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Human herpesvirus 7, Epstein–Barr virus and human cytomegalovirus in periodontal tissues of periodontally diseased and healthy subjects

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Abstract

Aims: To evaluate (i) the presence of human herpesvirus 7 (HHV-7), Epstein–Barr virus (EBV) and human cytomegalovirus (HCMV), and (ii) the transcription pattern of HHV-7 in gingival biopsies from patients affected by periodontitis (P) and periodontally healthy subjects (H).

Material and Methods: Thirty-seven subjects (P: $n = 24$; H: $n = 13$) were included. Each P patient contributed two gingival biopsies (representative of a clinically affected and non-affected site) and each H subject contributed one gingival biopsy. After DNA extraction, nested polymerase chain reaction was used to identify the viruses.

Results: HHV-7 was detected in 91.7% of P patients and in 61.5% of H subjects ($p = 0.02$), EBV in 50.0% samples of P patients and 7.7% of H subjects ($p = 0.005$) and HCMV only in one sample from H group. EBV was more frequently detected in biopsies from affected sites (50.0%) than from non-affected sites (16.7%) ($p = 0.008$). HHV-7 transcription was detected in 15.4% of affected and 15.4% of non-affected sites.

Conclusions: The results indicate that (i) gingival tissues can be considered a potential reservoir for HHV-7; (ii) when present, HHV-7 persists in a latent state in the majority of cases; (iii) the presence of EBV seems to be associated with the diseased state of the patient and site.

Key words: Epstein–Barr virus; herpesvirus; human cytomegalovirus; human herpesvirus 7; periodontal disease

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Conflict of interest and source of funding statement

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Human periodontal diseases represent several pathological entities, which exhibit a variety of clinical presentations, polymicrobial infections and pathophysiologic mechanisms (Slots 2004a). It is now scientifically accepted that although specific subgingival bacterial pathogens are causally necessary for the development of periodontitis, the mere amount of bacterial plaque does not seem to provide a sufficient basis for explaining important clinico-pathologic features of the disease (Saygun et al. 2002, Slots et al. 2003, Yapar et al. 2003, Kubar et al. 2005).

Recently, various human herpesviruses (HHVs) have emerged as putative pathogens in destructive periodontal disease (Slots 2002, Slots et al. 2006). An ''HHV-bacterial pathogen'' model has been proposed in which HHVs contribute to periodontal pathosis by impairing local host response, resulting

in an increased virulence of the resident bacterial pathogens, or by inducing the release of cytokines and chemokines from inflammatory and connective tissue cells (Slots & Contreras 2000, Slots 2007).

On the basis of these considerations, we previously investigated the presence of the novel HHV-6, HHV-7 and HHV-8 in gingival biopsies from patients affected by chronic periodontitis (ChP) (Cassai et al. 2003). As control, gingival biopsies from periodontally healthy subjects were also analysed. We reported that gingival tissues may act as a reservoir for HHV-7. High prevalence of HHV-7 was detected in both ChP and periodontally healthy individuals (Cassai et al. 2003). However, the role of HHV-7 DNA in periodontal lesions still needs to be determined. In fact, HHV-7, like all other HHVs, may persist in the infected host in a latent state. Latent (non-productive) or active (productive) replications are characterized by specific and typical transcription patterns. In the case of HHV-7, we showed that analysis of viral transcription by reverse transcriptase (rt)-polymerase chain reaction (PCR) is a useful tool to ascertain or dismiss pathogenic associations between persistence of viral DNA and specific diseases (Gonelli et al. 2001). Differences in viral transcription between gingival biopsies from periodontally healthy and diseased subjects as well as from clinically affected and non-affected sites would be suggestive of a potential association of HHV-7 with periodontal disease status.

Among HHVs, also human cytomegalovirus (HCMV) and Epstein–Barr virus (EBV), infections may be implicated in the pathogenesis and progression of periodontal lesions (Slots et al. 2002, Sabeti et al. 2003, Saygun et al. 2005, Botero et al. 2007, 2008, Teughels et al. 2007). The subgingival presence of both EBV and HCMV was reported to be associated with the major periodontopathic bacteria and the severity of periodontal disease (Saygun et al. 2008, Sunde et al. 2008b). The hypothesis of a correlation between HCMV and EBV infection and the pathogenesis and progression of aggressive periodontitis (AgP) have been proposed by previous studies (Kamma & Slots 2003, Kubar et al. 2004, Saygun et al. 2004a). In this respect, several findings suggested that periodontitis occurs more frequently and progresses more rapidly in sites infected by EBV and HCMV rather than in non-infected sites (Ling et al. 2004, Slots 2004b). Moreover, EBV–HCMV co-infection has been associated with inflammation and destruction of periodontal tissues (Wu et al. 2007).

The present study was designed in order to evaluate (i) the presence of HHV-7, EBV and HCMV and (ii) the transcription pattern of HHV-7 in gingival biopsies from ChP, AgP and periodontally healthy subjects.

Material and Methods

Study population

The investigation was performed in accordance to the requirements of the ''Declaration of Helsinki'' as adopted by the 18th World Medical Assembly in 1964 and revised in Edinburgh (2000). Each subject provided a verbal informed consent before participation.

The participants were selected among periodontally healthy (H), ChP and AgP subjects seeking care at the Research Centre for the Study of Periodontal Diseases, University of Ferrara. The clinical diagnosis of ChP and AgP at the time of the initial visit was based on a recent classification (Armitage 1999). ChP, AgP and H subjects were included in the study according to the following inclusion criteria: (i) systemically healthy subjects not assuming antivirals in the past 6 months; (ii) ChP patients: at least five sites with clinical attachment level (CAL) $\geqslant 5$ mm and eight sites with pocket probing depth (PPD) $\geqslant 6$ mm; AgP patients: CAL $\geqslant 5$ mm in more than four teeth apart from first molars or incisors; H subjects: no sites with PPD $\geqslant 4$ mm or inter-dental CAL loss $\geqslant 2$ mm.

Sampling procedure

Before specimen collection, each subject received single or multiple sessions of periodontal debridement and oral hygiene instructions. Biopsy specimens were obtained during osseous resective surgery in ChP and AgP patients, and during tooth extraction procedures or periodontal plastic surgery in H subjects. Each ChP and AgP patient contributed two biopsies involving the epithelium and connective tissue facing the sulcus/periodontal pocket (Cassai et al. 2003). One biopsy was collected from a site having PPD $\geqslant 5$ mm and presenting with bleeding upon probing (clinically affected site) at the time of biopsy collection. The other biopsy was collected from a site with PPD $\leqslant 3$ mm and without bleeding on probing (clinically non-affected site). Each H subject contributed one biopsy involving the epithelium and connective tissue facing the sulcus.

Immediately after removal, all tissue specimens were divided into two parts (one for DNA and the other one for RNA extraction) and put in RNA later solution at 4°C (Ambion, Applied Biosystems, St. Austin, TX, USA) to avoid nucleic acid degradation (especially RNA). Before freezing, RNA later solution was removed, samples were weighted, frozen in liquid nitrogen and stored at -135°C until nucleic acid extractions.

Laboratory procedures

All samples were processed under code, and periodontitis samples were identified only after all the experiments were performed. As the analyses were performed with extremely sensitive nested amplification procedures, the highest care was applied to avoid false positives. Handling of samples, extraction of nucleic acids, PCR amplification and detection of amplified products were carried out in different rooms, with dedicated equipment and reagents. Blank samples (consisting of extraction buffer, water or reaction buffer) were interspersed between samples in order to avoid cross-contamination. Particular care was taken to ensure that negative results were not due to experimental procedures.

Extraction of DNA and PCR analysis

Of the two samples from the same patients, the one lower in weight was utilized for DNA extraction. DNA was extracted by conventional procedures, as previously described (Rotola et al. 1992) and according to manufacturer's instructions. Briefly, biopsies were finely minced with scissors in the lysis solution [60 mM Tris-HCl pH 8.0, 100 mM ethylenediaminetetraacetic acid (EDTA) pH 8.0, 0.5% Sodium dodecyl sulphate] containing 500 μg/ml proteinase K (Roche Diagnostics, Mannheim, Germany) and incubated for 4 h at 55°C. After phenol and phenol–chloroform extractions, DNA was recovered by ethanol precipitation, resuspended in TE buffer (10 mM Tris, 1 mM EDTA) and stored at -20°C until PCR analysis. Particular care was taken to avoid

Table 1. Primers and PCR/nested PCR of Herpesviruses genes

ORF	Oligonucleotide sequences (5′–3′)	Amplicons (bp)		[$MgCl_2$] (mM)	PCR conditions
		DNA	cDNA		
HHV-7 U16/17	CgTgATCgATAgACgTTAggA CgCgATTCTCTTAgTgACTTC	610		2.5	5 min. at 94°C, 30 s at 94°C, 30 s at 58°C, 30 s+3 s/cycle at 72°C for 35 cycles and 10 min. at 72°C
	gCTgCTgTTgTTACgTCgTT AACCgAgCTgCAAgACCTAT	171		1.0	
HHV-7 U42	AAgCTgCAAgACggAgTTgT AgTATTCCggTgAAgCACgA	856		1.5	5 min. at 94°C, 30 s at 94°C, 30 s at 58°C, 30 s+3 s/cycle at 72°C for 35 cycles and 10 min. at 72°C
	CgCgTATgAACTgAggTTgT CCAgCTCATAggATTCgAgA	574		1.0	
HHV-7 U98/100	ggACATTATgAAgggCATCC CgTgTTgTTCTgCCgTATTg	887	793 720	2.5	5 min. at 94°C, 30 s at 94°C, 30 s at 56°C, 30 s+3 s/cycle at 72°C for 35 cycles and 10 min. at 72°C
	TTATgAAgggCATCCTAgAg AgTTgAgAACgACCTTgAgA	563	469	1.0	5 min. at 94°C, 30 s at 94°C, 30 s at 58°C, 30 s+3 s/cycle at 72°C for 35 cycles; 10 min. at 72°C
LMP-1 EBV	gCgACTTCTgCTggAAATgATg TgATTAgCTAAggCATTCCCA	315		1.5	5 min. at 95°C, 30 s at 94°C, 30 s at 55°C, 30 s at 72°C for 40 cycles and 10 min. at 72°C
	AAAATTgACggAAgAggTTgA TgATTAgCTAAggCATTCCCA	283			5 min. at 95°C, 30 s at 94°C, 30 s at 57°C, 30 s at 72°C for 20 cycles and 10 min. at 72°C
HCMV MIE	AAgCggCCTCTgATAACCAAgCC AgCACCATCCTCCTCTTCCTCTgg	435		1.5	5 min. at 94°C, 30 s at 94°C, 30 s at 57°C, 30 s at 72°C for 35 cycles and 10 min. at 72°C
	AgTgTggATgACCTACgggCCATCg ggTgACACCAgAgAATCAgAggAgC	110			5 min. at 94°C, 30 s at 94°C, 30 s at 59°C, 30 s at 72°C for 25 cycles and 10 min. at 72°C

HHV, human Herpesvirus; EBV, Epstein–Barr virus; HCMV, human Cytomegalovirus.

contamination of the samples, and blank reactions, consisting of the extraction mixture alone, were interspersed with every third experimental sample (to control for possible cross-contamination of DNA samples). DNA concentration was determined by reading optical density at 260 nm. To ensure that all biopsies were suitable for DNA amplification, PCR specific for human β-actin gene (Rotola et al. 1998) was performed on 10 ng of each DNA sample.

To determine which samples harboured HHV sequences, 1 μg of DNA (corresponding to 150,000 cells) was analysed by nested PCR. The presence of HHV-7 DNA was searched by nested PCR specific for U16/17 and U42 genes (Di Luca et al. 1995), as already described (Cassai et al. 2003) (Table 1). To investigate HCMV presence in collected biopsies, a nested PCR which amplified a fragment of 110 bp within the major immediate-early gene (MIE) of HCMV Ad169 strain (Fenner et al. 1991) was utilized (Table 1). The exons 1–4 of this region encode the IE1 transactivator which appears in infected cells 1 h post infection and has an important function in the regulation of HCMV genes (Pignatelli et al. 2004). EBV was searched using primers specific for type-1 and type-2 EBV LMP-1 region (Dolcetti et al. 1997) (Table 1), which encode an integral membrane protein of 386 amino acids able to exert pleiotropic effects essential for EBV-induced B-cell transformation (Eliopoulos & Rickinson 1998). This region is highly heterogeneous across diverse isolates, showing clustered point mutations, insertions and in frame 30 or 69 bp deletions (Hudson et al. 1985, Weiss et al. 1987, Carbone et al. 1993). Primers were chosen within regions of the LMP-1 carboxy terminus (Dolcetti et al. 1997).

PCR analysis was performed in triplicate on individual biopsies for each set of primers. The sensitivities of all PCR reactions were determined by amplification of known amounts of cloned target sequences. PCR products were run on 2–3% agarose gels (FMC, Rockland, ME, USA), according to the expected fragment size, and UV visualized after ethidium bromide staining. Particular care was taken to avoid sample-to-sample contamination: different rooms and dedicated equipment were used for DNA extraction and processing, for PCR set-up and gel analyses, all pipette tips had filters for aerosol protection and all experimental samples were interspersed with blank reactions.

Extraction of RNA and rtPCR analysis

To ascertain the replicative state of HHV-7, RNA was extracted and analysed by RT-PCR for the presence of viral transcripts specifically associated to active replication. The absence of contaminating DNA was checked by PCR amplification of human β-actin gene before retrotranscription. Negative PCR results for β-actin ensured that the RNA sample was completely free from DNA sequences, and that positive amplification after retrotranscription was positively associated to viral transcripts. Productive infection is associated to the simultaneous presence of mRNAs belonging to the immediate-early, early or late transcriptional classes (Menegazzi et al. 1999). Therefore, we searched by nested RT-PCR the presence of HHV-7 transcripts utilizing three set of primers belonging to immediate-early (U16/17 and U42) or late (U98/100) genes. Total RNA was extracted with RNAzol B (Biotecx, Houston, TX, USA), following the protocol provided by the manufacturer. After DNAse treatment (4 U/μg RNA, 3 × 20 min. at room temperature), aliquots of RNA were stored at − 135°C in ethanol, until utilized for reverse transcription and

PCR amplification. First strand cDNA synthesis was carried out with MuLV reverse transcriptase and random hexamer primers (Applera Corporation, Norwalk, CT, USA), following the manufacturer's instructions, retrotranscribing 2 μg of total RNA from all the samples (either periodontitis and controls RNA). The complete absence of contaminating DNA was verified by PCR amplification of samples before retrotranscription with primers specific for β-actin gene. Primers and PCR conditions for HHV-7 genes have been previously described (Menegazzi et al. 1999) and are summarized in Table 1. Efficiency of retrotranscription was assessed by analysis of dilutions of cDNA with PCR specific for human β-actin gene. Nested PCR on 1/10 of total cDNA was performed for HHV-7 U16/17, U42 and U 98/100 genes.

Statistical analysis

Fishers's exact test was employed to analyse the results. The level of significance was fixed at 0.05.

Results

Study population

ChP group consisted of 13 patients (nine men, four women; mean age 50.8 $\pm$ 6.2 years), AgP group of 11 patients (three men, eight women; mean age 40.9 $\pm$ 4.0 years) and H group of 13 subjects (five men, eight women; 25.8 $\pm$ 12.0 years). All the subjects included in the present study were caucasians.

Sensitivity of PCR analysis

The sensitivity of PCR was consistent in all the experiments, detecting as few as 1000 target molecules in the first round of amplification and down to 10 target molecules after nested amplification, as shown by reconstruction experiments (data not shown). All PCR reactions for the different viral types had the same sensitivities (data not shown). All negative controls (extraction blanks and blank reactions without DNA) were negative at PCR.

HHVs prevalence

The results on the presence of HHV DNA in biopsies from periodontitis patients (ChP+AgP) and H subjects are shown in Table 2. All samples were negative after the first PCR step, and positivity was detected only after nested amplification, suggesting that positive specimens harboured less than 1000 target molecules in 150,000 diploid cells (Fig. 1). HHV-7 DNA sequences were detected in 22/24 (91.7%) biopsies from periodontitis patients and in 8/13 (61.5%) from H subjects ($p = 0.02$). EBV DNA sequences were detected in 12/24 (50.0%) samples from periodontitis patients and in 1/13 (7.7%) from H subjects ($p = 0.005$). HCMV DNA was detected only in one sample from H subjects (1/13, 7.7%). This sample contained both EBV and HHV-7 (Fig. 1).

Table 2. Presence of Human Herpesvirus 7, Human Cytomegalovirus and Epstein–Barr Virus DNA in the periodontally-affected patients (Chronic and Aggressive Periodontitis) and periodontally-healthy controls

	ORF HHV-7		Total	HCMV	EBV
	U16/17	U42	HHV 7	MIE	LMP1
ChP+AgP patients	17/24 (70.1%)	18/24 (75.0%)	22/24 (91.7%)	0/24	12/24 (50.0%)
Periodontally healthy subjects	6/13 (46.2%)	5/13 (38.5%)	8/13 (61.5%)	1/13 (7.7%)	1/13 (7.7%)

HHV, human Herpesvirus; EBV, Epstein–Barr virus; HCMV, human Cytomegalovirus; ChP, chronic periodontitis; AgP, aggressive periodontitis.

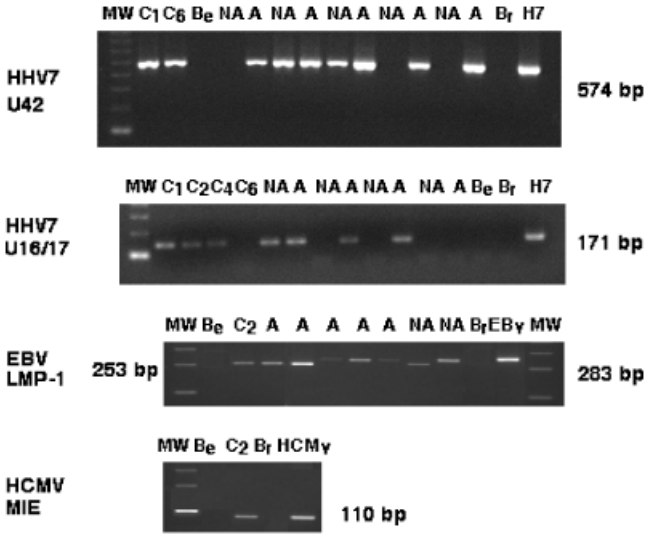

Fig. 1. Presence of Herpesviruses by nested PCR in biopsies from periodontitis patients and controls. MW, 123 bp DNA ladder; C, controls; A, affected site; NA, non-affected site; B_e and B_r, controls of extraction and of reaction respectively; H7, HHV-7 amplicon; EB_V, EBV amplicon; HCM_V, HCMV amplicon.

Table 3. Prevalence of Herpesvirus 7 and Epstein–Barr Virus according to the diseased state of the patient (Chronic or Aggressive Periodontitis, Periodontally Healthy) and biopsied site

Diagnosis	HHV-7		EBV		Total*	
	A	NA	A	NA	HHV7	EBV
Chronic periodontitis	8/13 (61.5%)	9/13 (69.2%)	6/13 (46.2%)	2/13 (15.4%)	11/13 (84.6%)	6/13 (46.2%)
Aggressive periodontitis	7/11 (63.6%)	8/11 (72.7%)	6/11 (54.5%)	2/11 (18.2%)	11/11 (100%)	6/11 (54.5%)
Total periodontitis	15/24 (62.5%)	17/24 (70.8%)	12/24 (50.0%)	4/24 (16.7%)	22/24 (91.7%)	12/24 (50.0%)
Periodontally healthy	8/13 (61.5%)		1/13 (7.7%)		8/13 (61.5%)	

*The column ''Total'' expresses the cumulative proportion of patients who presented the virus in at least one site (either affected or non-affected).
HHV, human Herpesvirus; EBV, Epstein–Barr Virus; A, affected site; NA, non-affected site.

Table 3 shows the prevalence of HHV-7 and EBV according to the diseased state of patients and sites. There was no difference in the presence of HHV-7 DNA either between ChP (11/13, 84.6%) and AgP patients (11/11, 100%) or affected and non-affected sites (15/24, 62.5% and 17/24, 70.8%). Similarly, there was no difference in the presence of EBV between ChP and AgP patients. However, when ChP and AgP patients were jointly considered, EBV was more frequently detected in biopsies from affected sites (12/24, 50.0%) than in those from non-affected sites (4/24, 16.7%) ($p = 0.008$). Five out of eight EBV positive samples (62.5%) (four affected and one non-affected) also contained HHV-7 sequences.

HHV-7 replicative state

Single-step PCR amplification of cDNAs did not reveal the presence of detectable levels of viral transcripts. A nested round of amplification resulted in the detection of U16/17 and/or U42 mRNA in 2/13 (15.4%) affected and 2/13 (15.4%) non-affected sites from ChP patients (Table 4). Only in one case both sites were positive. No samples from AgP or H subjects revealed viral transcription with any HHV-7 primers, but all cDNA samples resulted positive for amplification of the eucaryothic gene (Table 4; Fig. 2).

Table 4. Presence of Human Herpesvirus 7 transcripts in periodontitis patients and periodontally healthy subjects (controls)

Samples	HHV-7 ORF								
	c-DNA β-actin		c-DNA U16/17		c-DNA U42		c-DNA U98/100		Total HHV7
	A	NA	A	NA	A	NA	A	NA	CDNA
Chronic periodontitis	13/13	13/13	0/13	2/13 (15.4%)	2/13 (15.4%)	2/13 (15.4%)	0/13	0/13	3/24 (12.5%)
Aggressive periodontitis	11/11	11/11	0/11	0/11	0/11	0/11	0/11	0/11	0/11
Periodontally healthy	/	3/13	/	0/13	/	0/13	/	0/13	0/13
Total	37/37		2/37 (5%)		3/37 (8%)		0/37		3/37 (8%)

HHV, human Herpesvirus; A, affected site; NA, non-affected site.

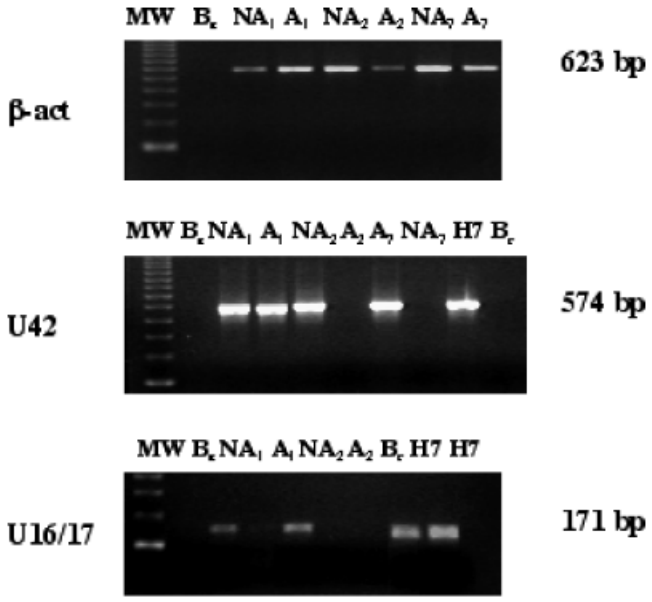

Fig. 2. Agarose gel stained with ethidium bromide showing the results of RT-PCR for eucaryothic gene (β-actin) or HHV-7 genes (U16/17 and U42) in CAP patients. RNA was extracted from CAP samples, retrotranscribed in cDNA and analysed by nested PCR using primers specific for human β-actin, U42 or U16/17 HHV7genes. MW, 123 bp DNA ladder; NA, non-affected site; A, affected site; numbers subscript indicate samples belonging to the same patient. B_e and B_r, controls of extraction and of reaction respectively; H7, HHV-7 amplicon.

Discussion

The present study was designed in order to evaluate (1) the presence of HHV-7, EBV and HCMV and (2) the transcription pattern of HHV-7 in gingival biopsies from ChP, AgP and H subjects. Our results indicate that the prevalence of EBV is significantly higher in periodontally affected patients and sites compared with the healthy controls. On the other hand, no correlation was detected for HHV-7 or HCMV. Prevalence of the investigated viruses resulted similar in both ChP and AgP patients (Fisher's exact test, $p > 0.05$). However, the sample size in the two different forms of periodontitis may have prevented the detection of statistically significant differences between ChP ($n = 13$) and AgP ($n = 11$) patients. Moreover, our study demonstrated that HHV-7, when present, persists in a latent state in the majority of cases.

Our data indicated a high tropism of HHV-7 for gingival tissues, which can be considered a potential reservoir for the virus. About 66% (40/61) of gingival biopsies contained HHV-7 DNA. These data are consistent with our previous studies where a high prevalence of HHV-7 was found by nested PCR in gingival biopsies from either ChP or H subjects (Cassai et al. 2003). It should be noted that in the present study, the presence of HHV-7 was detected only after the more sensitive nested PCR, the samples being sometimes positive only with one of the two copies of HHV-7-specific primers. These observations implicate that low amounts of virus were harboured in the biopsy specimen. Moreover, the mere presence of viral DNA does not necessarily imply an etiologic role of the virus, either as a direct or as a co-infective agent. In fact, HHV-7 is able to establish latent infections in the majority of infected individuals (Menegazzi et al. 1999). PCR analysis does not allow to discriminate among latent, persistent and productive infections. As it has been suggested that HHV infection might be associated with low levels of replication (Kempf et al. 1998), it is important to determine precisely the state of viral transcription. Recently, we showed that analysis of viral transcription by rtPCR is a useful

tool to ascertain or dismiss pathogenic associations between the persistence of viral DNA and specific diseases (Gonelli et al. 2001). rtPCR, as performed in the present study, revealed that HHV-7 persists in a latent state in the majority of patients. In fact, all samples from AgP and H subjects were negative for HHV-7 transcripts using primers which amplified both immediate-early and late gene, which are normally expressed during lytic infection (Menegazzi et al. 1999). Unlike HHV-6 (Rotola et al. 1998), it is not yet identified as a molecular marker of latency for HHV-7. Thus, the absence of transcripts normally detectable in the infected cells is probably indicative that the virus is present in a non-replicative state. In biopsies from ChP patients, viral transcripts were detected with low prevalence (3/24, 12.5%). No significant differences in viral transcription were detected between affected and non-affected sites in periodontally diseased patients. This finding may be ascribed to a reactivation of HHV-7 in lymphocytes, which represent a relevant cell component of the inflammatory infiltrate of the periodontitis lesion.

In our subject sample, EBV exhibited a significantly higher prevalence in periodontitis patients when compared with healthy subjects (50.0% and 7.7%, respectively). Moreover, affected sites in periodontitis patients were characterized by a significantly higher prevalence of EBV with respect to non-affected sites (50% and 16.7%, respectively). These data are corroborated by the findings of a previous study (Saygun et al. 2005), where a high prevalence of EBV was found in ChP and AgP patients. In that study, a strong correlation between PPD and EBV counts was reported (Saygun et al. 2005). The potential association between EBV and periodontitis is strengthened further by studies on viral transcription. A recent study showed that the EBV DNA present in tissues with periodontitis are transcriptionally active and synthesize virus-specific mRNA molecules, thus supporting productive infection (Sabeti et al. 2003). In a recent case report, the eradication of EBV through systemic antiviral therapy in a patient affected by recurrent periodontitis was shown to be associated with a dramatic improvement of periodontal status, both short and long term (Sunde et al. 2008a).

In our material, HCMV was only detected in one healthy subject, who was also positive for HHV-7 and EBV. In contrast, several studies suggested a significant association between HCMV infection and periodontitis (for review, see Slots 2005). In our opinion, this discrepancy could be partly explained by differences in the study population. Even though HCMV infection is endemic throughout the world, the overall prevalence of infection varies greatly among different populations. HCMV prevalence is higher and occurs at earlier stages of life in developing countries and in the lower socioeconomic strata (reviewed by Pass 1985). Moreover, several studies which reported a positive association between HCMV and periodontitis had been conducted in specific ethnic groups, including Turkish population (Saygun et al. 2002, Yapar et al. 2003, Kubar et al. 2004, 2005, Saygun et al. 2004a, b, Saygun et al. 2008). Interestingly, HCMV infection is highly prevalent in Turkey, with an HCMV prevalence at the highest end of worldwide ranges (Hizel et al. 1999). Previous studies, investigating a potential association between HCMV and periodontitis in an Italian population, reported prevalence values ranging from 9% to 27% in periodontitis patients with no statistically differences with controls (Santangelo et al. 2004). Another potential explanation for the differences in HCMV prevalence among studies could be found in the timing of the sampling procedure with respect to the phases of periodontal treatment. In our material, biopsy specimens were harvested at the time of periodontal surgery, i.e. after multiple sessions of non-surgical periodontal therapy had been performed. It was demonstrated that active periodontal therapy can induce substantial reductions of HCMV counts in subgingival plaque and saliva (Saygun et al. 2005).

In conclusion, the results from the present study, although confirming the high prevalence of HHV-7 in gingival tissues, do not support an active pathogenic role of the virus in the onset of destructive periodontal diseases. Data on EBV prevalence suggest a potential involvement of this virus in the aetiology of periodontal disease. Within the limit of this study population, our findings failed to demonstrate an association between the presence of HCMV and the periodontitis status.

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Clinical Relevance

Scientific rationale for the study: Recent studies hypothesized a link between human HHVs infection and periodontal disease.

Principal findings: HHV-7 and EBV were more prevalent in gingival biopsies from periodontitis patients when compared with healthy subjects. EBV was detected more frequently in affected sites than in non-affected sites. HCMV was detected only in one healthy subject. HHV-7 active transcription was detected sporadically in periodontitis patients but not in healthy subjects.

Practical implications: Data seem to suggest that EBV may play a role in periodontal disease pathogenesis and/or progression.