1. Introduction and Specific Aims of the Project

Herpesviruses have recently been implicated in the pathogenesis of periapical pathosis, acute periodontitis, and acute inflammation of the gingiva and oral mucosa (1-3). Endodontic pathosis has been hypothesized to be a multi-microbia, multi-mechanism and multi-stage inflammatory disease initiated by migration of pathogenic microorganisms, including bacteria, fungi and viruses, and their by-products, into the dental pulp and consequently leading to an influx of inflammatory cells to cause pulpitis and periapical periodontitis. Those inflammatory cells might contain latent herpesviruses, and the disease progression might be moderated by potential reactivation of latent herpesviruses to cause additional impairment of host immune response, which in turn cause acute endodontic inflammation. Herpesviruses appear to participate in the pathosis of symptomatic periapical lesions (1-3). Herpesviruses are composed of three groups: 1) Alphaherpesvirinae (herpes simplex virus group), 2) Betaherpesvirinae (cytomegalovirus group) and 3) Gammaherpesvirinae (lymphoproliferative group including Epstein-Barr virus), and appear to participate in the pathosis of symptomatic periapical lesions (1-3). Human cytomegalovirus (HCMV) appears to reside in periodontal macrophages and T lymphocytes and latent Epstein-Barr virus (EBV) in periodontal B lymphocytes, and both herpesviruses are often co-residents of oral virus disease. Herpes simplex virus-1 (HSV-1) establishes latency in the nerve ganglia. The EBV latent membrane proteins (LMPs) are similar to the receptors of the tumor necrosis factor (TNF) receptor superfamily and can activate immune signaling pathways to produce several cytokines (4). In addition, LMP-1 variants have been identified in the oral cavity and may have variations in EBV transmission and persistence (5). Previous studies have demonstrated late-stage HCMV and EBV infections in endodontic symptomatic periapical lesions (1,2). Other studies have shown that herpesviruses and bone resorption-inducing cytokines are present in periapical lesions of deciduous teeth (6).

The objectives of this study are to ascertain the presence of HCMV, HSV-1 and EBV in the irreversible pulpitis, apical periodontitis and periapical abscess, and to determine the possible correlation of the herpesviruses with the clinical symptoms, such as acute pain, etc. The specific aims of this project are: 1) To test the hypothesis that HCMV, HSV-1 and EBV are present in endodontic lesions, and are associated with the severity of the symptoms; 2) To identify potential HCMV, HSV-1 or EBV variants that may be responsible for severe endodontic pathosis. The presence of specific EBV variants may help determine the origin of infection of the dental pulp, by transmission of saliva through caries and traumatic pulpal exposure, or alternatively by entry via the periapical blood supply.

Based upon the results of this study, our future alternate strategies will be to further investigate the possible roles and mechanism of herpesviruses in the endodontic pathoses. Our next step will be to test the hypothesis that HCMV, HSV-1 and EBV infection are associated with the expression of pro-inflammatory cytokines in endodontic lesions and that these cytokines may also influence the severity of the endodontic pathosis. We will explore the possible interaction of herpesviruses and bacterial pathogens in the endodontic biofilm formation, as well as their synergetic pathogenesis. In addition, we plan to utilize dental pulp cultures and an immortalized dental pulp cell line and interleukin-1 beta (IL-1β) promoter recombinants and test the hypothesis that Herpesviruses may induce the expression of specific immune factors or cytokines leading to endodontic inflammation. Moreover, we plan to study the potential strategy of anti-virus therapy for endodontic infection in the future.

2. Previous Work and Background of the Project

Classification. Replication, and Latency of Herpesviruses: There are eight human herpesvirus species in three major classifications: 1) Alpha-herpesvirinae, including herpes simplex
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Identification of latent and reactivated Herpesviruses in endodontic pathoses.
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herpesvirus-1, herpes simplex virus-2, and Varicella-zoster virus, 2) Beta-herpesvirinae, including human cytomegalovirus, human herpesvirus 6, and human herpesvirus 7, and 3) Gamma-herpesvirinae, including Epstein-Barr virus and human herpesvirus 8. All eight herpesviruses have the ability to enter a latent state following infection of the host, and can be reactivated at a later time, although the exact mode of reactivation can vary between species. The Alpha-herpesvirinae group has rapid and cytocidal growth cycles and can establish latency in nerve ganglia. The Beta-herpesvirinae group has slow replication cycles and form multinucleate giant host cells upon primary infection. Latency is primarily established in nonneural tissue, including lymphoreticular cells and glandular tissue. Gamma-herpesvirinae replicate and establish latency in mucosal epithelium. Herpesviruses replicate in the nucleus and similar to other DNA viruses undergo 1) virus adsorption and penetration, 2) viral DNA replication and nucleocapsid assembly, including expression of immediate early, delayed early, and late gene expression, 3) acquisition of the nuclear envelope, and 4) latency or quiescent state. The cell type where latency occurs is not usually the same cell type for productive infection and replication. Latency for HSV-1 occurs in the trigeminal ganglia, where copies of the viral genome are present as nonintegrated circular molecules of DNA. Reactivation of HSV-1 can occur through hormonal changes or physical damage to the neurons. Newly synthesized HSV-1 can then be transported down the axon to the nerve ending and can infect adjacent epithelial cells (7,8, 9, 10).

Proposed Model of Herpesvirus Infection in Periodontal and Endodontic Disease and Mechanism of Cytokine Release: HCMV, HSV-1 and EBV have been identified in periodontal and endodontic diseases, and have been proposed to increase the virulence of resident bacterial pathogens by enhancing bacterial adherence and invasiveness into epithelial cells and other mammalian cells (11, 12). Destruction of epithelial cells by herpesvirus infection can facilitate the penetration of pathogenic bacteria into connective tissue. HCMV also inhibits the expression of macrophage surface receptors that recognize lipopolysaccharides (LPS), components in the Gram – negative bacterial cell wall, and thus deter macrophages from destroying invasive gram-negative bacteria. Furthermore, herpesviruses destroy components of the major histocompatibility complex pathway within macrophages and impair the immune defense in its ability to present antigen, silence natural killer cells, stop cell death, and divert antiviral cytokine responses (11, 12). Therefore, herpesvirus infections can promote bacterial colonization and can enhance the growth and strength of abscess-producing bacteria by disrupting several aspects of the host immune defense. Bone resorption-inducing cytokines, specifically “receptor activator of nuclear kappa B ligand” (RANKL) are involved in odontoclastogenesis activation and physiological root resorption (13). RANKL is produced by activated T lymphocytes and osteoblastic lineage cells and belongs to the tumor necrosis factor superfamily. RANKL has been implicated in the pathogenesis of periodontal disease (13).

Figure 1: Proposed molecular mechanism for synergistic interaction of HCMV and periodontopathic bacteria. (Obtained from: Wara-aswapati N. et al. (2003) see reference 14 in the Literature Cited section).

EBV Variants and Localization in the Oral Cavity: EBV is an important human pathogen that is contained in greater than 90% of the world population, and is the causative agent for infectious mononucleosis (14,15). EBV transmission occurs via infected saliva or blood, resulting in the establishment of latent or persistent infection of B lymphocytes and chronic asymptomatic shedding of virus particles (14,15). Latent EBV infection results in the restricted expression of viral genes and therefore escapes immune surveillance (14,15). EBV types 1 and 2 contain sequence divergence in the EBV nuclear antigen 2 (EBNA2) and EBNA3 genes (4,5,16). EBV strains have also been distinguished by changes in the coding
sequences for the EBNA1, BLZF1, and LMP1 genes. The profile of strains and patterns of abundance in the oral cavity and blood provide evidence for compartmentalization of specific EBV strains and potential progression of disease (17).

**Molecular Activation of IL-1 Transcription by HCMV immediate Early (IE) Gene Products:**
HCMV plays a significant role in the initiation and progression of periodontal disease that may be moderated by the ability of HCMV to activate IL-1, a major cytokine produced during inflammation (18). Expression of the IL-1 gene is controlled by two independent elements: 1) a TATA-containing cell-specific promoter and 2) a lipopolysaccharide- (LPS-) responsive enhancer element. Bacterial LPS activates NF-IL6 and enables this molecule to bind to both the enhancer element and to the Spi-1 site contained in the IL-1 promoter. HCMV IE gene products interact directly with the Spi-1 site, and in conjunction with LPS, can synergistically up-regulate IL-1 gene expression (14).

**Preliminary Data**

**Collection of Endodontic Specimens at OHSU:** We have a large collection (60+) of abscess and pulpitis, apical periodontitis specimens from symptomatic and asymptomatic patients exhibiting pain and diverse sizes of apical radiolucency. Full records, including descriptions of patients’ signs and symptoms, scale of pain, results of clinical tests, size of apical radiolucency, size of abscess, presence of sinus tract, and character of the drainage, etc. have been recorded for each patient and each collection of abscess, pulpitis, and apical periodontitis specimens.

**Preliminary Results Verifying Presence of HCMV, EBV in Endodontic Lesion Specimen:**
In a pilot study, using PCR, RT-PCR and nested PCR, we have identified HCMV, EBV DNA and RNA in selected patients exhibiting symptomatic pulpitis, apical periodontitis and periapical abscess.

We have also assembled a number of control reagents, including 1) recombinant plasmids containing HCMV, EBV, and human actin sequences (provided by M. Stinski, University of Iowa; and R. Longnecker, Northwestern University), 2) genomic DNA from HCMV-transformed lymphoid cells (provided by S. Chou, VAMC, Portland, OR), 3) genomic DNA and cell pellets (for RNA extractions) of EBV-transformed lymphoblastoid cell lines and matching non-transformed controls (obtained from ATCC), 4) HCMV stocks and permissive endothelial cell line to be used as immunocytochemistry controls (provided by X. L. Wang, Baylor College of Medicine), and 5) PCR primer pairs recognizing diverse regions of the HCMV, HSV and EBV genomes. Using nested reverse transcriptase (RT) – PCR, we have successfully identified HCMV RNA in the pulpitis specimen from an endodontic patient exhibiting severe pain (Figure 2).

**Figure 2:** Nested RT-PCR demonstrating presence of HCMV in pulpitis specimen. Lane 1: MW standards (bp), Lanes 2 and 3: HCMV large fragment (308 bp) in lane 2 and HCMV small fragment (220 bp) in lane 3 were amplifi ed using HCMV pp65 plasmid DNA in standard PCR. Lanes 4-12: Pulpitis RNA was reverse transcribed using random amplifiers (lanes 4-7) or oligo dT (lanes 9-12). cDNAs were then used as templates for PCR with primers for actin (366 bp), HCMV large fragment and HCMV small fragment (small fragment is nested within the large fragment sequence). Nested RT-PCR of the HCMV small fragment was conducted using DNA generated from the HCMV large fragment amplification (lane 7).

In addition, we also identified EBV positive samples in symptomatic pulpitis, symptomatic apical periodontitis, and acute apical abscess patients, but not in the healthy pulp tissue. Ms. Vicky Chen, a dental student working in our lab, has demonstrated that HCMV was positive in 9 samples, and EBV was positive in 2 out of 32 pus samples collected from acute apical abscess.
3. Significance of Research in Relation to the AAE Research Priorities

Our research project directly addresses the AAE Research Priority C. Biology of pulpal and periradicular disease, sublist 5. Contribution of microorganisms to the pathogenesis of pulpal and periradicular disease.

The etiology and mechanism of endodontic pathosis are multi-factorial and may involve several microorganisms. Herpesviruses, more specifically HCMV, HSV-1 and EBV, may be actively involved in the inflammatory cascade leading to endodontic pathoses and may represent key determinants in the development of acute symptoms, including intolerable pain, and abscess. Therefore, defining the precise involvement of these herpesviruses in endodontic lesions is required to fully understand the pathogenesis of endodontic disease and may help develop antiviral treatment strategies. The current clinic strategy for treatment of endodontic disease is based on gross debridement and apical and coronal sealing of the canal. By understanding the pathogenesis of endodontic disease, treatment strategies may be developed to rescue or regenerate the infected pulp. Also, by drawing analogies of the role of EBV genotypes in infectious mononucleosis, we will determine if there is a progression and/or potential selection of specific EBV genotypes in endodontic pathosis. We also expect to provide the beginning framework for understanding the role of the bone resorption-inducing cytokines and HCMV gene activation in periapical pathosis, and for determine the potential correlation between cytokine expression and disease severity.

This research is innovative because almost all of the published research on HCMV, HSV-1 and EBV in the oral cavity is related to periodontitis and infectious mononucleosis and not endodontic diseases. The previous studies regarding the herpesviruses and endodontic diseases are scarce and inconclusive. The lone published manuscript on HCMV, HSV-1 and EBV in endodontic disease is limited to periapical tissue from persistent apical periodontitis, which did not identify herpesviruses in the other endodontic lesions, and did not address the correlation between the herpesviruses and clinical signs and symptoms. Furthermore, this study will provide the knowledge to identify components of HCMV, HSV-1 and EBV induction of the inflammatory cascade and the initiator molecular events leading to periapical pathoses, and to provide a foundation for future study about the relationship of bacteria, viruses and host immune system.

4. Experimental Method

Rationale and Experimental Design:

To test the hypothesis that HCMV, EBV, and HSV-1 are present in symptomatic endodontic pathoses, we will conduct a 3-stage series of PCR experiments. 1) The presence of HCMV, EBV, and HSV-1 particles will be determined with genomic PCR using viral nucleic acid extracted from the aqueous portion of the abscess/cellulitis fluid specimens. Hematoxylin-eosin staining reveals no immune cells within the abscess specimens. Therefore, detection of herpesviral DNAs will be indicative of the presence of virus particles and active / reactivated herpes virus infection. 2) The presence of latent herpesvirus infections that have not undergone reactivation will be detected with genomic PCR using DNA extracted from the pulpal and apical tissues specimens. We propose that the herpesviral DNA extracted from tissues would be nonintegrated viral genomic DNA. 3) The presence of an active herpesvirus infection and late-stage viral gene expression will be detected with RT-PCR using mRNA extracted from the pulpal and apical tissue specimens. With genomic PCR and RT-PCR, we can also determine if asymptomatic lesions contain latent HCMV, EBV, or HSV-1 DNA, but not HCMV, EBV, or HSV-1 RNA, which would indicate an active viral infection, and whether symptomatic endodontic pathoses and pain can be correlated with the presence of reactivated herpesviruses. In addition, immunocytochemistry using specific HCMV, HSV-1 and EBV antibodies will further confirm the presence of viral infection.
1). Specimen collection. We are collecting endodontic specimens from patients obtaining dental care at the OHSU School of Dentistry. Human subjects study for collecting extracted teeth and endodontic specimens has been approved by the OHSU Institutional Review Board (IRB; see attached letter). First, we review the patient’s medical history, dental history, chief complaint, associated past and present symptoms such as duration, pain scale, swelling, and other factors. Clinical exam and tests including percussion, palpation, probing depth, mobility, thermal response, electronic pulpal vitality test, and x-ray will be conducted. From those data, pulpal and periapical diagnosis will be performed and the patient will be classified into the following groups. The inclusion criteria will be patients who are generally healthy with no systemic diseases and patients who need teeth extraction or endodontic treatment due to the presence of endodontic diseases. The exclusion criteria will be periodontally involved teeth (probing depth is >4mm, with periodontal bone loss), immature teeth with open apexes, or cracked or fractured teeth. The pulpal and periapical tissues will be collected during endodontic treatment or after extraction. For pulpal specimens, the access will be disinfected with 5.25% NaOCl and inactivated with sterile 5% sodium thiosulphate, and the pulp tissue will be subsequently extirpated. For extracted teeth, after disinfection with 5.25% NaOCl, and deactivation with 5% sodium thiosulphate, the roots will split after vertical grooves have been cut around the roots, and the pulp tissue collected and stored at -85°C. For the periapical tissue specimens, the attached periapical tissue will be removed from the root apexes by scraping with a blade and stored in -85°C. Abscess specimens will be aspirated with syringe needles after surface disinfection and subsequently stored at -85°C.

There will be 9 groups of specimens with 15-20 samples of each group. Due to no previous data available, power analyze will be carried out and the exact sample numbers for each group will be determined at a later stage. The diagnostic terminology is based on the new guidelines from American Board of Endodontics (2007).

(1) Pulp tissues from symptomatic irreversible pulpitis: the tooth has lingering thermal pain, spontaneous pain and referred pain, and subjective and objective findings indicating that the vital inflamed pulp is incapable of healing.

(2) Pulp tissue from asymptomatic irreversible pulpitis: the tooth has no clinical symptoms but inflammation produced by caries, and the vital inflamed pulp is incapable of healing.

(3) Periapical tissues from symptomatic apical periodontitis: the tooth has inflammation, usually of the apical periodontium, producing clinical symptoms including painful response to biting and percussion. It may or may not be associated with an apical radiolucent area.

(4) Periapical tissues from asymptomatic apical periodontitis: the tooth has inflammation and destruction of apical periodontium that is of pulpal origin, appears as an apical radiolucent area and does not produce clinical symptoms.

(5) Periapical tissues from persistent apical periodontitis: the tooth has previous endodontic treatment but still has persistent signs and symptoms indication failed RCT.

(6) Periapical tissue from acute apical abscess: the tooth has an inflammatory reaction to pulpal infection and necrosis characterized by rapid onset, spontaneous pain, and tenderness of the tooth to pressure, pus formation and swelling of associated tissues.

(7) Pus specimen from acute apical abscess: same as group 6. Pus specimens will be aspirated from the abscess.

(8) Periapical tissues from chronic apical abscess: the tooth has an apical inflammation to pulpal infection and necrosis characterized by gradual onset, little or no discomfort and the intermittent discharge of pus through an associated sinus tract.

(9) Pulp tissues from healthy third molars or bicuspids as control: the tooth has no caries or restoration. The tooth needs to be extracted due to orthodontic treatment.

2). Genomic DNA and RNA extraction. The tissue specimens will be minced with scalpels and commercial DNA and RNA extraction kits will be used. For genomic DNA isolation, we are going to use PureLink Genemic DNA Kits from Invitrogen. For RNA isolation, RNeasy Mini Kit from Qiagen will be used.
3). Viral Nucleic acid isolation. The HCMV, HSV-1 and EBV viral particles from the aqueous portion of the abscess fluid samples will be isolated with the EasyXpress viral Nucleic Acid release kit from the Express Biotech International.

4). PCR and RT-PCR. The presence of latent herpesvirus infections that have not undergone reactivation will be detected with PCR using genomic DNA extracted from the pulpitis, apical periodontitis specimens. The presence of an active herpesvirus infection and late-stage viral gene expression will be detected with RT-PCR using mRNA extracted from the pulpitis, apical periodontitis specimens. With genomic PCR and RT-PCR, we can also determine if asymptomatic lesions contain latent HCMV, HSV-1 and EBV DNA, but not HCMV, HSV-1 and EBV RNA indicative of an active infection, and whether symptomatic endodontic disease and pain can be correlated with the presence of reactivated herpesviruses. RT-PCR analyses with primers recognizing cytokines mRNAs (see Table 1) will also be performed to test the hypothesis that HCMV, HSV-1 and EBV infection may induce the expression of bone resorption-inducing cytokines, and shed insight on the importance of these cytokines in the severity of the endodontic pathosis. The band intensities of PCR and RT-PCR products will be compared and quantitated with band intensities obtained from standard curves using recombinant and HCMV- and EBV-transformed genomic DNA.

SuperScrip III CellsDirect cDNA Synthesis System from Invitrogen will be used to synthesis the first stain of cDNA from the RNA preparation. Table 1 illustrated the primers sequences of representative HCMV, HSV-1 and EBV, and bone resorption-inducing cytokines and other major immune factors.

### Table 1: Primer sequences of representative HCMV, HSV and EBV.

<table>
<thead>
<tr>
<th>Target molecule</th>
<th>Location</th>
<th>PCR Primer</th>
<th>Size of amplicon</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCMV</td>
<td>347-366</td>
<td>5’ TGCCGCTCAAGATGCTGAAC 3’</td>
<td>308</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5’ TCACCTGCATCTTTGGTTGC 3’</td>
<td></td>
</tr>
<tr>
<td>HCMV Saygun</td>
<td>126958-126977</td>
<td>5’ GAGCGCGGCTCCAAAGTCTA 3’</td>
<td>263</td>
</tr>
<tr>
<td></td>
<td>127221-127202</td>
<td>5’ GTGATCCGACTTGGGCGAAA 3’</td>
<td></td>
</tr>
<tr>
<td>HCMV Yildrim</td>
<td>197042-197066</td>
<td>5’ GGATCCGATGCGATTCAGTGATGT 3’</td>
<td>406</td>
</tr>
<tr>
<td></td>
<td>197448-197424</td>
<td>5’ GAATTCAGTGGATAACCTCGGCA 3’</td>
<td></td>
</tr>
<tr>
<td>HSV-1</td>
<td>120655</td>
<td>5’ CCAAACACAGACGGGAAAG 3’</td>
<td>345</td>
</tr>
<tr>
<td></td>
<td>121000</td>
<td>5’ GGAACATGTCTTGTCCACAG 3’</td>
<td></td>
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<tr>
<td></td>
<td>120703</td>
<td>5’ AGACAGCAAAAAATCCCTGAG 3’</td>
<td></td>
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<tr>
<td></td>
<td>120898</td>
<td>5’ ACGAGGGAAAACAAATAGG 3’</td>
<td></td>
</tr>
<tr>
<td>EBV</td>
<td>76775</td>
<td>5’ CAGCTCCACGCAAGTCAATG 3’</td>
<td>481</td>
</tr>
<tr>
<td></td>
<td>77256</td>
<td>5’ ATCAGAAATTTGACTTTTTT 3’</td>
<td></td>
</tr>
<tr>
<td></td>
<td>76814</td>
<td>5’ TTGACATGACATGGAAGAC 3’</td>
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<td></td>
<td>77176</td>
<td>5’ CCTGTTGTTGTGTTTTGCTAC 3’</td>
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<tr>
<td>β-Actin</td>
<td>1130-1151</td>
<td>5’ CAGCAGATGGTGACTAGCAAGC 3’</td>
<td>366</td>
</tr>
<tr>
<td></td>
<td>1495-1474</td>
<td>5’ AGGATGGCAAGGACTTCTGT 3’</td>
<td></td>
</tr>
</tbody>
</table>

HCMV: human cytomegalovirus, HSV: herpes simplex virus, EBV: Epstein-Barr virus,

5). DNA sequencing of the PCR products. DNA sequencing of EBV PCR fragments will be conducted to identify potential EBV variants that may be responsible for severe endodontic pathosis. The identification of EBV variants may also determine whether endodontic infection of dental pulp occurs by transmission of saliva through carious pulpal exposure, or alternatively by entry via the periapical blood supply.

6). Immunocytochemistry to detect viral antigens. Specific antibodies for HCMV, EBV, and HSV-1 will be used for direct immunocytochemistry of the endodontic specimens and will potentially
identify the cell types for each herpesviral infection (15). The pulp and periapical tissue samples will be fixed in 4% paraformaldehyde at 4°C for 24 hr, rinsed successively in PBS for 24 hr, and subsequently cryoprotected in 30% sucrose for 5 hr. Frozen sections will then by collected on poly-lysine coated slides, dried for 1 hr at room temperature and stored at -85°C until ready to immunostain. The embedded tissues were mounted on a cryostat block and sectioned in increments of 10 microns. The slices of tissues were washed with PBS three times and incubated in blocking buffer, which was made with PBS/0.05% triton X, 10% goat serum, and sodium azide, for an hour. After incubating in blocking buffer for an hour, the tissue slices were incubated with different primary antibodies overnight in 4 degree (anti-EBV monoclonal IgG1 in 1:50 dilution and anti-HCMV monoclonal IgG2Ack in 1:100 dilution). The tissues were washed with PBS/0.05% Triton X for 10 minutes three times the next day. After that, the tissues were incubated with secondary antibodies (isospecific or general goat anti mouse secondary antibodies for an hour in room-temperature). The specimens were covered with aluminum foil while being incubated in secondary antibodies. After secondary antibody incubation, the tissues were rinsed with PBS/0.05% triton X for 3 to 4 times and were mounted in slowfade mounting media with clover slip before viewing under the confocal microscope.

**Expected Results and Critique:**

We propose to validate the presence of HCMV, HSV and EBV in endodontic patients who exhibit acute symptoms, especially pain, acute abscess, and the presence of large apical radiolucency in excess of 5 mm in diameter. Although the endodontic disease observed in our study is comparable to the disease published by the Slots group, the patient specimens are not exactly equivalent. We will collect dental pulps, periapical tissues, and abscess fluid, while the Slots group has collected tissue specimens from persistent chronic apical periodontitis lesions. We assume, but have not proven, that the HCMV, HSV-1 and EBV infections have progressed from the site of the dental pulp to apical periodontitis and apical abscess, and believe that infected cells or released virus, if present, can migrate to these distal sites.

5. **Data Analysis**

We plan to use ANOVA to analyze whether there are statistic differences between different groups regarding the HCMV, HSV-1 and EBV positive infections.

6. **Resources and Environment**

Dr. Machida and Dr. Baumgartne’s laboratories, and the Department of Integrative Biosciences at the OHSU School of Dentistry, have all the necessary equipment to conduct the experiments of this study. Clinical samples can be obtained at the Endodontic, Urgent care and Oral surgery clinics of School of Dentistry, OHSU. The clinical samples can be precessed in Dr. Baumgartner’s laboratory (room 830 D; 400 sq. ft), equipped for DNA and RNA extraction, quantitation, and electrophoresis (PicoGreen dsDNA quantitation reagent and Fluorescent HPLC monitor). Dr. Machida’s laboratory (room 405/407; 700 sq ft) is fully equipped for conducting DNA and RNA extraction and reverse transcriptase-PCR, and has substantial experience manipulating recombinant viruses. Dr. Machida’s laboratory also contains thermal cyclers for PCR analysis, agarose gel electrophoresis equipment, gel documentation equipment (Biorad Gel Doc.), and image analysis software (Biarad Quantity One), as well as two laminar flow containing hoods, tissue culture incubator, inverted phase microscope, phosphorimager (Biorad Molecular Imager FX), luminometer, ultra-low and mid-ranger freezers, low-speed table-top refrigerated centrifuge, hybridization incubator, bacterial incubators, water baths and temperature recirculators, and sequencing apparatuses. In addition, other large equipment, including ultra-centrifuges, mid-range centrifuges, scintillation counter, and autoclaves are located in the School of Dentistry, and a fully-equipped darkroom, including automatic film developer, is available in an adjacent building (Casey Eye Institute, OHSU). The Department of Integrative Biosciences has also recently purchased a
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Stratagene MX300P multiplex quantitative PCR machine that is available for this project. A large walk-in cold room is adjacent to Dr. Machida’s laboratory and is also readily available. Furthermore, the molecular biology research core facility at OHSU has advanced technique support and fully equipped with primer syntheses and auto sequencing, as well as other supplies.

Histology facilities are available in the Department of Integrative Biosciences. A Bright-Hacker Cryostat/Microtome is available for taking frozen tissue sections. Microtomes and associated supplies for sectioning and processing paraffin-embedded tissue and plastic-embedded tissue are also available in the department. A confocal microscope facility, located in the School of Dentistry, is available for use at $35/hour. It consists of a BioRad Radiance (now zeiss) 2100 laser scanning confocal microscope attached to a Nikon Eclipse E800 upright fluorescent microscope fitted with 3 lasers: argon ion (457,477,488,518 nm) for Alexa Fluor 488, green helium neon (543 nm) for Alexa 546 and rhodamine, and a red diode (630 nm) for Cy 5 or Alexa 647. Water immersion and dry objectives include10x, 40x and 60x. 3 PMTs (detectors) allow simultaneous 3-channel fluorescence detection and laser-stroking mode makes it possible to do simultaneous multi-channel image capture at different wavelengths with zero bleed-through between channels.

Other support for applicant and sponsors: The applicant is not currently supported by any extramural funds or fellowships. Dr. Baumgartner is supported by the OHSU School of Dentistry, and he has been awarded with numerous grants, including a few AAE grants. Dr. Machida is supported by the NIH, Oral Biotech, and the OHSU School of Dentistry. He has been awarded 5 NIH grants, including one grant on retroviral vectors.

Human subjects: A human subject protocol describing collecting extracted teeth and tissue specimens to identify the HCMV, HSV-1 and EBV in endodontic pathoses has been reviewed and approved by IRB at OHSU. (see attached).

Recombinant DNA: This protocol has been submitted to the OHSU Institutional Biosafety Committee and has received an exemption status. Documentation verifying the exempt status is included with this application. Dr. Machida was a former member of the OHSU Recombinant DNA Review Committee (during period 1989-2004), more recently renamed as the OHSU IBC. This project adheres to the current NIH Guidelines for Research Involving Recombinant DNA Molecules. Only partial viral genomes (<20% of complete genome) will be manipulated in this project and no infectious recombinant virus will be generated.

7. Time Schedule For Research


