Higher Expression of Galectin-3 and Galectin-9 in Periapical Granulomas than in Radicular Cysts and an Increased Toll-like Receptor-2 and Toll-like Receptor-4 Expression Are Associated with Reactivation of Periapical Inflammation

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Abstract

Introduction: Cysts and periapical granulomas are inflammatory reactions that develop in response to periapical infection by microbial species in dental root canal. It is known that toll-like receptors (TLRs) are pathogen recognition molecules and that galectins are lectins that can be associated with the inflammatory process, stimulating or inhibiting the immune system. The objective of this study was to evaluate the *in situ* expression of TLRs and galectins in radicular cysts and periapical granulomas. **Methods:** We analyzed 62 cases (30 radicular cysts, 27 periapical granulomas, and 5 control cases). Indirect immunohistochemistry was used to evaluate the expression of TLRs (TRL-2 and TLR-4) and galectins (Gal-3 and Gal-9). **Results:** The expression of Gal-3 and Gal-9 was significantly higher in periapical granulomas and radicular cysts than in the control group. Similarly, both Gal-3 and Gal-9 were expressed significantly more in periapical granulomas than in radicular cysts. The expression of TLR-2 was significantly higher in periapical granulomas and radicular cysts than in the control group, and it was also significantly higher in radicular cysts with sinus tract than in the cases without sinus tract. Furthermore, the expression of TLR-4 was significantly higher in the cases of periapical granulomas with sinus tract than in the cases without sinus tract. **Conclusions:** Gal-3/Gal-9 and TLR-2/TLR-4 expression in the periapical granulomas and radicular cysts is associated with reactive periapical inflammation. Pathobiology of periapical disease is a very complex interplay of many bioactive molecules involved in immunoinflammatory responses. Up-regulation of these bioactive molecules might be an important modulator of inflammatory periapical lesions. *(J Endod 2014;40:199–203)*

Key Words

Galectins, radicular cysts and periapical granulomas, TLR

Cysts and granulomas are chronic periapical lesions mediated by a combination of inflammatory mediators that develop to prevent periapical infection in response to microbial species in dental root canal (1). Innate recognition of bacterial products is a major bulwark of host defense against infection. Moreover, innate mechanisms, including phagocytic leukocytes and cytokines, play a central role in the pathogenesis of oral infections (2). The recently identified family of toll-like receptors (TLRs), homologous to *Drosophila* Toll, are key participants in innate recognition of pathogens. Exogenous danger signals are usually microbe-derived and can also be referred to as pathogen-associated molecular patterns, which are absent from normal host tissues but common to broad classes of potential pathogens (3). These are sensed by a number of specialized transmembrane receptors as well as by cytoplasmic receptors, the best studied of which belong to the TLR family (4), which is currently known to consist of 10 members in humans. TLR-2 and TLR-4 are implicated in the recognition of various bacterial cell wall components, such as lipopolysaccharide (LPS) including peptidoglycan (5) and lipoteichoic acid (6). The role of TLRs in responses to oral pathogens and in alveolar bone destruction is unknown. The presence of TLR-expressing cells in periapical granulomas and in cysts provides further evidence that periapical cysts are likely to be sustained by the immune system via reaction to bacterial antigens (7). Mammalian lectins also play an important role in the innate and adaptive immune system. Among these, galectins are members of a β-galactoside–binding lectin family involved in the regulation of host immune responses during physiological and pathologic conditions, including inflammation, cancer, and infection (8–10). Extracellular and intracellular galectins have multiple functions, including cell adhesion, signaling, proliferation, differentiation, survival, and apoptosis (8). Galectin-3 (Gal-3) is expressed in many inflammatory cells, including monocytes, dendritic cells, macrophages, eosinophils, mast cells, natural killer cells, and activated T and B cells (11, 12). Because Gal-3 is capable of binding polylactosamine structures on endogenous ligands (13), it interferes with several cellular processes *in vitro*. It has been recently demonstrated that Gal-3 binds to several pathogens, such as mycobacteria, protozoan parasites, and yeast (14), and,

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thus, it participates in the phagocytosis of some microorganisms by macrophages, as well as it triggers host responses to pathogens, at least *in vitro* (15). Other members of the galectin family, such as Galectin-9 (Gal-9), are thought to be essential for regulating cell homeostasis and inflammation (16). Previous studies demonstrated that Gal-9 induces various biological reactions, such as cell aggregation, adhesion, chemoattraction, activation, and apoptosis (17). The expression of Gal-3 and Gal-9 in periapical granulomas and cysts could not be found in the literature. It is unclear whether galectins contribute to signaling events in accessory cells during infection *in vivo*. Likewise, their role in the signaling pathways triggered by TLRs, which are key sensors in innate immune cells (18), is still elusive. The present study aimed to evaluate the expression of galectins (Gal-3 and Gal-9) and TLRs (TLR-2 and TLR-4) in human periapical granulomas and radicular cyst capsules in comparison with control tissues.

Materials and Methods

Tissue Samples

Thirty cases of human radicular cysts and 27 cases of human periapical granulomas were studied. Periapical lesions were diagnosed on the basis of clinical and histopathologic findings. Radicular cysts were defined as follows:

1. A lesion in the periapical region of a nonvital tooth
2. A cavity with a fluid or semisolid content detected during surgery
3. Histologic evidence of nonkeratinizing stratified squamous epithelium completely or partially lining a cystic cavity or tissue fragments

For dental granulomas, in addition to the clinical features mentioned above, we also observed a chronic inflammatory reaction in the biopsy specimen detected by histopathologic analysis. The following data were obtained from the patients' records: age, gender, location of the lesions, and symptoms such as palpation or spontaneous tenderness and swelling at the time of diagnosis and surgical intervention. Radiographs were evaluated for the evidence of previous endodontic treatment and the size of the periapical radiolucency. Furthermore, the radiographic limits of the lesions were analyzed and classified as defined (with or without a radiopaque line around the lesion and no visible bone resorption) or diffuse (no distinct limits and clearly visible bone resorption). Dental pulp samples were obtained from a group of impacted third molars recommended for extraction. These tissues did not show any inflammation and were used as control samples. The samples were collected during surgery at the Dental Clinic of UNIUBE, Uberaba, Minas Gerais, Brazil and were fixed in 10% buffered formalin for 24 hours. The sections were then embedded in paraffin, and histologic sections of approximately 5 mm were performed. The control group consisted of pulp tissues obtained from 5 patients with a surgical indication for extraction of impacted healthy third molars. The study was approved by the Ethics Committee of UNIUBE under protocol CAAE–0003.0.227.000-06.

Immunohistochemistry

For immunohistochemistry, deparaffinized sections were treated with 3% hydrogen peroxide in methanol for 10 minutes and incubated at 90°C for 30 minutes for antigen detection. The sections were incubated in 2% bovine serum albumin at room temperature for 30 minutes to reduce nonspecific binding. Then the specimens were individually incubated with polyclonal antibodies specific for human anti–galectin-3 (1:75) (AF-1154) (R & D Systems, Minneapolis, MN), monoclonal antibodies specific for human anti–galectin-9 (1:75) (AF-2045) (R & D), monoclonal antibodies specific for human anti–TLR-2 (1:200) (PRS-3135) (Sigma-Aldrich, St Louis, MO), and anti–TLR-4 (1:50) (AF-1478) (R & D), diluted in 2% bovine serum albumin before use at 37°C for 2 hours. The specimens were then incubated with secondary biotinylated anti-mouse immunoglobulin (Ig), anti-rabbit Ig, and anti-goat Ig antibodies by using Link System 002488 (Dako, Carpinteria, CA) at 37°C for 30 minutes. The sections were washed and then incubated with streptavidin-peroxidase conjugate (Dako) for 30 minutes. The reaction was developed by incubation with diaminobenzidine (Sigma-Aldrich). The sections were counterstained with hematoxylin.

Morphometric Analysis

For morphometric analysis, immunopositive cells were quantified by using images of the histologic sections captured with a digital system and analyzed by using Image J software (National Institutes of Health, Bethesda, MD). For this purpose, each field to be quantified was captured with a camera coupled to a common light microscope and to a computer to digitize the image. The number of cells in each field was determined as well as the area of each field (0.091575 mm^2). The density of positive cells was expressed as the number of cells per square millimeter.

Statistical Analysis

The data were analyzed by using Statview software (Abacus Concepts, Berkeley, CA). After analysis for normality and variance of the data, Mann-Whitney and Kruskal-Wallis tests were performed. *P* values $<.05$ were considered to be statistically significant.

Results

The expression of Gal-3 was significantly higher in the periapical granulomas and in the radicular cysts in comparison with the control group. By comparing the 2 periapical lesions, it was observed that Gal-3 was expressed significantly more in periapical granulomas (Fig. 1*A*) (Kruskal-Wallis, $P < .001$).

Similarly, the expression of Gal-9 was significantly higher in periapical granulomas and in radicular cysts than in the control group. When comparing both periapical lesions, we noticed that Gal-9 was expressed significantly more in periapical granulomas (Fig. 1*A*) (Kruskal-Wallis, $P = .009$). Both the granuloma and the radicular cyst were connected with an increase in the expression of the 2 galectins studied herein, and the expression was more intense in periapical granuloma. There was no difference in the expression of Gal-3 and Gal-9 when the cases were grouped according to the presence of sinus tract (data not shown).

Expression of TLR-2 was significantly higher in periapical granulomas and radicular cysts than in the control group (Fig. 1*B*) (Kruskall Wallis, $P < .001$). However, there was no significant difference when comparing TLR-2 expression among the 2 periapical lesions.

The expression of TLRs in periapical lesions was further compared in each lesion, according to the presence of sinus tract. In radicular cysts, the expression of TLR-2 was significantly higher in lesions with sinus tract when compared with those without sinus tract (Fig. 1*C*) (Mann-Whitney, $P = .03$). There was no significant difference when comparing TLR-2 expression with sinus tract in the cases of granulomas.

On the other hand, the expression of TLR-4 was significantly higher in the cases of periapical granulomas with sinus tract than in the cases of granulomas without sinus tract (Fig. 1*D*) (Mann-Whitney, $P = .016$). Nonetheless, there was no significant difference when comparing the expression of TLR-4 in radicular cysts grouped according to the presence of sinus tract. In periapical granulomas, there was no difference when comparing TLR-4 among sinus tract on samples. Figure 2*A–H* illustrates typical immunohistochemical findings of each marker.

Discussion

Granulomas and cysts represent a spectrum of inflammatory sequestration responses that may be triggered by a variety of agents,