Journal of
PERIODONTAL RESEARCH

J Periodont Res 2012; 47: 114–120

JOURNAL OF PERIODONTAL RESEARCH
doi:10.1111/j.1600-0765.2011.01413.x

Correlation of cytomegalovirus and human herpesvirus 7 with $CD3^+$ and $CD3^+CD4^+$ cells in chronic periodontitis patients

R. L. Thomasini[1,2], S. H. Bonon[2], P. Durante[2], S. C. B. Costa[2]

Department of Clinical Medicine, State University of Campinas, UNICAMP, Campinas, São Paulo, Brazil and [2]Laboratory of Clinical Pathology, Hermínio Ometto Foundation, UNIARARAS, Araras, São Paulo, Brazil

Thomasini RL, Bonon SH, Durante P, Costa SCB. Correlation of cytomegalovirus and human herpesvirus 7 with $CD3^+$ and $CD3^+CD4^+$ cells in chronic periodontitis patients. J Periodont Res 2012; 47: 114–120. © 2011 John Wiley & Sons A/S

Background and Objective: Human chronic periodontitis is an inflammatory process characterized by dense accumulation of immune cells in the periodontal tissue. The periodontitis can lead to loss of teeth in the patient and the pathogenesis of this disease is not completely known. This study tested the hypothesis that chronic periodontitis-affected sites can harbor betaherpesviruses and that viruses are linked to a profile of the inflammatory infiltrate.

Material and Methods: Biopsies of periodontal tissue were taken from periodontitis-affected patients and from healthy subjects. Immunohistochemistry was performed to count $CD19^+$ B cells, $CD3^+$ total T cells, T-$CD4^+$ and T-$CD8^+$ cell subsets, and PCR was performed to detect cytomegalovirus and human herpesvirus 6 and 7 in the samples. One slide of each sample was stained with Giemsa for histopathological examination and to evaluate the quality of the cellular infiltrate.

Results: As expected, tissues collected from healthy subjects presented no significant level of inflammatory infiltration and were therefore excluded from immunostaining procedures. Results showed that $CD19^+$ B cells were in higher number than $CD3^+$ T cells in the periodontitis-affected tissue, but this was not statistically significant. The T-$CD4^+$ lymphocyte subset was significantly higher than the T-$CD8^+$ lymphocyte subset ($p = 0.004$) in the samples. Cytomegalovirus and human herpesvirus 7 were found at periodontitis-affected sites, but not in tissue collected from healthy subjects ($p = 0.04$ and $p = 0.04$, respectively). Human herpesvirus 6 was rarely detected. We found a correlation between cytomegalovirus and lower $CD19^+/CD3^+$ ratios (ratio < 0.9, $p = 0.003$) and between human herpesvirus 7 and lower $CD19^+/CD3^+$ ratios (ratio < 0.9, $p = 0.003$) and higher $CD4^+/CD8^+$ ratios (ratio > 1.1, $p = 0.002$).

Conclusion: This study shows that cytomegalovirus and human herpesvirus 7 can be present at periodontitis-affected sites but are uncommon at healthy periodontal sites. Moreover, our data suggest that cytomegalovirus can be related to an inflammatory infiltrate with predominance of $CD3^+$ T cells, whereas human herpesvirus 7 can be associated with an infiltrate with predominance of T-$CD4^+$ cells. However, further studies are necessary to support this hypothesis. Herpesviruses could play a role in human chronic periodontitis by modulation of the T cell response.

Professor Ronaldo L. Thomasini, MSc, Professor, Department of Clinical Medicine, Faculty of Medicine, State University of Campinas-UNICAMP, CEP 13081-970, Campinas, SP, Brazil
Tel: +55 19 8136 6851
Fax: +55 19 3544 9155
e-mail: ronaldothomasini@yahoo.com.br

Key words: chronic periodontitis; cytomegalovirus; human herpesvirus 6; human herpesvirus 7

Accepted for publication July 27, 2011

Periodontitis is an inflammatory disease of the support tissues of the teeth that is clinically differentiated from gingivitis by the loss of the connective tissue attachment to the teeth, in the presence of concurrent gingival inflammation (1).

Although periodontitis is considered to be a process that involves a multifactorial interaction between microbial, host and environmental modulating factors (2), it is well accepted that periodontitis is associated with specific bacterial species and polymicrobial colonization of the teeth surfaces (3,4); however, the amount of bacterial plaque *per se* does not completely explain the clinical and pathological features of periodontitis (5–8).

Periodontal diseases are inflammatory processes characterized by dense accumulation of immune cells, including polymorphonuclear neutrophils, T and B lymphocytes, plasma cells, mast cells, monocytes and macrophages (9,10). The T cells have a very important role in the immune response, regulating the polyclonal activation of the B cells and plasma cells in the periodontitis-affected sites. The interaction between host immune system and periodontopathogens is relevant to the pathogenesis of periodontitis (11).

Some authors have also suggested that the presence of herpesviruses in the periodontal sites could play a role on the pathogenesis of human periodontitis (12–14). Herpesviruses are ubiquitous and after primary infection can persist latently in the host in several types of cells, including cells of the immune system. The cytomegalovirus (CMV) is the most-studied member of the betaherpesviruses in periodontal sites. Recently, the remaining two betaherpesviruses, known as human herpesvirus 6 (HHV-6) and human herpesvirus 7 (HHV-7), have been investigated because these viruses are frequently detected in saliva (12–15). Betaherpesviruses have also been studied after organ transplantation, and some authors have suggested that viruses can act directly or indirectly by modulating immune mechanisms and influencing the immune response due to viral replication in lymphocytes and monocytes/macrophages (16–19).

Inflammatory cells harboring herpesviruses present at sites of periodontal inflammation could contribute to the development and course of periodontitis (20–22). Cytomegalovirus can induce direct cytopathic effects on fibroblasts, keratinocytes, endothelial cells and inflammatory cells, including polymorphonuclear leukocytes, T lymphocytes and macrophages, and can also possibly affect bone cells. In periodontitis patients, T cells are activated and specific lymphocyte responses are driven by the nature of the initial antigenic stimuli. This process is supported by a complex cascade of events involving cytokines, chemokines and other inflammatory mediators, which could be altered due to CMV infection. Proinflammatory and anti-inflammatory balances controlled by different subsets of lymphocytes are thought to be crucial in the pathogenesis of periodontitis (23).

The expression of different cellular antigens can be dramatically altered in HHV-6-infected tissues, in which the viral infection can induce CD4 upregulation and CD3 downmodulation in the T cells. Human herpesvirus 6 can severely affect the physiology of secondary lymphoid organs through direct infection of T lymphocytes and modulation of key membrane receptors and chemokines (24). In addition, cytotoxicity and immunomodulatory effects caused by HHV-7 infection in T-CD4$^+$ lymphocytes *in vitro* and *in vivo* have been described as an adaptative mechanism to host immune system evasion (25).

Local immunomodulatory effects caused by herpesviral infection could facilitate bacterial proliferation and virulence or induce the release of cytokines and chemokines from inflammatory and connective tissue cells (22,26). In addition, viruses and bacteria could act in synergy to produce pathology.

As the CD19$^+$ B lymphocytes, CD3$^+$ T lymphocytes and the helper (T-CD4$^+$) and cytotoxic (T-CD8$^+$) subsets are involved in the pathogenesis of periodontitis and these cells can be infected by herpesviruses, studies to evaluate the association of herpesviruses with immune cell subpopulations in the inflammatory infiltrate could contribute by clarifying the interaction between viruses and the immune system in the periodontal sites. However, few studies have reported any association of the presence of herpesviruses with profiles of inflammatory infiltrates (20,27).

The aim of this study was to determine the prevalence of CMV, HHV-6 and HHV-7 in biopsies obtained from periodontitis-affected patients and periodontally healthy subjects (control group). In addition, immunophenotyping to determinate CD19$^+$ B cells, CD3$^+$ total T cells and the T-CD4$^+$ and T-CD8$^+$ cell subsets was carried out to evaluate which profile of the inflammatory infiltrate was more frequently associated with the presence of CMV, HHV-6 and HHV-7.

Material and methods

Subjects

Twenty chronic periodontitis-affected patients were included in this study. Patients who presented with chronic periodontitis based on clinical and radiographic findings (28) were eligible to participate in this study. All patients were systemically healthy and had not received periodontal treatment or antibiotics for at least 6 mo prior to the clinical examination and sampling.

Each patient contributed one biopsy, collected from a clinically affected site involving epithelium and connective tissue facing the sulcus and periodontal pocket (13). In 12 healthy subjects (healthy controls), tissue samples were harvested from periodontally healthy sites. All healthy subjects presented probing depth ≤ 3 mm, with no attachment loss or gingival bleeding on probing; gingival biopsies were obtained from the sulcular region during tooth extraction procedures. The demographic data of the patients and healthy subjects included in this study are shown in Table 1.

The tissue fragments were rinsed several times with sterile saline and divided into two parts; one part was fixed in 10% buffered formalin and the other was stored frozen (−20°C) in sterile flasks for DNA extraction.

Table 1. Demographic and clinical characteristics of the chronic periodontitis patients and control subjects included in this study

Patient group	Total (*n*)	Sex [male/female] *n* (%)	Age (years)		Average probing depth (mm)	Average clinical attachment loss (mm)
			Range	Median		
Chronic periodontitis	20	7/13 (35/75)	39–69	52	5.6	6.2
Control group	12	6/6 (50/50)	20–40	34	—	—

Formalin-fixed tissue fragments were destined for immunohistochemical analysis and histopathological examination. The DNA extraction was carried out using nonfixed tissue fragments on the same day as sampling. The protocol was designed in accordance with the requirements for research involving human subjects, was approved by the institutional Ethics Committee and complies with Declaration of Helsinki.

Extraction of DNA

Briefly, DNA was extracted from tissue fragments (finely minced) using a phenol–chloroform protocol after incubation overnight in lysis buffer (10 mM Tris–HCl pH 8.0, 10 mM EDTA, 10 mM NaCl, 0.2% dodecyl sodium sulfate and 100 μg proteinase K) at 56°C, followed by DNA precipitation with cold ethanol (13). The resulting DNA pellet was eluted in 50 μL of TE buffer (10 mM Tris and 1 mM EDTA) and stored frozen (−20°C) until PCR analysis.

PCR

Five microliters of DNA extracted following the protocol described in the previous subsection were used for the nested PCR using a mixture containing specific primers to CMV and employing a previously described technique (29,30). Primers and the protocol used for HHV-6 and HHV-7 nested PCR have also been described previously (31,32). The amplifications were performed using a Peltier Thermal Cycler (MJResearch, Waltham, MA, USA). Positive and negative controls for each virus were included systematically. Genome amplifications were carried out using the primers with DNA fragments containing 159, 258 and 122 bp of CMV, HHV-6 and HHV-7, respectively. The nested PCR product was analyzed under ultraviolet light after electrophoresis in 2% agarose and stained with ethidium bromide. All nested PCRs were performed in duplicate using a second fresh aliquot. A PCR with primers for beta-globin gene amplification in the DNA extracted from samples was performed to detect possible false-negative results, which were not included in this study (33).

Tissue preparation and antigen retrieval

The formalin-fixed fragments of tissue were dehydrated following standard histochemical protocols and then paraffin embedded. The blocks containing tissue fragments were sectioned with a microtome (6 μm), and sections were then transferred to silane-pretreated slides (Sigma-Aldrich, St Louis, MO, USA). The slides were dewaxed by immersion in xylol, rehydrated and washed with phosphate-buffered saline (PBS). The antigen retrieval was carried out by immersion and incubation of the slides in citrate buffer (0.01 mM sodium citrate, pH 6.0) at 96°C for 50 min in water-bath. The slides were washed in distilled water, PBS and immediately submitted to immunohistochemistry following the protocol described in the next subsection. A slide for each sample was also stained with Giemsa for histopathological examination.

Immunostaining procedure

The slide containing the tissue section was fixed with cold methanol–acetone (1:1) for 15 min and incubated with 1% bovine serum albumin in PBS for 15 min to avoid interference from nonspecific binding. The tissue section was covered with fluorescein isothiocyanate-conjugated mouse monoclonal antibodies (Biolegend, San Diego, CA, USA) against human CD19 (clone HIB19), CD3 (clone HIT3a), CD4 (clone RPA-T4) or CD8 (clone HIT8a) and incubated for 2 h at 37°C in the dark. The slides were washed three times with PBS and counterstained with 4′,6-diamidino-2-phenylindole (300 nM in PBS) for 5 min in the dark. The slides were washed several times with PBS, mounted and immediately observed under an ultraviolet photomicroscope (Leica DM2000, Wetzlar, Germany). The intensity of light and filter was adjusted to provide perfect visualization and contrast of the cells among tissues. Cells that presented a specific light green fluorescence were considered to be positive. The nuclei of cells presented a light blue fluorescence after 4′,6-diamidino-2-phenylindole staining.

Morphological and immunohistochemical analysis

Each sample was examined by light microscopy for determination of quality and intensity of infiltrate. The intensity of the infiltrate was graded according to the following scale: 0, absence of infiltration; 1, discrete infiltration; 2, moderate infiltration; and 3, intense infiltration.

The numbers of $CD19^+$ B cells, $CD3^+$ T cells and its T-$CD4^+$ and T-$CD8^+$ cell subsets were estimated by counting the total areas of the slide. The area of infiltration on the slide was focused under ultraviolet light at a magnification of ×400, and round positive cell (light green fluorescent cells) counts were performed. The total nuclei (stained light blue) counts were also performed in the same area of the slide. All counting procedures were performed in triplicate. The percentage of positive cells was calculated by dividing the number of positive cells by the total number of cells and multiplied by 100. The ratios of $CD19^+/CD3^+$ and $CD4^+/CD8^+$ were calculated by dividing the number of $CD19^+$ cells by the number of $CD3^+$ cells, and the

number of $CD4^+$ cells by the number of $CD8^+$ cells, respectively.

Statistical analysis

The statistical analysis of continuous variables was performed using Student's paired *t*-test, and categorical variables were analyzed by the chi-square test or Fisher's exact test. A *p*-value of ≤ 0.05 was considered to be statistically significant.

Results

Morphological and immunohistochemical analysis

As expected, none of the 12 samples collected from healthy subjects presented any significant level of infiltration and therefore these samples were excluded from immunohistochemical procedures. Among the 20 samples collected from the chronic periodontitis-affected sites, all presented chronic inflammatory infiltrates with scores ranging from 1 to 3. In the chronic periodontitis-affected tissues, the percentages of scores 1, 2 and 3 were 21.4, 21.4 and 57.2%, respectively.

Evaluation of the distribution of each marker showed that $CD19^+$ B cells, $CD3^+$ total T cells and its T-$CD4^+$ and T-$CD8^+$ cell subsets were frequently found in the infiltrate of chronic periodontitis sites, although in different amounts and ratios. In most cases (60%), $CD19^+$ B cells were predominant in the sample (CD19/CD3 ratio >1.1); however, the number of cells expressing CD19 was not significantly different from the number of cells expressing CD3 in the total number of the samples (Fig. 1A).

In nine of 20 (45%) patients, the T-$CD4^+$ cell subset were predominant in the sample (CD4/CD8 ratio >1.1). No sample presented any predominance of T-$CD8^+$ cells. The number of cells expressing CD4 was significantly higher than the number of cells expressing CD8 ($p = 0.004$) in the total number of samples (Fig. 1B).

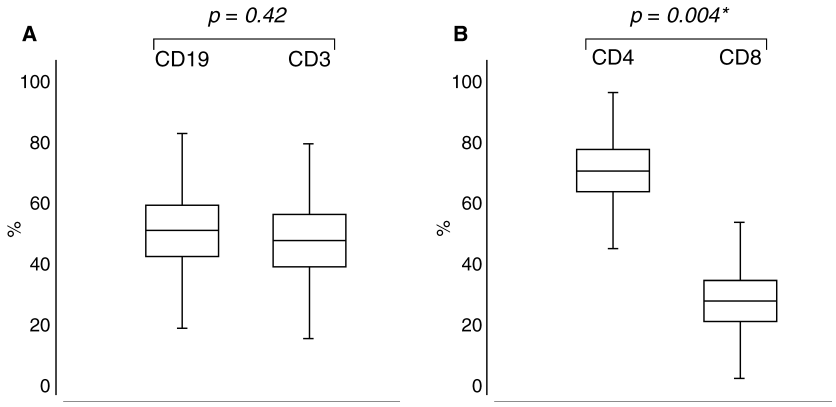

Fig. 1. (A) Relative frequencies of $CD19^+$ cells (B cells) and $CD3^+$ cells (T cells) in the overall samples obtained from chronic periodontitis patients. No significant difference was found between these subpopulations. (B) Relative frequencies of T-$CD4^+$ and T-$CD8^+$ subsets. Significant predominance of T-$CD4^+$ cells over T-$CD8^+$ cells in the samples (paired *t*-test). Whiskers indicate the standard deviation.

Table 2. Distribution of cytomegalovirus (CMV), human herpesvirus 6 (HHV-6) and human herpesvirus 7 (HHV-7) as detected by nested PCR in biopsies collected from periodontitis-affected sites and healthy subjects

	Affected sites [*n* (%)]	Healthy control [*n* (%)]	*p*-Value
CMV	6 of 20 (30)	0 of 12 (0)	0.04*
HHV-6	0 of 20 (0)	1 of 12 (5)	NS
HHV-7	6 of 20 (30)	0 of 12 (0)	0.04*

* Fischer's exact test. NS, not significant.

Nested PCR analysis for CMV, HHV-6 and HHV-7

Six samples collected from chronic periodontitis-affected sites (30%) presented positive nested PCR for CMV DNA detection. Cytomegalovirus nested PCR was negative in healthy subjects. Human herpesvirus-6 was not detected in any chronic periodontitis patients, while one sample collected from a healthy subject was positive for HHV-6. In chronic periodontitis-affected sites, HHV-7 was detected in six of 20 (30%) sites and was not detected in samples collected from healthy subjects. Cytomegalovirus and HHV-7 were significantly more frequent in chronic periodontitis-affected sites than in other sites ($p = 0.04$ and $p = 0.04$, respectively). Table 2 shows the frequency for each herpesvirus in each type of subject. Cytomegalovirus and HHV-7 were not correlated with the severity of cases nor with probing depth.

Cytomegalovirus, HHV-7 and immunophenotypic features of the infiltrate

Cytomegalovirus was most frequently found in samples when $CD3^+$ T cells were predominant in the sample (CD19/CD3 ratio <0.9, $p = 0.003$). No correlation was found between CMV and T-$CD4^+$ and/or T-$CD8^+$ cell subsets.

Human herpesvirus-7 was also most frequently found when $CD3^+$ T cells were predominant in the sample (CD19/CD3 ratio <0.9, $p = 0.003$). Moreover, HHV-7 was associated with predominance of T-$CD4^+$ in the samples (CD4/CD8 ratio >1.1, $p = 0.002$). Table 3 shows the frequency of detection of each virus in different profiles of CD19/CD3 and CD4/CD8 ratios found in the total number of samples studied.

Discussion

In the present study, tissues biopsied from periodontitis-affected patients

Table 3. Frequencies of each virus as detected by nested PCR and distributed by each profile of CD marker (ratios) on the samples analyzed

CD marker			CMV positive			HHV-7 positive		
	n	(%)	*n*	(%)	*p*-Value	*n*	(%)	*p*-Value
CD19/CD3 ratio								
0.9–1.1	4 of 20	(20)	2 of 4	(50)	0.34	2 of 4	50	0.34
> 1.1	12 of 20	(60)	0 of 12	(0)	NS	0 of 12	0	NS
< 0.9	4 of 20	(20)	4 of 4	(100)	0.003*	4 of 4	100	0.003*
CD4/CD8 ratio								
0.9–1.1	11 of 20	(55)	4 of 11	(36.3)	0.15	0 of 11	0	NS
> 1.1	9 of 20	(45)	2 of 9	(22.2)	0.66	6 of 9	100	0.002*
< 0.9	0 of 20	(0)	—	—	—	—	—	—

* Fischer's exact test. NS, not significant.

and from healthy subjects were analyzed to determinate the profile of the inflammatory infiltrate and the possible association between the profile and the presence of CMV, HHV-6 and HHV-7 in the samples.

The results found in this study demonstrated a discrete predominance of $CD19^+$ B cells in chronic periodontitis-affected sites, although $CD3^+$ T cells were also frequently found. The T-$CD4^+$ cell subset was significantly predominant compared with the T-$CD8^+$ cell subset. Nakajima *et al*. (34) found a dominance of $CD19^+$ B cells over $CD3^+$ T cells and a similar dominance of the T-$CD4^+$ subset in samples collected from chronic periodontitis patients. Yamazaki *et al*. (11) also found a dominance of $CD19^+$ B cells, although $CD3^+$ T cells were found in significant numbers and, similar to the present study, T-$CD4^+$ cells were present in higher numbers than T-$CD8^+$ cells. In contrast, Cardoso *et al*. (10) found a predominance of $CD3^+$ T cells and no significant difference between T-$CD4^+$ and T-$CD8^+$ cell subsets; however, several lymphocyte subpopulations were present in the majority of cases, although the ratio of $CD19^+$ B cells, total $CD3^+$ T cells and T cell subsets may be variable.

Several studies have demonstrated a significant role of T and B lymphocytes in the progression of chronic periodontitis. Conversion of a stable T cell lesion to a progressive B cell lesion has been hypothesized as being associated with the physiopathology of chronic periodontitis. Also, the conversion of gingivitis to periodontitis involving different phenotypes of T cell infiltration has been studied (11).

The present study revealed that B cells and T cells, especially the T-$CD4^+$ cell subset, were an important component of the chronic inflammatory infiltration in chronic periodontitis patients. Data reported by Lima *et al*. (35), regarding T-$CD4^+$ cells in chronic and aggressive periodontitis, showed that T-$CD4^+$ cells from chronic periodontitis patients are more committed to interleukin-4 production and the T-$CD4^+$ cells from aggressive periodontitis patients are more committed to tumor necrosis factor-α production. This signifies that the cytokine pattern may be different between different clinical forms of the disease. T cells can be a common reservoir of betaherpesviruses, and the present study reports that T cells are present in significant numbers in the infiltrate of chronic periododontitis sites and that cells may be harboring herpesviruses.

In the present study, CMV and HHV-7 were significantly more frequent in periodontitis-affected sites than in samples from healthy subject; however, HHV-6 was not frequently found in the periodontal tissue. Some authors have reported the presence of CMV DNA in periodontitis-affected patients. In a Brazilian population, Casarin *et al*. (36) found 37–39.1% positivity for CMV in type 2 diabetic patients, and Imbronito *et al*. (37) described 50% positivity in nondiabetic subjects. Chalabi *et al*. (38) described the presence of viral CMV DNA in 59% of patients, but only when probing depth was ≥6 mm. In the present study, we did not find any correlation between probing depth and the presence of CMV. Bilichodmath *et al*. (2) found a 26.3% level of CMV DNA in chronic periodontitis-affected sites. Grenier *et al*. (4) and Watanabe *et al*. (39) found lower frequencies of 11 and 6% of CMV DNA, respectively.

Few studies in the literature have reported the presence of HHV-6 and HHV-7 DNA in periodontitis-affected patients. Cassai *et al*. (13) did not find HHV-6 DNA in affected sites, and found HHV-6 DNA in 10% of tissue biopsied from healthy subjects, demonstrating reasonably similar results to this study. Contreras *et al*. (12) described 21% positivity for HHV-6 DNA in affected sites, with no detection in nonaffected sites by nested PCR. Contreras *et al*. (15) detected HHV-6 DNA in 21% of periodontitis-affected patients, but did not include nonaffected control samples or periodontally healthy subjects. In the present study, only one sample collected from a healthy subject was positive for HHV-6 DNA. It is important to mention that our protocols were combined with a PCR protocol for beta-globin gene amplification as an internal quality control for DNA extraction and the presence of inhibitors (31,33).

In the present study, HHV-7 DNA was detected in 30% of the periodontitis-affected sites and was not detected in healthy subjects. Contreras *et al*. (12) reported 43% positivity for HHV-7 by PCR in periodontitis patients and did not detect HHV-7 DNA in periodontally healthy subjects. Another study from the same authors (15) reported that 29% of periodontitis patients were positive for HHV-7, which is similar to the present study. In contrast, Cassai *et al*. (13) found a high prevalence of HHV-7 DNA in both periodontitis-affected and healthy subjects (77 and 70%, respectively).

Naturally, to compare studies many types of different factors must be considered, such as the type and number of samples, sensitivity of primers used for PCR, presence of systemic disease, immunosenescence, ethnic origin and whether the patient presents with

aggressive or chronic periodontitis. In addition, apical and marginal periodontitis should be considered separately from each other. However, with regard to the differences between studies, our present data confirm those reported in other studies.

Although the presence of betaherpesviruses related to the profile of the inflammatory infiltrate in periodontitis-affected sites has been documented (20,27), there are few reports in the literature concerning this association until the present study.

Association of the herpesviruses with the immune cell subpopulation could help our understanding of the involvement of these viruses with human periodontitis. Contreras *et al.* (20) found that, in 20% of biopsies obtained from periodontitis-affected sites, $CD3^+$ T cells harbored CMV, and $CD3^+$ T cells from healthy gingiva did not contain this virus. Sabeti *et al.* (27) also found CMV in $CD3^+$ T cells of 54% of periapical lesions. Although, hypothetically, $T\text{-}CD4^+$ cells are the main target for HHV-7 infection in the chronic periodontitis inflammatory infiltrate, there is a lack of data available to support this hypothesis.

Herein, we have focused on the presence of betaherpesviruses in association with the phenotype and number of each lymphocyte subpopulation in the inflammatory infiltrate. In the present study, CMV correlated with samples that presented a predominance of $CD3^+$ T cells, while HHV-7 correlated with samples in which the $T\text{-}CD4^+$ cell subset was predominant. Although associations were observed between CMV and $CD3^+$ T cells and between HHV-7 with the $T\text{-}CD4^+$ predominance in the samples, the viruses could act as cofactors, modulating the activity of these cells. The release of different types of cytokines and B lymphocyte activation orchestrated by T lymphocytes has been shown to be an important feature in the chronic periodontitis process (10,11). In addition, T-regs cells (T regulatory $CD4^+CD25^+$) may be involved in modulation of the local immune response in chronic periodontitis patients (10). Cells infected by viruses suffer complex changes, leading to the up- or downregulation of different factors that could contribute to the pathogenesis of the inflammatory process.

Cytomegalovirus has the potential to suppress the function of antigen-specific T cytotoxic cells and can cause a reduction in the number of $T\text{-}CD8^+$ cells, leading to decreased cell-mediated immune function. Immunossupressive effects that involve direct impairment of lymphocytes, inducing the production of auto-antibodies and inflammatory mediators, have been described (20). In the present study, we failed to find a correlation between specific T lymphocyte subsets with CMV, possibly due to the fact that this virus can infect types of cells such as monocytes/macrophages, dendritic cells, epithelial cells, fibroblasts and endothelial cells (9) which may be present in the periodontal tissue.

Human herpesvirus 7 infects most specifically the $T\text{-}CD4^+$ cells, which can result in cytotoxicity and immunomodulatory activities in the host (25). It has also been demonstrated that the downmodulation of human leukocyte antigen (HLA) and β2-microglobulin expression by HHV-7 is linked to viral replication and is not merely the consequence of the interaction of virions with the cell surface. Infected cells can therefore escape efficiently from host immune pressure, which might explain the persistence of HHV-7-positive cells in several types of tumors and chronic infectious diseases (40).

Although HHV-7 has restricted tropism to $CD4^+$ cells, it should be noted that HHV-7-infected $T\text{-}CD4^+$ cells kill uninfected $T\text{-}CD8^+$ cells *in vitro*. Moreover, HLA class I and β2-microglobulin are also downmodulated in the $T\text{-}CD8^+$ cells in the presence of HHV-7-infected leukocytes *in vitro* (25). In the present study, we found lower numbers of $T\text{-}CD8^+$ cells compared with $T\text{-}CD4^+$ cells in the samples, and this could be the result of CMV and/or HHV-7 infections in the host cells.

A 'herpesvirus–bacterial pathogen' model has been proposed, in which the herpesviral infection could facilitate bacterial proliferation and virulence or induce the release of cytokines and chemokines from inflammatory and connective cells (6,22).

Cytopathogenic effects, immune evasion, immunopathogenicity, latency, reactivation and cell tropism are important aspects related to herpesviruses, with regard to periodontitis. Herpesviral infection causes a release of proinflammatory cytokines, which can activate T lymphocytes and osteoclasts (41). As the $CD3^+$ T cells and $T\text{-}CD4^+$ lymphocyte subpopulation could harbor CMV and/or HHV-7, these viruses could play a role in the pathogenesis of chronic periodontitis. Immunohistochemical methods or flow cytometry could be performed to help to confirm herpesviral infections and co-localization of viral antigens and CD markers. These methods are under investigation in our center.

In conclusion, this study shows that CMV and HHV-7 can be present at periodontitis-affected sites but are uncommon at healthy periodontal sites. Moreover, our data suggest that CMV can be related to an inflammatory infiltrate with predominance of $CD3^+$ T cells, while HHV-7 can be associated with an infiltrate with predominance of $T\text{-}CD4^+$ cells; however, further studies are necessary to support this hypothesis. Herpesviruses could play a role in human chronic periodontitis by modulation of the T cell response.

Acknowledgements

We thank the State University of Campinas for the use of the laboratory facilities, and Ms Renata Barbieri and Dr Grasiela D. C. S. Aguiar of the laboratory of Micromorphology of Herminio Ometto Foundation, UNIARARAS, and Dr Ana Veiga from State University of Campinas, UNICAMP, for technical assistance. We also thank Dr Norma G. S. Mota for a very useful discussion.

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