Distribution of Mast Cells and Macrophages and Expression of Interleukin-6 in Periapical Cysts

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Abstract

Introduction: Mast cells and macrophages are important components of the inflammatory infiltrate found in inflammatory periapical diseases. Several cytokines participate in the mechanisms of inflammation, tissue repair, and bone resorption associated with periapical cysts. The aim of the present study was to evaluate the distribution of mast cells and macrophages and the expression of interleukin-6 (IL-6) in periapical cysts.

Methods: Thirty periapical cysts were selected for the study, and clinical, demographic, and gross information from the cases was obtained from the laboratory records. Five-micrometer sections stained with hematoxylin-eosin were reviewed for analysis of the microscopic features of the cysts, and 3-μm sections on silanized slides were used for immunohistochemical reactions with anti-tryptase, anti-CD68, and anti–IL-6.

Results: There was no statistically significant difference in the mean number of mast cells and macrophages when comparing superficial and deep regions of the fibrous capsule of the cysts. Mean number of mast cells on the superficial region of the fibrous capsule was higher in cysts showing intense superficial inflammation and exocytosis. Macrophages were more commonly found in areas showing IL-6 expression, and IL-6 was less expressed in deep regions of the fibrous capsule in cysts showing greater gross volume. Conclusions: The results reinforced the participation of mast cells and macrophages in the pathogenesis of periapical cysts and suggested that IL-6 is not the major bone resorption mediator in larger periapical cysts. (J Endod 2014;40:63–68)

Key Words

Interleukin 6, macrophage, mast cell, periapical cyst

Periapical cysts and periapical granulomas are the most common inflammatory jaw lesions, representing up to two-thirds of all radiolucent jaw lesions diagnosed in oral pathology laboratories. Pertapical cysts (PCs) usually develop from inflammation and necrosis caused by the presence of microorganisms in the pulp tissue. Without adequate treatment, this aggression induces a periapical response, which is characterized by the presence of inflammatory cells and their products. Analysis of the cellular content of inflammatory periapical diseases (IPDs) has shown that their number, proportion, and distribution differ in distinct lesions, reflecting the histologic heterogeneity of the different stages of development and progression of the inflammatory response and repair in these specific processes. Mast cells and macrophages, essential cells in early and late immune responses, respectively, compose the inflammatory infiltrate in PCs and are associated with the production of several inflammatory mediators. Among them, interleukin-6 (IL-6), a cytokine with broad effects, can act in several scenarios, including proinflammatory effects and regulation of bone deposition and resorption. Despite the evidence of the participation of these cells in IPDs, their exact distribution on the different tissue compartments in PCs and their relationship with the expression of IL-6 are not well-understood. Thus, the aim of the present study was to evaluate mast cell and macrophage distribution and compare these findings with IL-6 expression in PCs.

Materials and Methods

Thirty PCs treated by complete surgical removal and diagnosed in the Oral Pathology Laboratory, Estácio de Sá University, from 2008–2010 were selected for the study. All PCs diagnosed in the period were reviewed, and 30 cases presenting typical microscopic characteristics, representative amount of tissue on the parafinn blocks, and sufficient clinical information on the laboratory charts were retrieved. Clinical and gross information was obtained from the laboratory charts. Five-micrometer sections were obtained from the parafinn blocks, stained with hematoxylin-eosin, and analyzed under light microscopy. Histologic features that were evaluated included intensity of the inflammatory infiltrate on the superficial and deep regions of the fibrous capsule (focal/weak or moderate/intense), presence of marked exocytosis (infiltration of several inflammatory cells on the epithelial lining, presence of isolated inflammatory cells was considered as negative), presence of cholesterol clefs, and distribution of the inflammatory infiltrate on the fibrous capsule (homogeneous or non-homogeneous). Division of the fibrous capsule in superficial and deep regions was done by calculating half the thickness from the epithelium surface to the external limit of the fibrous capsule. The same division was applied when analyzing the distribution of mast cells and macrophages and IL-6 expression.

For the immunohistochemical reactions, deparaffinized 3-μm sections on silanized slides were submitted to antigen retrieval with citrate buffer pH 6.0 on microwave. After inactivation of endogenous peroxidase, slides were incubated with primary antibodies against mast cell tryptase (monoclonal mouse anti-human mast cell tryptase, clone AA1, dilution 1:10,000; Dako, Copenhagen, Denmark), CD68 (monoclonal mouse anti-human CD68, clone PG-M1, dilution 1:500; Dako), and IL-6 (monoclonal mouse anti-human IL-6, dilution 1:500; Santa Cruz Biotechnology, Santa Cruz, CA). Secondary antibodies conjugated with streptavidin–biotin system (LSAB + system HRP; Dako, Glostrup, Denmark) were used, followed by diaminobenzidine as chromogen (DAB; Dako). The slides were counterstained with Carazzi’s hematoxylin, mounted, and...
analyzed under light microscopy. Negative and positive controls were used for all reactions. Antibodies against CD68 and mast cell tryptase were selected because of their well-known effectiveness in the adequate immunostaining of macrophages and mast cells, respectively (12, 15).

Mast cell and macrophage counting was performed by the selection of 10 high-power fields (original magnification, \( \times 40 \)) showing areas in which the total thickness of the cysts could be evaluated (superficial and deep regions of the fibrous capsule). For mast cells, after counting the number of positive cells in 10 fields, the mean number per region (superficial and deep) was obtained. The presence of mast cells in areas of cholesterol clefts and marked exocytosis was also recorded. For macrophages, counting was performed by using a number scale including 0 (no macrophages), 1 (mild, 1–5 macrophages), 2 (moderate, 6–10), and 3 (intense, more than 10) per field. This scale was used because of the difficulty in counting all macrophages in some areas of the capsule where they were numerous. After using the values of the scale for the 10 fields, the mean value was registered for the superficial and deep regions for each individual case. IL-6 expression was graded as present or not, and the location of the positive staining was recorded. All cases were descriptively analyzed regarding the pattern of distribution of IL-6–positive cells on the epithelium lining, superficial and deep fibrous capsule, and areas of marked exocytosis and cholesterol clefts. All slides were simultaneously analyzed by 2 observers, and the results were obtained by consensus.

Data were analyzed with the Statistical Package for Social Sciences (17.0; SPSS Inc, Chicago, IL), considering 5% as the significance level \( (P < .05) \). Categorical variables are presented as absolute numbers and proportions, and continuous variables are presented as means. Continuous variables were compared by using unpaired Student t test for independent data and paired Student t test for dependent data, and categorical variables were analyzed by using \( \chi^2 \) tests.

Results

Clinical data from the 30 PCs revealed that men and women were equally affected, with a mean age of 44.9 years and ranging from 19–68 years. There was no statistically significant difference in the mean age of affected men (45.5 years) and women (44.2 years \( (P = .772) \). Anterior and posterior teeth were associated with 12 (40%) and 18 cysts (60%), respectively. There was also no statistically significant difference in the mean age of patients affected by PCs associated with anterior (46.8 years) and posterior teeth (43.6 years \( (P = .495) \). Mean gross volume of the specimens was 1183.7 mm\(^3\) (range, 48–7875 mm\(^3\)) and the mean volume of the PCs associated with anterior teeth (1727.5 mm\(^3\)) was higher than the mean volume of the PCs associated with posterior teeth (821.17 mm\(^3\)), but this difference was not statistically significant \( (P = .255) \).

Analysis of the microscopic features on hematoxylin-eosin–stained slides revealed that inflammation in the superficial region was considered moderate/intense in 24 cysts (80%) and focal/weak in 6 (20%). In the deep region, inflammation was characterized as moderate/intense in 13 cysts (43.3%) and focal/weak in 17 (56.7%). Distribution of the inflammatory infiltrate on the fibrous capsule was classified as homogeneous in 12 cysts (40%) and non-homogeneous in 18 (60%). Twenty-two cysts (73.3%) presented as marked exocytosis (Fig. 1B), and 10 (33.3%) showed the presence of cholesterol clefts.

Immunohistochemical analysis showed that the mean number of mast cells and the mean presence of macrophages in the superficial region in comparison with the deep region of the fibrous capsule showed no statistically significant differences (Table 1). Distribution of the presence of macrophages was uniform when comparing superficial and deep
regions, and grades 1+2 represented 61% of the superficial fields and 62% of the deep fields. The mean number of mast cells in the superficial regions with focal/weak inflammation was lower than the mean number in PCs with moderate/intense inflammation (P = .02) (Fig. 1C and D); in deep regions, mean number of mast cells in areas of focal/weak inflammation was also lower, but the difference was not statistically significant (P = .086). Mean presence of macrophages in both superficial and deep regions showing focal/weak inflammation was similar to their means in areas showing moderate/intense inflammation (Fig. 2A and B). In areas showing marked exocytosis, the mean number of mast cells was higher than in areas without exocytosis (P = .001), but the mean presence of macrophages was similar in both groups (Table 1).

IL-6 expression was evaluated in 27 of 30 cysts (90%), because it was not possible to rule out unspecific expression/background in 3 cases. IL-6 expression was particularly found in macrophages, endothelial cells, fibroblasts, and cells from the epithelial lining (Fig. 2C and D and Fig. 3). IL-6 expression was positive in superficial regions in 15 PCs (56%) and in deep regions in 17 PCs (63%). The mean number of mast cells in IL-6–positive and IL-6–negative superficial and deep regions was similar. On the other hand, the mean presence of macrophages in IL-6–positive superficial and deep areas was higher than in IL-6–negative areas, and the difference was statistically significant in deep regions (P = .03) (Table 1). Mean volume of the specimens was greater in PCs showing superficial IL-6 expression (1821.87 mm³) than in IL-6–negative PCs (614.83 mm³) (P = .063). On the contrary, mean volume of the specimens was greater in PCs with IL-6–negative deep regions (1584.88 mm³) than in IL-6–positive PCs (776.3 mm³) (P = .161). IL-6 expression did not significantly correlate with the presence of exocytosis (P = .080) and cholesterol clefts (P = .190).

### Table 1. Mean Number of Mast Cells and Mean Presence of Macrophages According to the Region on the Capsule, Intensity of Inflammation, Presence of Exocytosis, and IL-6 Expression

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean number of mast cells (mean/range)</th>
<th>P value</th>
<th>Mean presence of macrophages (mean/range)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Periapical cyst region*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fibrous capsule, superficial region</td>
<td>15 ± 11.4</td>
<td>.302</td>
<td>1.19 ± 0.1</td>
<td>.917</td>
</tr>
<tr>
<td>Fibrous capsule, deep region</td>
<td>13.9 ± 9.2</td>
<td></td>
<td>1.17 ± 0.1</td>
<td></td>
</tr>
<tr>
<td>Fibrous capsule, superficial region†</td>
<td>9.2 ± 3.7</td>
<td>.02</td>
<td>1.4 ± 0.6</td>
<td>.393</td>
</tr>
<tr>
<td>Moderate/intense inflammation</td>
<td>16.4 ± 12.2</td>
<td></td>
<td>1.1 ± 0.7</td>
<td></td>
</tr>
<tr>
<td>Fibrous capsule, deep region†</td>
<td>11.4 ± 8.2</td>
<td>.086</td>
<td>1.2 ± 0.7</td>
<td>.935</td>
</tr>
<tr>
<td>Moderate/intense inflammation</td>
<td>17.2 ± 9.8</td>
<td></td>
<td>1.2 ± 0.7</td>
<td></td>
</tr>
<tr>
<td>Marked exocytosis‡</td>
<td>Yes</td>
<td>17.8 ± 11.9</td>
<td>.001</td>
<td>1.2 ± 0.7</td>
</tr>
<tr>
<td>IL-6 expression, superficial region†</td>
<td>No</td>
<td>7.2 ± 3.9</td>
<td></td>
<td>1.2 ± 0.6</td>
</tr>
<tr>
<td>IL-6 expression, deep region†</td>
<td>Yes</td>
<td>14.8 ± 11.3</td>
<td>.738</td>
<td>1.38 ± 0.8</td>
</tr>
<tr>
<td>IL-6 expression, deep region†</td>
<td>No</td>
<td>13.5 ± 7.9</td>
<td></td>
<td>0.98 ± 0.5</td>
</tr>
<tr>
<td>Mean number of mast cells</td>
<td>Yes</td>
<td>12.7 ± 8.7</td>
<td>.560</td>
<td>1.43 ± 0.7</td>
</tr>
<tr>
<td>Ly 6 expression, superficial region†</td>
<td>No</td>
<td>14.8 ± 10.1</td>
<td></td>
<td>0.78 ± 0.8</td>
</tr>
</tbody>
</table>

*Paired Student t test.
†Unpaired Student t test.

**Discussion**

Activated mast cells degranulate and release several preformed substances (histamine, serotonin, heparin, and proteases) in the extracellular environment (10). In addition to these molecules, activated mast cells can synthesize new vasoactive mediators, such as platelet-activating factor, chemotactic agents, and several proinflammatory cytokines, including IL-1α, IL-3, IL-4, IL-5, IL-6, IL-8, IL-10, interferon-gamma, macrophage colony-stimulating factor, tumor necrosis factor-α, and transforming growth factor-β. These cells are also an important source of proteolytic enzymes, such as trypsin and chymase, substances associated with degradation of the connective tissue (10–12). Because of their capacity of degranulation and production of various substances, mast cells are directly associated with inflammatory events, bone resorption, and interaction with other cells from the immune system (11, 12).

Macrophages are considered the main source of IL-1α, IL-1β, and tumor necrosis factor-α, cytokines involved in starting and regulating inflammatory processes, and are also capable of producing several other proinflammatory and anti-inflammatory substances, such as matrix metalloproteinases, prostaglandins, and IL-6 (13). Some of these products act directly in the connective tissue, whereas others indirectly activate bone resorption cells (osteoclasts); on the other hand, other substances act in tissue repair by stimulating fibroblast activation and proliferation and collagen production (13). Macrophages are essential cellular components in the pathogenesis of IPDs, and their presence, together with their secretion products, is essential for both development and repair of PCs (14, 15).

In the present study, mast cells were more frequently found in areas showing marked exocytosis, in accordance with Netto et al (12), who showed that these cells are associated with the presence of inflammation in odontogenic cysts, including PCs. Cholesterol clefts can be found in 29%–43% of the PCs, and they are associated with the induction of a multinucleated giant cell reaction, which is supposedly associated with some endodontic treatment failures (17, 18). Mast cells and macrophages were frequently located in close association with cholesterol clefts in the present studied PCs, but there were no differences in the mean total number of these cells in PCs with and without cholesterol clefts.

Mast cells have also been found in greater number in areas of intense inflammation and at the periphery of the fibrous capsule of PCs (12, 19). The present results did not show statistically significant differences in the mean number of mast cells and macrophages when comparing the superficial and deep regions of the fibrous capsule of PCs. However, in superficial areas, the mean number of mast cells was greater when intense inflammation was present, reinforcing their importance in the pool of inflammatory cells that participate in the response to the local damage in PCs.

IL-6 is produced and secreted by several cell types, including macrophages, B lymphocytes, and other inflammatory cells (20, 21). Apart
from its importance in the inflammatory process, IL-6 is also involved in the regulation of endocrine and metabolic functions such as bone remodeling, stimulating bone resorption both \textit{in vitro} and \textit{in vivo}, and acting directly in osteoclast differentiation and activation (9, 14, 22). It has been considered a key cytokine associated with the development of osteoporosis and other bone diseases mediated by

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure2.png}
\caption{Macrophages in area of chronic and acute inflammation (A and B) (immunoperoxidase; original magnification, $\times 40$). (C) IL-6 expression in superficial region of fibrous capsule in area close to epithelial lining and to cholesterol clefs (immunoperoxidase; original magnification, $\times 20$). (D) IL-6 expression in cytoplasm of macrophages (immunoperoxidase; original magnification, $\times 40$).}
\end{figure}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure3.png}
\caption{(A) IL-6 expression in macrophages and multinucleated giant cells surrounding cholesterol clefs (immunoperoxidase; original magnification, $\times 40$). (B) IL-6 expression in epithelial lining (immunoperoxidase; original magnification, $\times 40$). (C) IL-6 expression in fibroblasts and endothelial cells (immunoperoxidase; original magnification, $\times 40$). (D) Detail of IL-6 expression in endothelial cells (immunoperoxidase; original magnification, $\times 40$).}
\end{figure}
bone resorption (23). Depletion of IL-6 also promotes failure in the recruitment of immune cells to infection sites and some defects in the differentiation of macrophages, directly affecting the mechanisms of host defense against infectious diseases (25). In IL-6–positive super-

ficial regions of the PCs presently studied, mean presence of macrophage was greater than in IL-6–negative areas. In deep regions, this mean was also greater, and the difference with IL-6–negative PCs was statistically significant, reinforcing the evidence that macrophages are the main IL-6–producing cells in PCs.

Apart from the production of IL-6 by inflammatory cells, other components of PCs, such as epithelial cells from the cyst lining, fibro-

blasts, and endothelial cells, can also produce IL-6 (24–27). Gervásio et al (28) have demonstrated that IL-6 was found in the cyst fluid and tissue samples of 92.8% and 86.4% of their studied PCs, respectively, inducing IL-6 production in a dose-dependent ratio (25). Ogura et al with the presence and intensity of the inflammatory infiltrate (33), and of IPD and IL-6 levels, suggesting its role in bone resorption associated have found a positive association between the size of the radiologic image inflammatory and bone resorption mechanisms in IPDs. Martinho et al (32) has induced IL-6 time-dependent and dose-dependent production, sug-

gesting that this relationship can induce an important effect on the inflam-

matory and bone resorption mechanisms in IPDs. Gervásio et al (31) have demonstrated that the treatment of periodontal ligament cells with lipopolysaccharides derived from Porphyromonas endodontalis has induced IL-6 time-dependent and dose-dependent production, suggest-

ing that this relationship can induce an important effect on the inflam-

matory and bone resorption mechanisms in IPDs. Martinho et al (32) have found a positive association between the size of the radiologic image of IPD and IL-6 levels, suggesting its role in bone resorption associated with these lesions. It has also been demonstrated that the proliferative ac-

tivity of the epithelial lining cells in periapical cysts is directly associated with the presence and intensity of the inflammatory infiltrate (35), and that inflammation can also modulate the expression of cytokines and tissue-degrading enzymes in PCs (34). The results from the present study showed that the mean gross volume of the PCs with IL-6–positive super-

ficial regions was greater than the mean volume of IL-6–negative PCs. On the other hand, the mean volume of the PCs presenting deep IL-6–posi-

tive regions was lower than that of the IL-6–negative PCs. These results show that in the deep regions of PCs presenting greater volume, IL-6 expression was lower than in smaller cysts, suggesting that IL-6 expres-

sion in deep regions decreased with evolution of the lesions. These find-

ings support the evidence that IL-6 can act as a proinflammatory cytokine in superficial regions of PCs, and that it is not the most important bone resorption mediator in larger PCs.

The results of the present study showed that in the studied PCs, the presence of mast cells was more evident in areas showing exocytosis and intense superficial inflammation. The presence of macrophages was greater in IL-6–positive regions, and this interleukin was less expressed in the deep region of the PCs presenting greater volume, supporting that IL-6 is not the most important bone resorption mediator on these lesions. These findings reinforce the participation of mast cells, macrophages, and IL-6 in the pathogenesis of PCs and support the evidence that IL-6 is mostly produced by macrophages, and that it seems to be less associated with bone resorption with progression of the lesions. Other studies including the evaluation of mechanisms of tissue repair and their relation-

ship to IL-6 expression would highlight additional evidence of its impor-

tance and participation in the pathogenesis and development of PCs.

Acknowledgments

The authors thank FAPERJ for the financial support for the study.


