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Immunohistochemical localization of oncostatin M in epithelialized apical periodontitis lesions

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Abstract

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Aim To investigate the *in situ* location of oncostatin M (OSM) in epithelialized apical periodontitis lesions.

Methodology Thirty periapical lesions of pulpal origin were collected with informed consent from patients at the time of apical surgery. Tissue specimens were fixed with 10% buffered formalin overnight, dehydrated in an ascending series of graded alcohol, and embedded in paraffin. Five micron sections from formalin-fixed, paraffin-embedded specimens were examined by immunohistochemistry. In addition, another section from each specimen was stained with haematoxylin and eosin to assess the presence of inflammatory infiltrates. Differences in OSM expression between tissue with low and high levels of inflammation were subsequently analyzed by Fisher's exact test.

Results Based on histological examination of haematoxylin and eosin stained sections, all specimens revealed the morphology of epithelialized apical periodontitis lesions. The results from immunohistochemistry demonstrated that OSM stain was detected in the inflammatory infiltrates, epithelium, connective tissue, and endothelium. The OSM signal was mainly expressed in endothelial cells (100%) followed by inflammatory cells (93.33%), epithelial cells (53.33%), and fibroblasts (16.67%). In addition, OSM expression was significantly higher in epithelialized apical periodontitis with higher levels of inflammatory infiltrates ($P < 0.001$).

Conclusions Oncostatin M was found to be expressed in epithelialized apical periodontitis lesions and would form part of the cytokine network involved in the disease process of apical periodontitis.

Keywords: epithelialized apical periodontitis, immunohistochemistry, inflammation, oncostatin M.

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Introduction

Chronic apical periodontitis is an inflammatory disorder of periradicular tissues caused by aetiological agents of endodontic origin, most frequently bacteria; the subject has been reviewed by Nair (2004, 2006). They are believed to result from inflammatory processes in the periapical tissues associated with bacterial infection and necrosis of the dental pulp in carious teeth. As a consequence of these processes and the inability of host defense mechanisms to eradicate antigens, periapical lesions such as apical abscesses, granulomas, and periapical cysts may be formed, with the aim of restricting microbial invasion (Nair 1997). Serial sectioning has shown that more than 50% of apical periodontitis lesions are epithelialized (Nair *et al.* 1996). Proliferating epithelium forms an irregular epithelial network in which vascular and infiltrated connective tissue is enclosed. Some chronic periapical lesions develop into cysts. More than half of cystic lesions are true apical cysts containing cavities completely enclosed in epithelial lining. The rest are apical pocket cysts with epithelium-lined cavities that open to the root canals (Nair *et al.* 1996, Nair 2003, 2006). Epithelialized apical periodontitis lesions grow within

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the periapical bone tissue; it is conceivable that intense bone resorption produced by activated osteoclasts may favor the intraosseous expansion of the lesion. However, very little is currently known about the precise mechanisms of the growth of epithelialized apical periodontitis lesions.

Oncostatin M (OSM), a 28 kDa glycoprotein produced predominantly by activated T lymphocytes and endotoxin stimulated macrophages, is a member of the interleukin (IL)-6 family which comprises IL-6, IL-11, OSM, ciliary neurotrophic factor, and leukemia-inhibitory factor (Heinrich *et al.* 2003). Similar to IL-6, OSM is pleiotropic and participates in diversified physiological processes such as differentiation, wound healing, cellular proliferation, and inflammatory response (Heinrich *et al.* 2003, Tanaka & Miyajima 2003). There is evidence to demonstrate that OSM can stimulate bone resportion (Palmqvist *et al.* 2002). In addition, the expression of increased OSM has been detected in inflammatory bone disease such as rheumatoid arthritis and chronic periodontitis (Hui *et al.* 1997, Lin *et al.* 2005, Lu *et al.* 2006).

Previously, Richards *et al.* (2000) have demonstrated the role of OSM in the induction of osteoclast differentiation and resorptive activity in the mouse. OSM is regarded as a proinflammatory cytokine that may induce osteoclastic activity in jaw bone. The present work was undertaken to identify the *in situ* localization of OSM expression in epithelialized apical periodontitis lesions as a contribution to the knowledge of the pathogenesis of such lesions.

Material and methods

Sample collection

Thirty periapical lesions of pulpal origin were collected with informed consent from patients at the time of apical surgery at the Oral Medicine Centre, Chung Shan Medical University Hospital, Taichung, Taiwan. All patients were without systemic diseases, had radiographic evidence of periapical lesions, including periapical alveolar bone loss. For haematoxylin and eosin-stained sections, tissue specimens were fixed with 10% buffered formalin overnight, dehydrated in an ascending series of graded alcohol, and embedded in paraffin.

Immunohistochemistry

Five micron sections from formalin-fixed, paraffin-embedded specimens were stained with the anti-OSM antibody [rabbit anti-human, OSM (N-1): sc-129, Santa Cruz Biotechnology, CA, USA] (1 : 50 dilution) using a standard avidin-biotin-peroxidase complex method as described previously (Tsai *et al.* 2005). Diaminobenzidine (Zymed, South San Francisco, CA, USA) was then used as the substrate for localizing the antibody binding. Negative controls included serial sections from which either the primary or secondary antibodies were excluded. Four biopsy specimens of inflamed gingiva were used as positive controls (Lu *et al.* 2006). The preparations were counterstained with haematoxylin, mounted with Permount (Merck, Darmstadt, Germany) and examined by light microscopy.

Statistical analysis

One section from each specimen was stained with haematoxylin and eosin to evaluate the magnitude of inflammation at the histological level. Each specimen was graded at 200× magnification as: grade low: inflammatory cells less than 50% per field and grade high: inflammatory cells higher than 50% per field. Grading of each specimen was based on the average inflammatory condition in three consecutive microscopic fields starting from the epithelial-connective tissue border and proceeding gradually deeper into lamina propria.

Processed immunohistochemically for OSM expression, sections graded as 'low' were represented by positive stained cells less than 50%; sections graded 'high' exhibited positive stained cells over 50% on three sections/tissue at 200× magnification. Fisher's exact test (two-tail) was applied for the statistical analysis of the results. A P-value of <0.05 was considered to be statistically significant.

Results

At light microscopic examination, all specimens revealed the morphology of epithelialized apical periodontitis lesions (Fig. 1). The underlying fibrous connective tissue wall was inflamed with variable degrees of inflammatory cell infiltration. Among 30 specimens, 15 cases exhibited low inflammation and 15 cases exhibited severe inflammation (Table 1).

Oncostatin M stain was detected in the epithelium, connective tissue, inflammatory infiltrates, and endothelium (Fig. 2). As shown in Table 2, the rank orders with respect to OSM positively stained cells were found

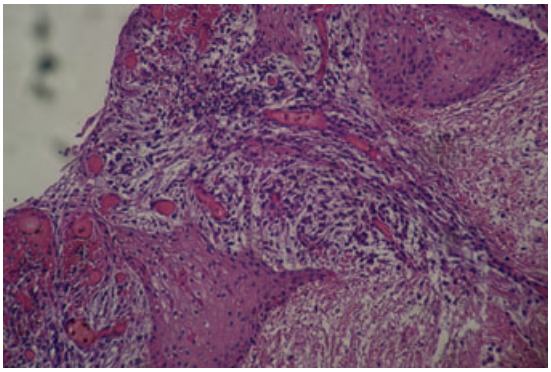

Figure 1 Photomicrograph represents a morphology of epithelialized apical periodontitis lesion. (H&E × 200).

Table 1 OSM expression in epithelialized apical periodontitis lesions

	Inflammation high	Inflammation low
OSM high	13	2
OSM low	2	13

A significantly greater OSM expression was noted in epithelialized apical periodontitis lesions by Fisher's exact test. $X^2 = 16.13$, $P = 0.0006$.
OSM, oncostatin M.

as follows: endothelial cells (100%) > inflammatory cells (93.33%) > epithelium (53.33%) > fibroblasts (16.67%). No signals were detected in the negative controls of epithelialized apical periodontitis lesions. In inflamed gingival specimens, OSM stain was detected in the epithelium, fibroblasts, inflammatory infiltrates, and endothelium (Fig. 3).

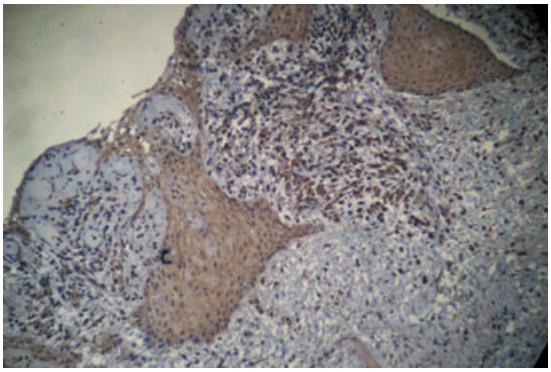

Figure 2 Immunolocalization of OSM in epithelialized apical periodontitis lesion by a peroxidase-labeled streptavidin-biotin technique. OSM stain was detected in the epithelium, connective tissue, inflammatory infiltrates, and endothelium. (×200). OSM, oncostatin M.

Table 2 Localization and number of OSM expression in 30 epithelialized apical periodontitis lesions

	Epithelial cells	Inflammatory cells	Fibroblasts	Endothelial cells
OSM positive	16 (53.33%)	28 (93.33%)	5 (17.67%)	30 (100%)

OSM, oncostatin M.

Oncostatin M expression in epithelialized apical periodontitis lesions with low or high levels of inflammation was listed in the Table 1. Differences in OSM expression between tissues with low and high levels of inflammation were subsequently analyzed using Fisher's exact test. A significantly greater OSM expression was noted in epithelialized apical periodontitis lesions with high levels of inflammation as compared to tissues with low levels of inflammatory cell infiltrates ($P = 0.0006$).

Discussion

Apical periodontitis develops as a response to infection, and in the chronic form a granuloma is formed with characteristics peculiar to the location and anatomy. In addition to the inflammatory cells, it typically contains fibrous tissue as well as proliferating strands of epithelium derived from the cells of Malassez. It may or may not develop a cyst cavity, which is lined in part or in full by epithelium (Huumonen & Orstavik 2002). Radicular cysts are common and comprise between

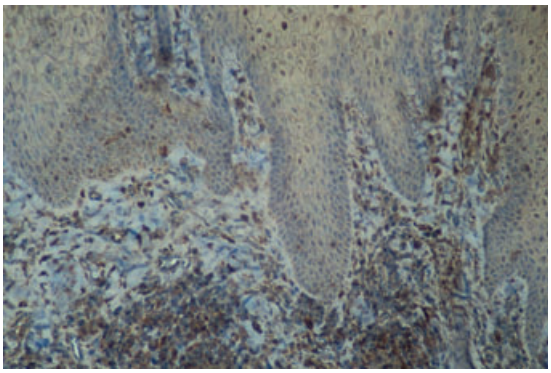

Figure 3 Photomicrograph shows a staining for OSM in an inflamed gingival tissue as positive control by a peroxidase-labeled streptavidin-biotin technique. (×200). OSM, oncostatin M.

52% to 68% of all the cysts affecting the human jaw (Shear 1992). However, accurate histopathological diagnosis of radicular cysts is possible only through serial sectioning or step-serial sectioning of the lesions removed in toto (Nair *et al.* 1996). Because of several reasons, such as incompleteness of the lesions, method of sectioning (serial or random sectioning) and the criteria of evaluation, it is unlikely that the lesions categorized as cysts were in fact cysts. In this study, a great majority of them simply might have been epithelialized apical periodontitis lesions.

In this study, all specimens revealed the histological evidence of epithelialized apical periodontitis lesions. OSM stain was detected in the epithelium, connective tissue, inflammatory infiltrates, and endothelium. The expression of OSM might play a role in the pathogenesis of epithelialized apical periodontitis lesions.

The mechanism responsible for the OSM expression in epithelialized apical periodontitis lesions may be explained as follows. Apical periodontitis lesions are believed to result from continuous antigenic stimulation from inflamed or necrotic root canals. Many studies have shown an association between bacteria and endodontic infection (Kakehashi *et al.* 1965, Sundqvist 1976). OSM was also found to be synthesized and secreted by bacterial lipopolysaccharide in dentritic cells (Suda *et al.* 2002), astrocytes, and astroglioma cells (Repovic *et al.* 2003). Previous studies have also shown that IL-1α, IL-6, TNF-α, and cyclooxygenase-2 were found to be expressed in radicular cyst specimens (Honma *et al.* 1998, Tsai *et al.* 2002). Therefore, OSM expression in the epithelialized apical periodontitis lesions may be induced either directly by bacteria from necrotic pulps or indirectly by inflammatory mediators generated by resident cells. Thus, these cells may play an important role in the pathogenesis of epithelialized apical periodontitis lesions by controlling the synthesis of inflammatory mediators and OSM.

Oncostatin M has been implicated as a mediator of bone destruction (Palmqvist *et al.* 2002) and was reported to induce tissue type plasminogen activator (t-PA) expression *in vitro* (Spence *et al.* 2002). Plasminogen activating system has shown to participate in degradation of bone-like matrix (Daci *et al.* 1999). Recently, studies have shown that t-PA were immunolocalized in epithelial cells, inflammatory cells, fibroblasts, and endothelial cells in radicular cyst (Tsai *et al.* 2004). Thus, the roles of OSM and t-PA expression in the pathogenesis of epithelialized apical periodontitis lesions are worthy of further investigation. Epithelialized apical periodontitis lesions are believed to result from continuous antigenic stimulation from inflamed or necrotic root canals. Since epithelialized apical periodontitis lesions grow within jaw bone, is conceivable that expansion of epithelialized apical periodontitis lesion is the result of bone destruction. Taken together, one of the possible mechanisms of epithelialized apical periodontitis lesions expansion *in vivo* may be *via* the OSM expression pathway.

The OSM has received considerable attention because of its participation in a wide variety of biologic activities in many inflammatory diseases. OSM could be detected in areas of the epithelialized apical periodontitis lesions, suggesting that OSM, stored in the cytosol of epithelial cells, inflammatory cells, fibroblasts, and endothelial cells, may represent a reservoir of OSM activity that can be released at certain stages of the inflammatory reaction. In addition, the expression of OSM increases with the grade of inflammation in epithelialized apical periodontitis lesions.

Conclusion

In the present study, immunoreactivity of OSM was expressed in the epithelium, connective tissue, inflammatory infiltrates, and endothelium in epithelialized apical periodontitis. The OSM signal was mainly expressed in endothelial cells followed by inflammatory cells, epithelial cells, and fibroblasts. The expression of OSM increases with the grade of inflammation in epithelialized apical periodontitis.

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