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Real-time polymerase chain reaction quantification of human cytomegalovirus and Epstein-Barr virus in periodontal pockets and the adjacent gingiva of periodontitis lesions

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Background: Genomic sequences of human cytomegalovirus (HCMV) and Epstein–Barr virus (EBV), two herpesviruses, can frequently be detected in periodontal pockets of progressive periodontitis lesions, but the prevalence and load of the two viruses in gingival tissue are unknown. This study determined levels of HCMV and EBV DNA in the periodontal pocket and in the adjacent gingiva of periodontitis lesions using a real-time polymerase chain reaction (PCR) assay.

Material and methods: A total of 20 systemically healthy periodontitis patients participated in the study. Nine patients below 35 years of age were tentatively diagnosed as having aggressive (early onset) periodontitis, and 11 patients 36–56 years of age as having chronic (adult) periodontitis. Clinical parameters were evaluated using established methods. Using periodontal curettes, specimens were harvested from 6–10 mm periodontal pockets and from the adjacent inflamed periodontal pocket wall. A 5′-nuclease (TaqMan®) real-time PCR assay was used to identify and quantify genomic copies of periodontal HCMV and EBV.

Results: HCMV DNA was detected in 78% of subgingival and 33% of gingival tissue samples from aggressive periodontitis lesions, but only in 46% of subgingival and 9% of gingival tissue samples from chronic periodontitis lesions. In aggressive periodontitis, HCMV subgingival and gingival tissue counts were positively correlated with periodontal pocket depth and probing attachment loss at sample sites ($p \leq 0.03$; Spearman's rank correlation coefficient test). EBV DNA was identified in 89% of subgingival and 78% of gingival tissue samples from aggressive periodontitis lesions, but only in 46% of both subgingival and gingival tissue samples from chronic periodontitis lesions. In aggressive periodontitis, positive correlations were found for EBV subgingival counts and periodontal pocket depth at sample sites ($p = 0.04$; Spearman's correlation) and for EBV gingival tissue counts and whole mouth mean gingival index ($p = 0.04$;

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Spearman's correlation). In chronic periodontitis, statistical significance was only found between EBV subgingival counts and periodontal pocket depth at sample sites ($p = 0.04$; Spearman's correlation). HCMV–EBV coinfection was revealed in 78% of aggressive periodontitis lesions but only 27% of chronic periodontitis lesions ($p = 0.03$; chi-squared test). Also, seven of nine aggressive periodontitis patients but only three of 11 chronic periodontitis patients revealed more than 10,000 copies of HCMV or EBV in subgingival or gingival tissue samples ($p = 0.03$; chi-squared test). Four of six patients having mean periodontal pocket depth at sample teeth (four study sites per tooth) > 6 mm, but none of 14 patients having mean pocket depth at sample teeth ≤ 6 mm revealed more than 100,000 copies of HCMV or EBV in subgingival or gingival samples ($p = 0.001$; chi-squared test). In periodontitis lesions demonstrating herpesviruses in paired subgingival and gingival tissue samples, the tissue samples showed the higher HCMV copy counts in three of four patients and the higher EBV copy counts in six of eight patients.

Conclusions: The elevated occurrence of HCMV and EBV DNA copies in periodontal pockets and in the gingival tissue of aggressive periodontitis lesions relative to chronic periodontitis lesions, and the increase in herpesvirus counts with increasing severity of periodontitis lend substantial support to a periodontopathic role of the two viruses. Real-time PCR determination of herpesvirus DNA in periodontal sites may become a promising marker to monitor the course of destructive periodontal disease. Herpesviruses and bacteria, now mostly studied in isolation, may cooperate synergistically in the development of periodontitis, and should probably be considered as a pathogenetic consortium in future investigations of periodontal infections.

The pathogenic process of periodontitis includes dynamic interactions among various infectious agents and interconnected cellular and humoral host responses. However, despite a long history of research into the pathobiology of periodontitis, a definitive statement about its probable causes on a molecular level remains elusive. Bacterial pathogens are causally necessary antecedents for the development of periodontitis but the mere amount of bacterial plaque does not seem to provide a sufficient basis for explaining important clinicopathologic features of the disease. Bacterial infection alone may not explain rapid tissue destruction around teeth exhibiting little plaque (1), the propensity of periodontitis to proceed with periods of exacerbation and remission (2), and the tendency of periodontal tissue breakdown to advance in a localized and bilaterally symmetrical pattern (3). In an attempt to accelerate progress in the field of periodontal infections, we have begun studying the importance of human viruses in destructive periodontal disease. The identification of a herpesvirus factor in the development of periodontitis may help clarify hitherto unexplained clinical and pathophysiologic characteristics of the disease (4).

Continually increasing evidence exists for an etiopathogenic role of human cytomegalovirus (HCMV) in progressive periodontitis (5, 6). HCMV-infected periodontal sites demonstrate a higher rate of disease-activity than sites showing no detectable HCMV, even when comparing HCMV-positive and HCMV-negative sites with similar pocket depth and degree of clinical inflammation (7). Epstein–Barr virus (EBV) has been related to periodontitis as well (8). In nonoral diseases, it is known that HCMV infection can increase the incidence of bacterial and fungal infections, aggravate the severity of concurrent microbial infections, and accelerate the tempo of infectious disease progression (9). Although less studied, EBV and bacterial pathogens may also act synergistically in nonoral infectious diseases (10). A similar theory for periodontitis focuses on the potential of periodontal HCMV and EBV to subvert local host defenses, thereby enhancing the aggressiveness of subgingival bacteria (4).

Molecular-based detection methods, especially polymerase chain reaction (PCR), have greatly facilitated investigations of herpesviruses in oral and nonoral diseases, but limitations of previous herpesvirus–periodontitis studies have tempered conclusions on the periodontopathic significance of herpesviruses. The absence of longitudinal data prevents an assessment of the extent to which lymphotropic herpesviruses are important risk factors for the later development of periodontitis, or merely secondary to a lymphocytic infiltration of inflamed gingiva. Also, herpesviruses pose a unique diagnostic challenge because of their ability to remain latent in lymphocytes without actively replicating and producing disease, and early PCR studies did not differentiate between herpesvirus latency and higher levels of replication. Viral activation may be assessed by means of molecular techniques to determine transcription of genes associated with viral

reactivation, immunologic methods to detect viral proteins, and electron-microscopic identification of intact virions to confirm viral assembly and egress. Moreover, misamplification and false-positive herpesvirus results could emerge in case of shared regions of nucleotide sequences between herpesvirus species and unknown infectious agents. However, since periodontal HCMV has been identified using a variety of primers in platforms of conventional PCR (8), nested PCR (7), reverse transcription PCR (11) and real-time PCR (6), the risk of a systematic misidentification of HCMV is small. EBV has also been identified in subgingival sites by a variety of PCR methods (8, 12–14). Finally, by merely providing a dichotomous outcome, early qualitative PCR methodologies were unable to differentiate between herpesvirus levels in disease-active and disease-stable periodontal sites. Recent real-time PCR methodologies permit a quantitative assessment of starting templates. Finding elevated herpesvirus levels in disease-active compared to disease-inactive periodontitis sites would strengthen the notion of a periodontopathic role of the viruses.

The 5′-nuclease (TaqMan®) real-time PCR assay has previously been used to quantify HCMV (7) and EBV (14, 15) genomes in periodontal pockets. The TaqMan assay is based on the ability of the 5′-to-3′ exonuclease activity of the *Taq* DNA polymerase to cleave a dual-labeled, fluorogenic, nonextendable hybridization probe, and directly relate the level of fluorescence emission to the amount of initial target gene (16, 17). The TaqMan fluorogenic probe hybridizes to the amplicon between the forward and reverse primers and is cleaved by *Taq* DNA polymerase during the extension step. Cleavage of the probe separates the reporter dye from the quencher, generating a fluorescent signal proportional to the number of amplicons produced. In the present study, the TaqMan PCR method was used to determine HCMV and EBV DNA copy numbers in the periodontal pocket and adjacent gingival tissue of periodontitis lesions in young and middle-age adults.

Material and methods

Subjects

Study subjects included three women and six men (ages 21–34 years) with aggressive (early onset) periodontitis, and four women and seven men (ages 36–56 years) with chronic (adult) periodontitis. The 20 subjects were scheduled for periodontal surgery at the Department of Periodontology, Gülhane Military Medical Academy, Ankara, Turkey. Patients diagnosed as having aggressive periodontitis exhibited probing attachment loss in excess of 5 mm on more than 14 teeth, at least three of which were not incisors or first molars. Chronic periodontitis patients had at least nine posterior teeth with 5–7 mm pocket depth and three teeth with 6 mm or more of probing attachment loss. All patients were systemically healthy and had not received periodontal treatment or antibiotics for at least 6 months prior to the study. The Institutional Internal Review and Ethics Board at the Gülhane Military Medical Academy, Sciences of Dentistry approved the study. Written informed consent was obtained from each study subject after all procedures had been fully explained.

Clinical procedures

Clinical periodontal evaluation included the plaque index (18), the gingival index (19), probing pocket depth, and probing attachment loss. Probings were carried out using a Williams probe calibrated in millimeters and were assessed at four sites per tooth, mesiofacial, midfacial, distofacial, and midlingual. At 2 weeks prior to periodontal surgery, supragingival plaque and calculus were removed by ultrasonic scaling. In each study patient, a subgingival and a gingival tissue specimen were obtained from the deepest pocket of the dentition (6–10 mm probing depth). Prior to sampling, the sample site was gently cleaned of supragingival plaque and saliva with sterile cotton pellets, isolated with cotton rolls, and air-dried. A sterile periodontal curette was gently inserted to the bottom of the test periodontal pocket, and subgingival material was removed by a single stroke. Gingival tissue specimens were obtained in conjunction with the modified Widman flap surgical procedure as described by Ramfjord and Nissle (20). A Bard-Parker knife blade was used for initial incision, and a mucoperiosteal elevator was used to raise buccal and palatal flaps. A periodontal curette was used to remove pocket epithelium, underlying connective tissue, and granulation tissue from the region of crestal alveolar bone destruction. A total of 36–50 mg (mean, 40 mg) of gingival tissue was collected in each patient. The subgingival and gingival tissue specimens were each suspended in 500 µl of TE buffer (10 mM Tris-hydrochloride, 1 mM EDTA, pH 8) and homogenized by vigorous mixing on a vortex.

Nucleic acid extraction

DNA was extracted from the clinical sample material using an alkali phenol–chloroform–isoamyl alcohol procedure (21). Briefly, 100 µl of specimen was placed in 10 µl of protease solution (65 mg/ml)(Sigma-Aldrich Corp, St. Louis, MO, USA) and 250 µl of potassium buffer for 60 min at 42°C. Following centrifugation at 10,000 *g* for 10 min at 12°C, DNA was extracted from the supernatant using a mixture of 250 µl alkali phenol and 250 µl chloroform–isoamyl alcohol (24 : 1), and then pelleted using 500 µl isopropyl alcohol. DNA was washed in 75% ethyl alcohol at 10,000 *g* for 5 min at 4°C, air-dried at 37°C, and dissolved in 100 µl distilled water.

Real-time TaqMan assay

PCR amplifications were performed as individual assays for each study virus. The sequences for the PCR primers and probes were designed using the Oligoware 1.0 software program (22). Primer sequences were 5′-TGAGCCC GGCGGTGGT-3′ and 5′-AGCTCAC CGATCACAGACAC-3′ for HCMV, and 5′-CCTGGTCATCCTTTGCCA-3′ and 5′-TGCTTCGTTATAGCCGTA GT-3′ for EBV. The TaqMan probes

were labeled with 6-carboxyfluorescein (FAM) reporter dye at the 5′ end, and with the 6-carboxytetramethyl-rhodamine (TAMRA) quencher dye at the 3′ end. TaqMan probes were 5′-FAM-AGAGAAGCGCCACATACAGCG C-TAMRA-3′ for HCMV and 5′-FAM-CAGTACGAGTGCCTGC GACCA-TAMRA-3′ for EBV.

Amplification, data acquisition and all analyses were carried out using the ABI PRISM 7700 Sequence Detection System (Applied Biosystems, Foster City, CA, USA). Standard procedures for the operation of the model 7700 system were followed, including the use of all default program settings. For each virus tested, the TaqMan PCR assay was performed with a final volume of 25 µl reaction mixture, containing 5 µl of extracted clinical sample, 12.5 µl TaqMan Universal PCR Master Mix (Applied Biosystems), 5 pmol primers and 4 pmol TaqMan probe. The PCR cycling program included 2 min at 50°C to eliminate carryover contamination, 10 min at 95°C to activate the hot start *Taq* DNA polymerase (AmpliTaq Gold® DNA Polymerase, Applied Biosystems), and then 40 cycles, with each cycle consisting of a step at 95°C for 15 s, followed by a step at 60°C for 1 min. Herpesvirus copy numbers were multiplied by 100 to adjust for sample dilution in the PCR assay. PCR quantification standards included the plasmids MC and ME containing HCMV and EBV genomes, which were cloned using the TOPO-TA Cloning Kit (Invitrogen Corp., Carlsbad, CA, USA). No cross-reactivity was observed among HCMV, EBV, herpes simplex virus types 1 and 2, human herpesvirus 6, and human herpesvirus 8 (data not shown). Also, a BLAST (National Institute for Biotechnology Information, Bethesda, MD, USA) search to check the specificity of the HCMV and EBV primers and probes showed no genomic cross-reactivity with other mammalian viruses or cells. The dynamic range of quantification of the TaqMan PCR assay was determined by serial dilution of the plasmid generated standards in the range 10^7–10^1 copies per ml.

Statistical analysis

Statistical evaluation was performed using the nonparametric Spearman's or Kendall's tau rank coefficient of correlation tests and chi-squared analysis (SPSS 10.0 statistical package; SPSS Inc., Chicago, IL, USA). *p*-values equal to or less than 0.05 were considered statistically significant.

Results

Figure 1 shows representative TaqMan real-time PCR amplification plots to determine HCMV and EBV DNA copy numbers in deep periodontal pockets and in the adjacent gingival tissue. HCMV showed higher detection rate in periodontal pocket than in gingival tissue samples, whereas EBV revealed a similar rate of occurrence in the two types of periodontal samples (Table 1). Two aggressive periodontitis patients and six chronic periodontitis patients did not reveal HCMV in either the periodontal pocket or the gingival tissue sample. Three chronic periodontitis patients failed to demonstrate EBV in the paired samples. One chronic periodontitis patient showed neither HCMV nor EBV in the periodontal pocket or the gingival tissue sample.

Table 2 shows the herpesvirus loads in the 20 periodontitis patients studied. Seven of nine aggressive periodontitis patients, but only three of 11 chronic periodontitis patients, yielded more

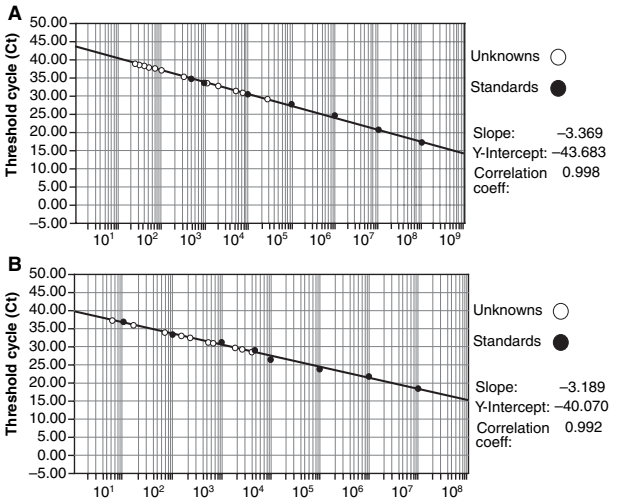

Fig. 1. Representative TaqMan real-time polymerase chain reaction amplification plots to determine the level of human cytomegalovirus (HCMV) and Epstein–Barr virus (EBV) DNA in periodontal pocket and gingival tissue samples from periodontitis lesions. Serial dilutions of HCMV and EBV plasmid standards were used as templates. Standard curves for HCMV (A) and for EBV (B) were produced from the amplification plots. Threshold cycle denotes the cycle number at which threshold fluorescence is reached. Linearity was achieved when plotting the threshold cycle against $\log_{10}$ of HCMV and EBV copy numbers.

Table 1. Distribution of human cytomegalovirus (HCMV) and Epstein–Barr virus (EBV) DNA in subgingival and gingival tissue samples from periodontitis lesions

Disease (no. of subjects)	HCMV, Subgingival samples	HCMV, Gingival tissue samples	EBV, Subgingival samples	EBV, Gingival tissue samples
Periodontitis (20, all subjects)	12 (60%)[a]	4 (20%)	13 (65%)	12 (60%)
Aggressive periodontitis (9)	7 (78%)	3 (33%)	8 (89%)	7 (78%)
Chronic periodontitis (11)	5 (46%)	1 (9%)	5 (46%)	5 (46%)

[a]No. (%) positive samples.

Table 2. Clinical data and human cytomegalovirus [HCMV] and Epstein-Barr virus [EBV] DNA loads in subgingival and gingival tissue samples from periodontitis lesions.

Subjects	Age (years)	Whole mouth mean plaque index	Whole mouth mean gingival index	Whole mouth mean periodontal pocket depth (mm)	Whole mouth mean probing attachment loss (mm)	Pocket depth (mm) at sample teeth (mean of 4 sites per tooth)	Probing attachment loss at sample teeth (mean of 4 sites per tooth)	Pocket depth (mm) at individual sample sites	HCMV counts/ml in subgingival samples	HCMV counts in gingival tissue samples	EBV counts/ml in subgingival samples	EBV counts in gingival tissue samples
Aggressive periodontitis												
1	34	1.5	2.0	4.3	4.3	6.3	6.8	8	34,000	750,000	2,400	17,000
2	27	1.8	2.1	5.1	5.1	6.8	7.0	8	2,800,000	110,000	10,000	7,700
3	34	1.3	2.0	4.0	4.0	5.5	5.5	6	0	0	2,800	0
4	32	1.8	1.9	5.1	3.4	3.5	3.,5	7	0	0	0	16,000
5	32	1.5	2.2	4.3	4.3	5.3	5.3	7	3,400	0	3,700	40,000
6	24	1.8	2.1	5.1	5.1	7.5	7.5	10	5,200	540,000	10,000	260,000
7	34	1.5	1.8	4.8	4.8	4.8	4.8	7	280	0	6,500	0
8	21	1.3	2.3	4.1	4.1	6.0	6.0	9	13,000	0	9,100	27,000
9	34	1.3	1.9	4.9	4.9	6.3	6.3	8	690	0	12,000	5,000
Chronic periodontitis												
10	49	2.5	2.0	3.7	4.2	5.3	5.8	6	3,200	7,300	5,100	0
11	36	2.3	1.8	4.4	4.9	5.3	5.3	7	0	0	2,900	0
12	51	1.8	1.9	2.9	3.4	5.3	5.8	6	0	0	0	6,400
13	40	1.5	2.1	4.0	4.8	6.3	6.3	8	3,400	0	730	0
14	36	1.8	1.8	3.7	3.7	4.3	4.3	6	0	0	0	3,400
15	36	2.3	2.0	3.7	3.7	5.8	5.8	7	0	0	0	28,000
16	45	2.3	1.8	3.4	3.7	4.0	5.0	6	0	0	0	0
17	56	2.0	2.0	4.0	4.8	5.3	5.5	6	2,600	0	0	0
18	56	2.5	2.2	4.1	4.4	5.8	6.0	7	0	0	3,200	13,000
19	40	2.3	2.5	6.8	7.8	7.5	7.5	10	24,000	0	20,000	820,000,000
20	47	2.0	1.8	3.4	3.9	5.5	5.5	6	770	0	0	0

than 10,000 copies of HCMV or EBV DNA in periodontal pocket or gingival tissue samples ($p = 0.03$; chi-squared test). Four of six patients having mean periodontal pocket depths at sample teeth (four study sites per tooth) > 6 mm, but none of 14 patients having mean pocket depths at sample teeth of ≤ 6 mm revealed more than 100,000 copies of HCMV or EMV in subgingival or gingival samples ($p = 0.001$; chi-squared test). Patient no. 1, 2, 6 and 19 demonstrated the highest herpesvirus counts as well as the most severe periodontal disease, as measured by pocket depth and the gingival index (Table 2).

Table 3 shows statistical correlations between clinical variables and herpesvirus counts using correlation coefficient analyses. In general, the HCMV and EBV counts in periodontal pockets and in gingival tissue were positively correlated with level of gingival inflammation and periodontitis disease severity, as assessed by pocket depth and probing attachment loss (Table 3). Positive correlations were found between herpesviruses and several clinical variables of aggressive periodontitis, whereas little relationship existed between the study viruses and clinical features of chronic periodontitis (Table 3).

Discussion

Clinical virology uses viral loads to assess disease severity and to monitor treatment efficacy (23). Herpesvirus infections can be evaluated by assays for viremia in cell culture, antigenemia, IgM and IgG specific antibody titers, and nucleic acid-based molecular techniques (23). The present study employed real-time quantitative PCR to begin determining the potential of HCMV and EBV DNA levels to differentiate disease-active and stable periodontitis sites. It may be assumed that most of the young adult study patients exhibited disease-active periodontitis, as inferred from the advanced type of periodontal destruction at an early age. Many of the middle-age patients most likely had a chronic form of the disease.

The present data further strengthen the plausibility of an important

Table 3. Spearman's statistical correlations among clinical variables, human cytomegalovirus (HCMV) and Epstein–Barr virus (EBV) in subgingival sites and adjacent gingival tissue of periodontitis lesions

Disease (no. of subjects)	Herpesvirus counts vs. clinical variables	Rho-values	*p*-values
Periodontitis (20, all subjects)	HCMV subgingivally vs. HCMV in gingival tissue	0.59	0.006
	HCMV subgingivally vs. EBV subgingivally	0.67	0.001
	HCMV subgingivally vs. pocket depth at sample sites	0.68	0.001
	HCMV in gingival tissue vs. pocket depth at sample teeth (mean of 4 sites per tooth)	0.43	0.06 (borderline)
	HCMV subgingivally vs. probing attachment loss at sample sites	0.68	0.001
	HCMV in gingival tissue vs. probing attachment loss at sample sites	0.53	0.02
	HCMV subgingivally vs. whole mouth mean gingival index	0.63	0.003
	EBV subgingivally vs. patient age	0.48	0.03 (negatively correlated)
	EBV in gingival tissue vs. patient age	0.46	0.04 (negatively correlated)
	EBV subgingivally vs. pocket depth at sample sites	0.63	0.001
	EBV in gingival tissue vs. pocket depth at sample sites	0.65	0.002
	EBV subgingivally vs. whole mouth mean attachment loss	0.61	0.004
	EBV in gingival tissue vs. probing attachment loss at sample sites	0.46	0.04
	EBV subgingivally vs. whole mouth mean gingival index	0.56	0.01
	EBV in gingival tissue vs. whole mouth mean gingival index	0.66	0.001
Aggressive periodontitis (9)	HCMV subgingivally vs. HCMV in gingival tissue	0.70	0.04
	HCMV subgingivally vs. pocket depth at sample sites	0.71	0.03
	HCMV in gingival tissue vs. pocket depth at sample teeth (mean of 4 sites per tooth)	0.73	0.03
	HCMV subgingivally vs. probing attachment loss at sample sites	0.75	0.02
	HCMV in gingival tissue vs. probing attachment loss at sample sites	0.78	0.01
	EBV subgingivally vs. pocket depth at sample sites	0.68	0.04
	EBV in gingival tissue vs. patient age	0.69	0.04 (negatively correlated)
	EBV in gingival tissue vs. whole mouth mean gingival index	0.68	0.04
Chronic periodontitis (11)	EBV subgingivally vs. pocket depth at sample sites	0.63	0.04

periodontopathic role for herpesviruses. The frequent presence of HCMV and EBV in deep periodontal pockets (5), and the clinical improvement after treatment-induced reduction of herpesviruses (13) are consistent with a periodontopathic contribution by the viruses. The present finding of high herpesvirus loads in gingival tissue in close proximity to the advancing front of periodontal breakdown reinforces the concept of herpesviruses possessing periodontopathic significance. In addition, as shown here and previously (7), HCMV and EBV periodontal coinfection tends to be associated with particularly severe forms of periodontal disease, suggesting multiple herpesvirus exposures act in concert in the pathogenesis of periodontitis, maybe as a result of the ability of HCMV to transactivate EBV and other herpesvirus genomes (24, 25).

Difficulty in obtaining relevant gingival tissue samples may partly explain the puzzling findings of a higher prevalence of HCMV in periodontal pockets than in gingival tissue but of higher HCMV counts in gingiva than in periodontal pockets in 75% of patients showing both a pocket and a gingival tissue HCMV infection. T-lymphocytes and macrophages, which are inflammatory cell reservoirs for HCMV (26), may be unevenly distributed in gingival tissue of periodontitis lesions (27). Some specimens may inadvertently have been harvested from gingival tissue areas with little or no HCMV presence, and missed gingival domains with significant HCMV occurrence. In comparison, the curette method of periodontal pocket sampling may constitute a relatively reliable means of obtaining subgingival HCMV. On the other hand, a curette also collects herpesvirus-containing pocket epithelial cells (28), which may cause an overestimation of the herpesvirus load in the periodontal pocket proper. The finding of a similar prevalence of EBV in periodontal pocket and gingival tissue samples may be due to a viral infection of B-lymphocytes (26), which are abundant in gingival tissue of advanced periodontitis lesions (29).

Overall, as discussed elsewhere (4), the relationship of HCMV and EBV with aggressive periodontitis seems to satisfy Hill's criteria of causation with respect to strength of association, consistency, biologic gradient, biologic cause-and-effect relationship, plausibility and analogy (30). However, it

remains to be shown that the selective removal of HCMV or EBV rather than a general suppression of pathogenic agents from periodontal sites halts, reverses, prevents or, at least, retards periodontitis. Moreover, HCMV and EBV are not the only viruses implicated in periodontitis and, as evidenced by the absence of detectable HCMV and EBV in one study patient, are not the sole requisite triggers of the disease. However, it is interesting to note that the herpesvirus-negative patient showed a maximal periodontal pocket depth of only 6 mm, suggesting a moderate type of the disease. As pointed out in this study, HCMV and EBV are typically associated with advanced types of periodontitis. It may also be that the PCR-negative patient harbored herpesviruses other than HCMV and EBV, such as the herpes simplex virus, which has been linked to periodontitis (31). Moreover, it is possible that prior antimicrobial therapy, maybe including the presurgical removal of supragingival deposits, had eliminated detectable HCMV and EBV from the periodontal study site (13).

The finding of high HCMV and EBV loads in aggressive periodontitis provides a reason for reconsidering current hypotheses on the etiopathogenesis of the disease, particularly since the dominant hypothesis of a genetic predisposition is still open to question (32, 33). The diagnosis of aggressive periodontitis denotes systemically healthy individuals of young age experiencing rapid loss of periodontal attachment (34). Aggressive periodontitis is typically associated with relatively little dental plaque, suggesting a low infectious agent stimulus triggering the progressive disease response. A localized form of aggressive periodontitis affecting young adolescents is particularly destructive but tends to be self-limiting and burns out after a few years (35). A generalized form of aggressive periodontitis affects mostly young adults and typically shows more accumulation of dental accretions (36). Aggressive periodontitis patients may experience intermittent disease flare-ups in adult life.

The clinicopathologic characteristics of aggressive periodontitis are consistent with a herpesvirus etiology of the disease. A unique feature of herpesviruses is their ability to infect host cells, followed by an extended period of latency that sporadically is interrupted by short intervals of viral reactivation. It is common for primary and recurrent episodes of herpesvirus clinical infections to exhibit considerably different signs and symptoms. Pathosis occurring at primary infection and during periods of herpesvirus reactivation tends to be severe in immunologically immature young people and in immunocompromised individuals, and mild-to-moderate in adults with preexisting herpesvirus immunity from past infection. For example, the varicella-zoster virus causes chickenpox during primary infection and shingles during viral recrudescence, herpes simplex virus may cause acute gingivostomatitis during primary infection and epidermal or mucosal ulcers during viral recrudescence, and EBV and HCMV can give rise to mononucleosis during primary infection and a variety of diseases during viral recrudescence (4). Similar to classic herpesvirus diseases, it can be theorized that herpesvirus-associated periodontitis has its most severe course in the initial phase of disease due to inadequate antiherpesvirus immunity, and then tapers off after the establishment of effective herpesvirus-specific cellular immune responses. Periodontitis disease relapses may preferentially occur in individuals who lack sufficient immunity against periodontal herpesviruses. In agreement with this hypothesis, virtually all established risk indicators/factors of periodontitis possess immunosuppressive capacity with the potential to activate latent herpesviruses (37). Herpesvirus infections can cause both cytopathogenic and immunopathogenetic effects (4, 38), and although the relative contribution of the two pathogenic mechanisms to destructive periodontal disease is not known, it is likely that the early stages of periodontitis in immunologically naive hosts mainly comprise cytopathogenic events, whereas most clinical manifestations in immunocompetent individuals are secondary to cellular or humoral immune responses. Clustering of aggressive periodontitis in families (34) may result from a transmission of herpesviruses among individuals in the same household rather than from a genetic predisposition, although the disease development may involve both pathogenetic components.

Nonetheless, despite intriguing similarity in pathogenic traits between classic herpesvirus diseases and periodontitis, the body of data pertinent to the herpesvirus hypothesis of aggressive periodontitis is still small, and several key questions on molecular aspects of the disease remain unanswered. The relationship between herpesvirus loads during the course of a periodontal infection and the severity of periodontitis remains to be elucidated. Studies are also needed to determine how sequential changes in herpesvirus periodontal load affect levels of immune activation markers in periodontal sites (39, 40). Findings from such studies may provide further insights into the pathogenesis of disease-active periodontitis.

In summary, the high HCMV and EBV loads in progressive periodontitis lesions support the notion that the two herpesviruses participate in the initiation or progression of the disease. Knowledge of the association of herpesviruses with periodontitis may not only be important for understanding the pathogenesis of the disease, but may also be of significance for diagnosis, monitoring and treatment. Active herpesvirus infection may serve as an important new marker to indicate progressive periodontitis and, in combination with other disease variables, form the basis for improved assessment of future disease risk. Data from this and our previous studies argue for further research into the role of HCMV, EBV and other herpesviruses in the pathogenesis of human periodontitis.

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