Correlation between Infections with Different Genotypes of Human Cytomegalovirus and Epstein-Barr Virus in Subgingival Samples and Periodontal Status of Patients

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Periodontal diseases are multifactorial infections elicited by a complex community of bacterial species that interact with host tissues and cells, causing the release of a broad array of inflammatory cytokines, chemokines, and mediators, some of which lead to the destruction of the periodontal structures, including the alveolar bone, periodontal ligament, and cementum around a tooth (34). Even though specific infectious agents are of key importance in the development of periodontitis, it is unlikely that a single agent or even a small group of pathogens are the sole cause or modulator of this heterogeneous disease.

Since the mid-1990s, herpesviruses have emerged as putative pathogens in various types of periodontal diseases. The present study was performed to examine infections with different genotypes of human cytomegalovirus (HCMV) and Epstein-Barr virus (EBV) in subgingival samples from a Chinese population and to analyze the correlation with periodontal status. A nested PCR assay was used to identify the presence of HCMV, EBV type 1 (EBV-1), and EBV-2; and the amplicons were further analyzed by restriction fragment length polymorphism analysis. HCMV was detected in 79.0% of 143 chronic periodontitis (CP) patients, 78.5% of 65 gingivitis patients, and 76.3% of 76 periodontally healthy individuals, while EBV was found in 63.6%, 32.3%, and 30.3% of the three groups of subjects, respectively. The HCMV-positive PCR products from all the samples were identified as corresponding to gB genotype I (gB-I) or gB-II. HCMV gB-II (62.9%), EBV-1 (43.4%), and EBV-2 (18.2%) were associated with CP at higher frequencies (P < 0.05), whereas HCMV gB-I was more often observed in gingivitis patients (40.0%) and healthy individuals (40.8%) (P < 0.05). Furthermore, a higher rate of coinfection with HCMV and EBV was shown in CP patients (52.4%), especially dual infections with HCMV gB-II and EBV-1 (30.8%) or HCMV gB-II and EBV-2 (12.6%), compared with the rates of single infections with HCMV or EBV (P < 0.05). Infection with HCMV gB-II, EBV-1, or EBV-2 was correlated with higher rates of bleeding on probing (P < 0.05). In patients infected with HCMV gB-II or both HCMV and EBV, including HCMV gB-II and EBV-1, a deeper probing depth or more serious attachment loss was found (P < 0.05). These findings clearly indicate that HCMV gB-II is the dominant genotype detected in subgingival samples in CP. HCMV gB-II infection and HCMV gB-II coinfection with EBV-1 are closely associated with periodontal tissue inflammation and destruction.

Accumulating evidence indicates that herpesviruses may be putative pathogens in various types of periodontal diseases. The present study was performed to examine infections with different genotypes of human cytomegalovirus (HCMV) and Epstein-Barr virus (EBV) in subgingival samples from a Chinese population and to analyze the correlation with periodontal status. A nested PCR assay was used to identify the presence of HCMV, EBV type 1 (EBV-1), and EBV-2; and the amplicons were further analyzed by restriction fragment length polymorphism analysis. HCMV was detected in 79.0% of 143 chronic periodontitis (CP) patients, 78.5% of 65 gingivitis patients, and 76.3% of 76 periodontally healthy individuals, while EBV was found in 63.6%, 32.3%, and 30.3% of the three groups of subjects, respectively. The HCMV-positive PCR products from all the samples were identified as corresponding to gB genotype I (gB-I) or gB-II. HCMV gB-II (62.9%), EBV-1 (43.4%), and EBV-2 (18.2%) were associated with CP at higher frequencies (P < 0.05), whereas HCMV gB-I was more often observed in gingivitis patients (40.0%) and healthy individuals (40.8%) (P < 0.05). Furthermore, a higher rate of coinfection with HCMV and EBV was shown in CP patients (52.4%), especially dual infections with HCMV gB-II and EBV-1 (30.8%) or HCMV gB-II and EBV-2 (12.6%), compared with the rates of single infections with HCMV or EBV (P < 0.05). Infection with HCMV gB-II, EBV-1, or EBV-2 was correlated with higher rates of bleeding on probing (P < 0.05). In patients infected with HCMV gB-II or both HCMV and EBV, including HCMV gB-II and EBV-1, a deeper probing depth or more serious attachment loss was found (P < 0.05). These findings clearly indicate that HCMV gB-II is the dominant genotype detected in subgingival samples in CP. HCMV gB-II infection and HCMV gB-II coinfection with EBV-1 are closely associated with periodontal tissue inflammation and destruction.

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Periodontal diseases are multifactorial infections elicited by a complex community of bacterial species that interact with host tissues and cells, causing the release of a broad array of inflammatory cytokines, chemokines, and mediators, some of which lead to the destruction of the periodontal structures, including the alveolar bone, periodontal ligament, and cementum around a tooth (34). Even though specific infectious agents are of key importance in the development of periodontitis, it is unlikely that a single agent or even a small group of pathogens are the sole cause or modulator of this heterogeneous disease.

Since the mid-1990s, herpesviruses have emerged as putative pathogens in various types of periodontal diseases (13, 15, 18). In particular, human cytomegalovirus (HCMV) and Epstein-Barr virus (EBV) seem to play important roles in the etiopathogenesis of severe types of periodontitis. The genomes of the two herpesviruses are present at a high frequency in adults with progressive periodontitis and patients with localized and generalized aggressive (juvenile) periodontitis, human immuno-
glycoprotein in the outer membrane of HCMV, the virus could arbitrarily be divided into four genotypes (gB genotype I [gB-I] to gB-IV) (3). HCMV gB-I does not infect T lymphocytes, whereas types II and III do (22). Infection with gB-II correlates with retinitis in AIDS patients, but infection with type I does not (5). In Japanese bone marrow transplant recipients, the predominant infecting gB genotypes were I, II, and III; but no cases of type IV infection were found (38). HCMV gB-I was common in an Italian population (4), while gB-II was more prevalent in India (21).

EBV, a double-stranded DNA virus, is one of the eight human herpesviruses (human herpesvirus 4). It is transmitted by salivary contact and establishes a lifelong latent infection that is usually asymptomatic (26). Two types of EBV exist, based on the allelic polymorphisms in the latent gene sequences encoding EBV nuclear antigen 2 (EBNA2) (24). It is frequently detected in blood samples from healthy individuals, and most EBV serum-positive healthy Caucasians are infected with EBV type 1 (EBV-1), while immunosuppressed individuals (HIV-infected and transplant patients) have a high rate of infection with EBV-2 (8, 14). An in vitro study showed that EBV-2 causes more lysis of B lymphocytes than EBV-1 (6).

Therefore, it is critical to identify the predominant genotypes of HCMV and EBV present in subgingival samples to further elucidate their roles in the etiology of periodontitis.

The purpose of this study was to investigate the frequency of subgingival infection with different genotypes of HCMV and EBV in Chinese patients with periodontal disease. A nested PCR method was established to detect the gB gene of HCMV and the EBNA2 gene of EBV in subgingival plaque samples from 143 chronic periodontitis (CP) patients and in 63 gingivitis patients and 76 healthy individuals with healthy periodontia. The amplicons were further characterized by restriction endonuclease digestion, and the relationship between infection with different genotypes of HCMV and EBV in subgingival samples and clinical parameters was analyzed.

MATERIALS AND METHODS

Subjects. The subjects in this study were 143 Chinese patients with CP (64 males aged 20 to 65 years [mean age, 41.7 ± 7.9 years] and 79 females aged 21 to 68 years [mean age, 42.5 ± 6.9 years]); 65 gingivitis patients (30 males aged 17 to 58 years [mean age, 38.2 ± 7.9 years] and 35 females aged 18 to 60 years [mean age, 37.1 ± 5.4 years]); and 76 individuals with healthy periodontia (35 males aged 22 to 64 years [mean age, 38.8 ± 7.1 years] and 41 females aged 21 to 63 years [mean age, 37.5 ± 6.9 years]) who were referred to the dental clinic in the Second Affiliated Hospital of the School of Medicine of Zhejiang University for dental or periodontal treatment or health monitoring. All the subjects were nonsmokers without any systemic disease and with at least 14 teeth remaining. Those who had received a professional cleaning or who had a history of antibiotic therapy during the preceding 3 months were excluded. All of the patients and the healthy individuals underwent a full-mouth examination. The criteria for the diagnosis of CP and gingivitis were based on the Classification of the Periodontal Healthy Individuals underwent a full-mouth examination. The criteria for the therapy during the preceding 3 months were excluded. All of the patients and the nonsmokers without any systemic disease and with at least 14 teeth remaining.

Second Affiliated Hospital of the School of Medicine of Zhejiang University for approved by the Ethics Committee of Zhejiang University.

Viral nucleic acid extraction. The specimens resuspended in 200 µl of TE buffer were vigorously mixed in a vortex mixer. The nucleic acid extraction technique was based on preferential binding to silica particles in the presence of a high concentration of guanidium thiocyanate (GuSCN) (25). Briefly, 200 µl of sample was mixed with 50 µl of silica particles (Sigma Chemical Co., St. Louis, MO) in 800 µl of lysis buffer (120 g of GuSCN; 100 ml of 0.1 mM Tris-HCl, pH 8.4; 22 ml of 0.2 M EDTA, pH 8.0; 2.6 g of Triton X-100), mixed in a vortex mixer for 10 s, and kept at room temperature for 10 min. The nucleic acid-silica complexes were recovered by centrifugation at 12,000 × g for 1 min and washed twice in buffer (GuSCN-Tris-HCl), twice in 70% ethanol, and once in acetone. The sample was then dried at 37°C. The nucleic acid pellet was resuspended in 100 µl of TE buffer containing 0.5 U/ml RNasin (Promega), and the DNA/RNA was separated from the silica particles by incubation at 56°C for 10 min. After centrifugation at 12,000 × g for 2 min, the supernatant was stored at −70°C.

PCR procedures. A nested PCR method was used to detect the DNA of HCMV (EBV-1, and EBV-2). The primers were designed for specificities and no cross-reactivity with the human genome, other viruses, or various microorganisms by other researchers (7, 13, 23, 25, 38). The sequences of the HCMV-specific outer primers were 5′ GGCT ATC AAC GAA AAA AAT CTG CTA C3′ (forward primer) and 5′-CAG TTG ACA AAT CTA CGA-3′ (reverse primer). The inner primers for HCMV were 5′-TGG AAC TGG AGT GGT TGG C3′ (forward) and 5′-GAA ACG CCG GAC AAT CGG-3′ (reverse primer). The sequences of the EBV-specific outer primers were 5′-AGG GAVGG GCG GGA AAC CAC AAG A3′ (forward primer) and 5′-TGG TGC TGC TGG TGG TCAAT C3′ (reverse primer). The inner primers for EBV-1 were 5′-CCT TGA TAG GGA CCT AGG GTA ATA-3′ (forward primer) and 5′-ACC GTG GTT CTG GAC TAT CTG GAT C3′ (reverse primer). The inner primers for EBV-2 were 5′-CAT GGG ATC CCT ACG ACA ATA-3′ (forward primer) and 5′-AGA CTI AGT AGT TGC TGC CCT AGT-3′ (reverse primer).

The first round of the PCR was carried out in a mixture with a total volume of 50 µl that included 5 µl of the template, 1 × PCR buffer (pH 8.3) and 2.5 U Ex-Taq DNA polymerase (TaKaRa), 0.2 mM each deoxynucleoside triphosphate, 1.5 mM MgCl2, and 1 µM outer primers. PCR amplification, which was performed in a Perkin-Elmer 2400 DNA cycler, included an initial denaturation step at 94°C for 5 min, followed by 30 cycles of denaturation at 94°C for 30 s, annealing at 56°C for 1 min, and extension at 72°C for 1 min. A second round of amplification was performed in a new tube with 2 µl of the first-round PCR product; 25 pmol of inner primers; and PCR buffer, Ex-Taq DNA polymerase, deoxynucleoside triphosphates, and MgCl2 at the concentrations described above. The program for the second PCR was 35 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 1.5 min, with a final extension step at 72°C for 7 min.

Positive controls included purified DNA from HCMV (Sigma) and Raji cells containing EBV-1 (American Type Culture Collection, Manassas, VA). For tests for sensitivity, 20 ng/µl of the HCMV or EBV-1 DNA was serially diluted 10-fold, and 5 µl of each dilution containing 10−6 ng to 100 ng DNA was used as the template in the PCR assay. Amplicons were detected by electrophoresis of 10 µl of sample in a 1.5% agarose gel containing 1 µg/ml of ethidium bromide. The expected sizes of the target fragments from HCMV, EBV-1 and EBV-2 were 299 to 305 bp, 497 bp, and 165 bp, respectively. The PCR assay was repeated once for each clinical sample.

Restriction endonuclease digestion analysis. The amplicons were further identified by restriction fragment length polymorphism analysis (RFLP). The HCMV gB gene fragments were digested with endonucleases Rsal and Hind I (TaKaRa). After Rsal digestion, the HCMV products were cleaved into two fragments (gB-I, 239 and 66 bp; gB-II, 239 and 63 bp) or three fragments (gB-III, 195, 63, and 41 bp) (gB-IV, 195, 66, and 44 bp). The HCMV products were separated into another two fragments (gB-II, 202 and 100 bp; gB-III, 202 and 97 bp) or three fragments (gB-I and gB-IV, 202, 67, and 36 bp) with endonuclease HindI. For further identification of EBV, Afla I was used to digest the 497-bp amplicon of EBV-1 into 355- and 142-bp fragments, while Stul was used to turn the 165-bp

Sample collection. Each CP patient contributed four subgingival samples from the four deepest periodontal pockets of the dentition, preferably one pocket from each quadrant. Each of the gingivitis patients and periodontally healthy individuals provided four subcultural samples from the mesial or distal buccal site of a tooth in each quadrant, and usually these four teeth included at least the two first molars. Prior to subgingival sampling, the supragingival plaque was removed with sterile cotton rolls. For all the subjects, three sterile endodontic paper points were placed into the depth of each site for 30 s and were then transferred to a microcentrifuge tube containing 200 µl TE buffer (10 mM Tris-HCl, 1.0 mM EDTA, pH 8.0) and stored at −70°C. The full-mouth clinical parameters recorded included bleeding on probing (BOP), probing pocket depth (PD), and attachment loss (AL) at six sites per tooth.
amplicon of EBV-2 into 118- and 47-bp fragments. The restriction enzyme digests were resolved in an 2.5% agarose gel containing 1 μg/ml of ethidium bromide.

Statistical analysis. The chi-square test and Fisher’s exact test were used to compare the rates of HCMV and EBV positivity in patients and periodontally healthy individuals. Odds ratios (OR) and 95% confidence intervals were calculated to determine whether there was a significant association between the occurrence of HCMV and EBV with periodontal status. An association between the frequencies of infection with HCMV or EBV and clinical parameters was examined by the Kruskal-Wallis test with Stata 8.0 software. A P value equal to or less than 0.05 was considered statistically significant.

RESULTS

Sensitivity and specificity of nested PCR assay. By using 10−6 ng to 100 ng HCMV or EBV-1 DNA as the template in the PCR assay, the nested PCR could detect as little as 0.01 ng of HCMV or EBV-1 DNA (Fig. 1). Repeated PCR results for EBV-1 and EBV-2 were identical for all the clinical samples. All the EBV-1 products could be digested into 355- and 142-bp fragments by AfaI, and Stul could digest all the EBV-2 amplicons into 118- and 47-bp fragments. For 95% of the samples, the results of the two PCR experiments for HCMV were consistent. For all the samples, RFLP analysis indicated that Rsal could cleave the target HCMV amplicons into two 239- and 66-bp fragments by comparison with the 100-bp marker. After Hinfl digestion, gB-I was cleaved into three fragments, while gB-II gave two fragments of the expected sizes (Fig. 2). All the HCMV gB gene-positive samples were type I or II. No HCMV gB type III or IV products were found.

Frequency of infection with HCMV and EBV in subgingival plaque samples from patients and healthy individuals. HCMV was detected in 79.0% of the CP patients, 78.5% of the gingivitis patients, and 76.3% of the periodontally healthy individuals, while EBV was detected in 63.6%, 32.3%, and 30.3% of the three groups of subjects, respectively (Table 1). The distributions of HCMV in these three groups were similar (χ2 = 0.01 to 0.21; P = 0.091 to 0.922). A majority of HCMV gB-II isolates (62.9%) were detected in CP patients, while the frequency of HCMV gB-I was much lower in CP patients than in gingivitis or periodontally healthy individuals (χ2 = 15.22 to 27.46; P = 0.000). EBV or EBV-1 was more frequently detected in CP patients than in gingivitis or periodontally healthy individuals (χ2 = 5.21 to 22.15; P = 0.000 to 0.022). The rate of EBV-2 detection was also higher in CP patients than in healthy individuals (P = 0.001), but the difference was not statistically significant for the frequency of EBV or of EBV-1 or EBV-2 between gingivitis patients and healthy individuals (P = 0.794, 0.685, 0.248) or for the frequency of EBV-2 between CP and gingivitis patients (P = 0.059).

As seen in Table 2, the rate of dual infection with HCMV and EBV was higher in CP patients than in gingivitis patients and individuals with healthy periodontia (P = 0.000), while in gingivitis patients and healthy individuals, infection with either HCMV or EBV predominated (P = 0.000). Among the CP patients, infection with HCMV gB-II and EBV-1 was most prevalent, followed by infection with HCMV gB-II and EBV-2 (P = 0.000 to 0.002).

A significant association between infection with specific genotypes of HCMV and EBV and the periodontal status was demonstrated (Table 3). HCMV gB-II was associated with CP (OR = 3.679), while HCMV gB-I was closely related to gingivitis (OR = 5.688) and a healthy periodontium (OR = 5.879). EBV-1 and EBV-2 were both found to be associated with CP (ORs = 2.000 and 8.222, respectively). In contrast to a single infection with HCMV or EBV, a higher rate of coinfection with HCMV and EBV was correlated with CP (OR = 10.938), especially dual infection with HCMV gB-II and EBV-1 (OR = 28.875) or HCMV gB-II and EBV-2 (OR = 8.250).

Association between infection with different genotypes of HCMV and EBV and clinical parameters. Table 4 describes the correlation between infection or coinfection with different genotypes of HCMV and EBV in CP patients with the mean values for BOP, PD, and AL in the mouth. Compared with CP patients who were negative for both HCMV and EBV, a higher percentage of BOP was observed in patients infected with HCMV gB-II, EBV-1, or EBV-2 or patients coinfected with HCMV gB-II and EBV-1, HCMV gB-II and EBV-2, or HCMV gB-I and EBV-1 (P < 0.05). Moreover, in patients infected with HCMV gB-II or...
both HCMV and EBV (especially with HCMV gB-II and EBV-1), a deeper probing depth or more serious attachment loss was found compared with the probing depths and attachment loss for patients who were not infected with either of the two viruses \( (P < 0.05) \).

**DISCUSSION**

In this study, a nested PCR-RFLP method was designed to detect gB-I to gB-IV of HCMV and EBV-1 and EBV-2 in subgingival samples with a detection limit of 0.01 ng of HCMV and EBV DNA template. The method provided a sensitivity sufficient to allow meaningful data to be obtained from a small volume of specimen removed from periodontal sites. Restriction endonuclease digestion analysis also confirmed the specificity of the method. Moreover, repeating the PCR analysis also showed that the method had a high degree of reproducibility.

Herpesviruses represent some of the most successful viruses in humans, infecting over 90% of humans and persisting for the lifetime of the individuals (8, 26). Of particular interest in the present study with a homogeneous demographic population, we found that the HCMV gB genotypes are distributed differently among subgingival samples from patients with different periodontal status. Four types of HCMV gB genes are known (3, 4), but only two types were detected in subgingival samples from the Chinese individuals. To determine if there are other types of HCMV gB genes in subgingival plaque samples, we expanded our research by recruiting more subjects. A higher frequency of HCMV gB-II was shown in CP patients, whereas HCMV gB-I was more often detected in gingivitis patients and periodontally healthy individuals. Statistical analysis revealed a significant relationship between HCMV gB-II infection and CP. In contrast, HCMV gB-I was found in patients with gingivitis and individuals with healthy periodontitis. This study thus represents the first description of the dominant genotypes of HCMV in subgingival samples from Chinese CP patients.

The rate of positivity for EBV-1 was higher in CP patients than gingivitis patients and periodontally healthy individuals, suggesting that EBV-1 infection is associated with the pathogenesis of CP (10, 19). In this study, we demonstrated that infection with either EBV-1 and EBV-2 correlates with CP. The relationship between EBV-2 infection and periodontitis still remains to be established. EBV-2 could be detected in gingival biopsy specimens from HIV-positive periodontitis patients but was not detectable in HIV-negative periodontitis patients (14). Other reports indicate that EBV-2 might be present in subgingival samples from HIV-negative periodontitis patients.

**TABLE 1. Distribution of the HCMV gB and EBV EBNA2 genotypes in subgingival samples**

<table>
<thead>
<tr>
<th>Group</th>
<th>Total No. of cases</th>
<th>HCMV/EBV positive</th>
<th>gB genotype</th>
<th>EBNA2 genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Type I</td>
<td>Type II</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>EBV-1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>EBV-1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>EBV-1 and EBV-2 mix</td>
</tr>
<tr>
<td>CP Patients</td>
<td>143</td>
<td>113/91</td>
<td>15(a)</td>
<td>90(b)</td>
</tr>
<tr>
<td>CP Samples</td>
<td>520</td>
<td>330/196</td>
<td>47(c)</td>
<td>283(d)</td>
</tr>
<tr>
<td>Gingivitis</td>
<td>65</td>
<td>51/21</td>
<td>26</td>
<td>22</td>
</tr>
<tr>
<td>Gingivitis Samples</td>
<td>260</td>
<td>128/52</td>
<td>69</td>
<td>59</td>
</tr>
<tr>
<td>Healthy Individuals</td>
<td>76</td>
<td>58/23</td>
<td>31</td>
<td>24</td>
</tr>
<tr>
<td>Healthy Samples</td>
<td>304</td>
<td>129/65</td>
<td>71</td>
<td>58</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(a P < 0.01\) compared with the results for gingivitis or periodontally healthy samples or patients.

\(b P < 0.05\) compared with the results for healthy periodontal samples.

**TABLE 2. Coinfection with different genotypes of HCMV and EBV in subjects**

<table>
<thead>
<tr>
<th>Virus</th>
<th>No. (%) of patients</th>
<th>CP</th>
<th>Gingivitis</th>
<th>Healthy</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCMV and EBV</td>
<td>75 (52.4)(a)</td>
<td>14</td>
<td>21.5</td>
<td>9 (11.8)</td>
</tr>
<tr>
<td>HCMV gB-II and EBV-1</td>
<td>44 (30.8)(a)</td>
<td>3</td>
<td>4.6</td>
<td>2 (2.2)</td>
</tr>
<tr>
<td>HCMV gB-II and EBV-2</td>
<td>18 (12.6)(a)</td>
<td>2</td>
<td>3.1</td>
<td>0</td>
</tr>
<tr>
<td>HCMV gB-I and EBV-1</td>
<td>9 (6.3)</td>
<td>7</td>
<td>10.8</td>
<td>4 (5.3)</td>
</tr>
<tr>
<td>HCMV gB-I and EBV-2</td>
<td>2 (1.4)</td>
<td>1</td>
<td>1.5</td>
<td>0</td>
</tr>
<tr>
<td>HCMV gB-I, HCMV gB-II, and EBV-1</td>
<td>2 (7.0)</td>
<td>1 (1.5)</td>
<td>3 (3.9)</td>
<td></td>
</tr>
<tr>
<td>HCMV or EBV alone</td>
<td>48 (33.6)</td>
<td>44</td>
<td>67.7</td>
<td>63 (82.9)</td>
</tr>
</tbody>
</table>

\(a P < 0.01\) compared with the results for gingivitis or periodontally healthy individuals.

**TABLE 3. Relationship between presence of HCMV and EBV and periodontal status**

<table>
<thead>
<tr>
<th>Virus</th>
<th>Periodontal status</th>
<th>OR</th>
<th>95% confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCMV gB-II</td>
<td>CP(a)</td>
<td>3.679</td>
<td>1.96–6.96</td>
</tr>
<tr>
<td>HCMV gB-I</td>
<td>Gingivitis(a)</td>
<td>5.688</td>
<td>2.59–12.67</td>
</tr>
<tr>
<td>HCMV gB-I</td>
<td>Healthy(a)</td>
<td>5.879</td>
<td>2.76–12.76</td>
</tr>
<tr>
<td>EBV-1</td>
<td>CP(a)</td>
<td>2.000</td>
<td>1.06–3.86</td>
</tr>
<tr>
<td>EBV-2</td>
<td>CP(a)</td>
<td>8.222</td>
<td>1.95–73.04</td>
</tr>
<tr>
<td>HCMV and EBV</td>
<td>CP(a)</td>
<td>10.938</td>
<td>4.78–27.05</td>
</tr>
<tr>
<td>HCMV gB-II and EBV-1</td>
<td>CP(a)</td>
<td>28.875</td>
<td>6.82–253.27</td>
</tr>
<tr>
<td>HCMV gB-II and EBV-2</td>
<td>CP(a)</td>
<td>8.250</td>
<td>1.78–76.22</td>
</tr>
</tbody>
</table>

\(a\) Compared with individuals with healthy periodontia.

\(b\) Compared with CP patients.

\(c\) Compared with gingivitis patients.
It has been proposed that periodontal herpesvirus activation could destroy the normal periodontal defense barrier and impair the local immune system, which would promote subgingival colonization and the proliferation of periodontal bacterial pathogens, finally resulting in periodontal tissue destruction (16, 19, 33, 35). Analysis of the association between infection with different genotypes of HCMV and EBV and clinical parameters indicated that infection with HCMV gB-II, EBV-1, or EBV-2 or coinfection with HCMV gB-II and EBV-1, HCMV gB-II and EBV-2, or HCMV gB-I and EBV-1 correlated with BOP, while HCMV gB-II infection or coinfection with HCMV gB-II and EBV-1 was associated with a deeper probing depth or more serious attachment loss. The results further demonstrated that HCMV gB-II was the disease-contributing genotype in CP patients and that exposure to multiple genotypes of HCMV and EBV acts in concert in the pathogenesis of periodontitis. Previous studies have demonstrated that HCMV and EBV are frequently detected in deep pockets (13, 20, 29), and HCMV infection is predictive of the presence of P. gingivalis (9, 29). EBV-1 may thus promote the development of periodontitis, probably through mechanisms involving periodontopathic species other than P. gingivalis (29). It would be useful to know why single infection with EBV-1 was not related to the risk of periodontal tissue destruction, but statistical significance was shown after the presence of HCMV gB-II was also considered.

In summary, the present study demonstrates that different HCMV genotypes are associated with disease, while others appear to be nonpathogenic. Coinfection with the HCMV gB-II genotype and EBV-1 was dominant in CP patients and was associated with periodontal tissue destruction and inflammation. Although a longitudinal study is still needed, infection with these genotypes of HCMV and EBV are likely to contribute to the onset and development of periodontitis. Future studies on the pathogenic mechanisms of these strains of HCMV and EBV should help to elucidate their role in the etiology of periodontitis.

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REFERENCES


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Correlation between Infections with Different Genotypes of Human Cytomegalovirus and Epstein-Barr Virus in Subgingival Samples and Periodontal Status of Patients

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