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Short communication

Cytomegalovirus-infected inflammatory cells in dental periapical lesions

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Introduction: As cytomegalovirus may be etiologically involved in periapical pathosis of endodontic origin, this study aimed to determine the cellular source of periapical cytomegalovirus.
Methods: Periapical granulomatous tissue was collected from 15 extracted teeth with symptomatic periapical lesions. Multi-color flow cytometry was used to identify cytomegalovirus-infected cells.
Results: Cytomegalovirus infection was identified in 10 of the 15 (67%) study lesions, and in periapical monocytes/macrophages (40% of lesions) and T lymphocytes (54% of lesions), but not in periapical B lymphocytes.
Conclusion: This study and previous polymerase chain reaction-based investigations show that cytomegalovirus is a frequent inhabitant of symptomatic periapical lesions.

Key words: cytomegalovirus; flow cytometry; endodontics; pathogenesis

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Herpesviruses play a role in the etiopathogeny of symptomatic periapical lesions of endodontic origin (16). Polymerase chain reaction-based studies have identified cytomegalovirus in seven (58% of study lesions) periapical symptomatic lesions of deciduous teeth in 2- to 8-year-old children (19), in five (100%) symptomatic periapical lesions of teeth with intact crowns (11), and in nine (32%) acute apical abscesses (3). Cytomegalovirus transcripts were detected in 25 (100%) symptomatic and in seven (37%) asymptomatic periapical lesions (15), and in 15 (54%) symptomatic and six (27%) asymptomatic periapical lesions (18). Complementary DNA identification of genes transcribed late during the infectious cycle of herpesviruses is indicative of an active viral infection. Cytomegalovirus has also been demonstrated in enlarged periapical cells, especially in subjects infected with human immunodeficiency virus (13), and in six (60%) odontogenic keratocysts and 18 (55%) periapical cysts, with the highest prevalence in cysts having a history of acute inflammation (1). A dual infection by cytomegalovirus and Epstein–Barr virus is frequently present in severe types of endodontic disease (11, 12). Herpes simplex virus-1 may also contribute to endodontic pathosis (6, 9, 12). Herpesviruses occur with low prevalence and copy-counts in the healthy pulp and in chronic pulpitis (6, 10, 17, 19).

The presence of cytomegalovirus in symptomatic periapical pathosis is consistent with an inflammatory cell source for the virus. Indeed, latent cytomegalovirus resides in various myeloid progenitor cell types and in more differentiated hematopoietic cell lineages, and cytomegalovirus translocation in the body occurs in monocyte/macrophages and dendritic cells (8). Also, cytomegalovirus in marginal periodontitis lesions exists in macrophages and T lymphocytes (4).

In this study, flow cytometry was employed to assess the presence of cytomegalovirus in periapical monocytes/macrophages and T and B lymphocytes. A total of 15 patients, 35–60 years old, with symptomatic teeth and periapical lesions were included in the study. Symptomatic teeth exhibited swelling, pain, discomfort on biting, or sensitivity on percussion or palpation. The periapical lesions ranged in radiographic diameter from 3.0 to 8.5 mm. The patients were systemically healthy and had not received endodontic treatment or antibiotics for at least 6 months before the start of the study. None of the study teeth demonstrated moderate or severe types of marginal periodontitis. The Institutional Review Board at the University of Southern California approved the study, and written informed consent was obtained from each study subject after all procedures had been fully explained.

Periapical samples were collected in conjunction with tooth extraction. Before administration of local anesthetics, the teeth, gingiva and mucosa of the sample area were washed with 0.12% chlorhexidine and patients rinsed with 0.12%

Table 1. Cytomegalovirus-positive inflammatory cells in 15 periapical symptomatic lesions[1]

Items	No. (%) of lesions
Study lesions (n = 15) with cytomegalovirus-positive cells	10 (67%)
Cytomegalovirus-positive CD3 cells (T lymphocytes)	8 (54%)
Cytomegalovirus-positive CD14 cells (monocytes/macrophages)	6 (40%)
Cytomegalovirus-positive CD3 cells (T lymphocytes) + cytomegalovirus-positive CD14 cells (monocytes/macrophages)	6 (40%)
No cytomegalovirus-positive reaction in any of the cellular fractions studied	5 (33%)

[1]No lesion showed the presence of cytomegalovirus in CD19 cells (B lymphocytes).

chlorhexidine mouthwash for 30 s. Periapical granulomatous tissue was rinsed with sterile saline, cut with a sterile blade, teased and passed through a laboratory sieve of 200 mesh size (74 μm) to obtain a single-cell suspension. The cell suspension was adjusted to 1 million cells per ml. Cellular viability was assessed by membrane-impermeant propidium iodine, which is generally excluded from viable cells.

A 200-μl subsample of the cell suspension was transferred to an empty vial, permeabilized, fixed for 10 min at ambient temperature using Permeafix (Beckman Coulter, Fullerton, CA), and washed once in IsoFlow Sheath Fluid (Beckman Coulter) to obtain good signal-to-noise ratio measurements during the flow cytometry analysis. Cells were then stained with multicolor-labeled mouse monoclonal antibodies for 30 min at ambient temperature, followed by washing twice with IsoFlow. The following antibodies were employed: phycoerythrin-Texas Red-conjugated (energy couple dye) CD3 (T lymphocytes), fluorescein isothiocyanate (FITC)-conjugated CD19 (B lymphocytes), phycoerythrin–cyanine 5-conjugated CD14 (monocytes) (all Beckman Coulter), FITC-conjugated CMV (cytomegalovirus; Millipore, Billerica, MA) and phycoerythrin-conjugated CD178 (Fas ligand; Biolegend, San Diego, CA). Corresponding positive and negative controls included infected and non-infected mononuclear cells. The specificity of the anti-cytomegalovirus antibody was confirmed by including unlabeled and FITC-labeled anti-cytomegalovirus antibody to show the extent of functional inhibition of the labeled anti-cytomegalovirus antibody.

Immunophenotyping was performed using the five-color Cytomics FC 500 Series Flow Cytometry System with FITC-labeled and phycoerythrin-labeled beads (Beckman Coulter). Cells of the experimental and the corresponding control samples were identified by forward vs. right-angle light scatter. A gate was placed around the events that exhibited the light-scatter properties of intact cells to permit a cellular analysis for events-versus-fluorescence intensity and a separation of non-specific unstained and specifically stained cells. The events were plotted in a linear mode on the y-axis and the fluorescence intensity was plotted in a log-scale on the x-axis. The fluorescence gains were set so that uninfected non-specifically stained cells occupied the first log of the fluorescence intensity scale on the x-axis, and antigen-positive cells appeared at least one log higher on the fluorescence intensity scale on the x-axis.

Table 1 shows the presence of cytomegalovirus in 10 of the 15 (67%) periapical lesions studied. It also reveals the distribution of cytomegalovirus infection in the various types of study cells. Cytomegalovirus-positivity was detected in monocytes/macrophages in 40% and in T lymphocytes in 54% of the study lesions. No periapical lesion revealed cytomegalovirus-positive B lymphocytes. As shown elsewhere (14), cytomegalovirus may also infect natural killer cells in periapical lesions.

The present findings were comparable with those of a previous study on cytomegalovirus presence in inflammatory cells of marginal periodontitis (4). Of 20 gingival biopsies obtained from marginal periodontitis, 65% were positive for cytomegalovirus, 55% demonstrated cytomegalovirus in monocytes/macrophages and 20% showed cytomegalovirus in T lymphocytes (4). No cytomegalovirus presence was detected in B lymphocytes from periodontitis lesions either (4). These data support the concept of a high degree of commonality between infectious aspects of periapical pathosis and marginal periodontitis.

CD178, or the Fas ligand, is expressed at the cell surface of T lymphocytes and phagocytes and is a member of the tumor necrosis factor (ligand) superfamily (5.7). The Fas/Fas ligand system, which is an important cellular pathway mediating apoptosis (7), can potentially eliminate cytomegalovirus-infected cells (2). In the present study, CD178 was expressed in both cytomegalovirus-infected and non-infected cells; however, the magnitude of expression of CD178 and cytomegalovirus tended to be inversely related. Forty-seven per cent of the lesions studied had a high CD178 expression level and low or barely detectable cytomegalovirus expression, and 33% of the lesions demonstrated a low level of CD178 expression and a high level of cytomegalovirus expression. It may be that a periapical cytomegalovirus infection can inhibit CD178 expression, the effect of which may be a failure of the host to successfully control or eliminate the viral infection and subsequently the bacterial infection.

In conclusion, the present flow cytometric analysis and previous histopathological and polymerase chain reaction-based findings have identified cytomegalovirus as a frequent inhabitant of symptomatic periapical lesions. As an active cytomegalovirus infection induces a multiplicity of interconnected immune reactions, incorporating cytomegalovirus and other herpesviruses into studies on infectious causes and causal mechanisms of periapical pathosis may provide important new insights into the etiopathogeny of the disease.

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