophoresis analysis. Oral Microbiol Immunol 2007: **22**: 14–18.
18. Márton IJ, Kiss C. Protective and destructive immune reactions in apical periodontitis. Oral Microbiol Immunol 2000: **15**: 139–150.
19. Möller AJ, Fabricius L, Dahlén G, Ohman AE, Heyden G. Influence on periapical tissues of indigenous oral bacteria and necrotic pulp tissue in monkeys. Scand J Dent Res 1981: **89**: 475–484.
20. Nair PN. Pathogenesis of apical periodontitis and the causes of endodontic failures. Crit Rev Oral Biol Med 2004: **15**: 348–381.
21. Olsen I, Dahlén G. Salient virulence factors in anaerobic bacteria, with emphasis on their importance in endodontic infections. Endodontic Topics 2004: **9**: 15–26.
22. Pande H, Campo K, Tanamachi B, Zaia JA. Human cytomegalovirus strain Towne pp65 gene: nucleotide sequence and expression in *Escherichia coli*. Virology 1991: **182**: 220–228.
23. Rams TE, Listgarten MA, Slots J. *Actinobacillus actinomycetemcomitans* and *Porphyromonas gingivalis* subgingival presence, species-specific serum immunoglobulin G antibody levels, and periodontitis disease recurrence. J Periodontal Res 2006: **41**: 228–234.
24. 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].
25. Saboia-Dantas CJ, Coutrin de Toledo LF, Sampaio-Filho HR, Siqueira JF Jr. Herpesviruses in asymptomatic apical periodontitis lesions: an immunohistochemical approach. Oral Microbiol Immunol 2007: **22**: 320–325.
26. Sakamoto M, Rôças IN, Siqueira JF Jr, Benno Y. Molecular analysis of bacteria in asymptomatic and symptomatic endodontic infections. Oral Microbiol Immunol 2006: **21**: 112–122.
27. Sandvej K, Zhou XG, Hamilton-Dutoit S. EBNA-1 sequence variation in Danish and Chinese EBV-associated tumours: evidence for geographical polymorphism but not for tumour-specific subtype restriction. J Pathol 2000: **191**: 127–131.
28. Siqueira JF Jr. Endodontic infections: concepts, paradigms and perspectives. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2004: **94**: 281–293.
29. Slots J. Herpesviruses in periodontal diseases. Periodontol 2000 2005: **38**: 33–62.
30. Slots J. Herpesviral–bacterial synergy in the pathogenesis of human periodontitis. Curr Opin Infect Dis 2007: **20**: 278–283.
31. 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.
32. Solache A, Morgan CL, Dodi AI et al. Identification of three HLA-A*0201-restricted cytotoxic T cell epitopes in the

cytomegalovirus protein pp65 that are conserved between eight strains of the virus. J Immunol 1999: **163**: 5512–5518.
33. Stashenko P, Teles R, D'Souza R. Periapical inflammatory responses and their modulation. Crit Rev Oral Biol Med 1998: **9**: 498–521.
34. Sunde PT, Olsen I, Enersen M, Grinde B. Patient with severe periodontitis and subgingival Epstein–Barr virus treated with antiviral therapy. J Clin Virol 2008: **42**: 176–178.
35. Sundqvist G. Associations between microbial species in dental root canal infections. Oral Microbiol Immunol 1992: **7**: 257–262.
36. Tal-Singer R, Lasner TM, Podrzucki W et al. Gene expression during reactivation of herpes simplex virus type 1 from latency in the peripheral nervous system is different from that during lytic infection of tissue cultures. J Virol 1997: **71**: 5268–5276.
37. Teughels W, van Essche M, Slieben I, Quirynen M. Probiotics and oral healthcare. Periodontol 2000 2008: **48**: 110–146.
38. Vossen MT, Westerhout EM, Söderberg-Nauclér C, Wiertz EJ. Viral immune evasion: a masterpiece of evolution. Immunogenetics 2002: **54**: 527–542.
39. Wu YM, Yan J, Ojcius DM, Chen LL, Gu ZY, Pan JP. Correlation between infections with different genotypes of human cytomegalovirus and Epstein–Barr virus in subgingival samples and periodontal status of patients. J Clin Microbiol 2007;**45**:3665–3670 [Erratum in: J Clin Microbiol 2008: 46: 836].