Immunohistochemical Analysis of Galectins-1, -3, and -7 in Periapical Granulomas, Radicular Cysts, and Residual Radicular Cysts

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Abstract

Introduction: Galectins play important roles in immunoinflammatory responses, but their participation in the development of periapical lesions remains unclear. This study aimed to evaluate the expressions of galectins-1, -3, and -7 in periapical lesions, correlating them with the intensity of the inflammatory infiltrate and the pattern of the cystic epithelium. **Methods:** Twenty periapical granulomas (PGs), 20 radicular cysts (RCs), and 20 residual radicular cysts (RRCs) were submitted to immunohistochemistry using anti–galectin-1, -3, and -7 antibodies. The percentage of immunopositive cells in epithelial and connective tissues was determined. **Results:** In connective tissue, PGs exhibited higher cytoplasmic/membrane expression of galectins-1 and -7 than RCs and RRCs ($P < .05$). There was higher nuclear expression of galectin-1 in PGs compared with RCs and RRCs ($P < .05$). The expression of galectins-1 and -7 in connective tissue was higher in lesions with grade III inflammation ($P < .05$). No significant differences in galectin-3 immunoexpression were observed for any of the parameters evaluated ($P > .05$). In the epithelial component, a higher nuclear expression of galectin-7 was detected in RRCs ($P < .05$), and a higher cytoplasmic/membrane expression of this protein was found in cysts with hyperplastic epithelium ($P < .05$). Positive correlations were observed between the nuclear and cytoplasmic/membrane expression of galectin-1 in connective tissue ($P < .05$) as well as between the nuclear and cytoplasmic/membrane expression of galectin-7 in epithelial tissue of cysts ($P < .05$). **Conclusions:** Galectins-1 and -7 may play important roles in the pathogenesis of PGs, RCs, and RRCs. On the other hand, the present results suggest only a minor involvement of galectin-3 in the development of these lesions. *(J Endod 2018;■:1–6)*

Key Words

Galectins, immunohistochemistry, jaw cysts, periapical granuloma, radicular cysts

Significance

This study provides additional insight into the mechanisms underlying the pathogenesis of periapical lesions. Considering the molecules involved in immunoinflammatory responses, galectins-1 and -7 may play important roles in the development of periapical granulomas, radicular cysts, and residual radicular cysts.

Infection of a root canal after pulp necrosis can spread apically and induce inflammation in periapical structures. This process can stimulate the destruction of periapical tissues and induce apical inflammatory root resorption (1). If the stimulus persists after the acute phase, the periapical lesion can progress to 1 of its chronic forms including a periapical granuloma (PG) or a radicular cyst (RC). Inflammatory mediators modulate the formation of these lesions, which develop in response to microbial components in the root canal (2, 3). In contrast, residual radicular cysts (RRCs) are RCs that remain in the gnathic bones after removal of the associated tooth (4, 5). These cysts histopathologically resemble RCs but have no communication with the anteriorly infected root canal system (5).

Various molecules are involved in the development and maintenance of chronic inflammatory processes and the modulation of the immune response, including galectins (6–8). These proteins exert different functions in biological processes, such as the regulation of cell growth and inflammatory responses as well as the maintenance of immune homeostasis (7–10). However, the role of galectins in the inflammatory response of periapical lesions remains unclear.

Galectins comprise a family of animal lectins, and 15 types have been identified in humans so far. All of them exhibit affinity for beta-galactosides but lack a classic signal sequence (10, 11). Galectins are predominantly intracellular proteins that are synthesized on ribosomes and then transported to cytosol, other cytoplasmic organelles, and the cytoplasmic membrane (9). In addition, these proteins can be expressed in the extracellular medium (12). Galectins play important roles in cell homeostasis during the innate and adaptive immune response including regulating cell survival, signaling, chemotaxis, and cell growth; mediating cell-cell and cell-matrix interactions; and modulating the secretion of cytokines (9, 13, 14).

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Despite their multifunctional role in immunoinflammatory responses, studies on the presence of galectins in periapical lesions are sparse in the literature. However, these molecules may represent an important mechanism for the regulation of inflammation in periapical lesions of inflammatory origin (14). Therefore, the objective of the present study was to evaluate the immunoexpression of galectins-1, -3, and -7 in PGs, RCs, and RRCs and their correlation with the intensity of the inflammatory infiltrate and the pattern of the epithelial lining in cystic lesions in order to verify the participation of these galectins in the pathogenesis of these lesions.

Materials and Methods

Sample Selection

Tissue samples of 60 specimens (20 PGs, 20 RCs, and 20 RRCs) stored in the archives of the Laboratory of Oral Histopathology, Department of Dentistry, State University of Paraíba, Campina Grande, Paraíba, Brazil, were selected. The PG and RC specimens were obtained from the periapex of human teeth without a history of endodontic treatment, and RRCs were collected at previous extraction sites of teeth affected by periapical lesions. For all cases, hematoxylin-eosin–stained slides (3–5 serial sections of 5-μm thickness) were re-evaluated by 3 oral pathologists. Only PGs without an odontogenic epithelium were selected for the study. All RCs and RRCs presented an unequivocal cystic cavity lined by a nonkeratinized stratified squamous epithelium. The study was approved by the Ethics Committee of the State University of Paraíba (approval no. 51330315.0.0000.5187).

Morphologic Analysis

For morphologic study, 5-μm-thick sections were stained with hematoxylin-eosin, and 2 previously calibrated observers analyzed the sections under a light microscope (Leica DM 500; Leica Microsystems Vertrieb GmbH, Wetzlar, Germany). The intensity of the inflammatory infiltrate was evaluated from the center of PGs and from the luminal portion of the cystic lesions toward the periphery. One microscopic field was examined at 200× magnification. Specimens whose inflammatory infiltrate was restricted to one third of the microscopic field were classified as grade I, lesions with inflammatory cells in up to two thirds as grade II, and lesions with an inflammatory infiltrate exceeding two thirds as grade III (15).

The pattern of the epithelial lining was determined based on the predominant type in each case. Cysts exhibiting a predominant epithelial lining consisting of 2 to 10 cell layers and a plane interface between the capsule and epithelium were classified as atrophic. A variable thickness (more than 10 cell layers) and an arcuate interface between the capsule and epithelium classified the epithelial lining as hyperplastic (16).

Immunohistochemistry

Three-micrometer-thick sections were mounted on glass slides prepared with organosilane adhesive. The sections were deparaffinized, submitted to antigen retrieval (Table 1), and immersed in 3% hydrogen peroxide to block endogenous peroxidase. The tissue sections were then washed with Tris buffer and incubated with the primary mouse monoclonal anti–galectin-1, rabbit polyclonal anti–galectin-3, and mouse monoclonal anti–galectin-7 antibodies (Table 1) in a moist chamber. The sections were washed twice with Tris buffer and treated at room temperature with a dextran polymer–based complex (Reveal; Spring Bioscience, Pleasanton, CA). Peroxidase activity was developed by immersing the sections in diaminobenzidine (Liquid DAB+; Dako, Carpinteria, CA), which resulted in a brown reaction product. Finally, the sections were counterstained with Mayer hematoxylin and coverslipped.

Histologic sections of normal human tonsils were used as the positive control for galectins-1 and -3, whereas sections of inflammatory fibrous hyperplasia specimens were chosen for galectin-7. Sections in which the primary antibody of the protocol was replaced with 1% bovine serum albumin in buffer served as the negative control.

Immunohistochemical Evaluation and Statistical Analysis

Under a light microscope (Leica DM 500), a previously calibrated observer identified 10 fields of the highest immunoreactivity at 200× magnification, 5 fields for analysis of the epithelial lining, and 5 for analysis of connective tissue. Photomicrographs of the areas were obtained at 400× magnification (ICC 50HD, Leica Microsystems Vertrieb GmbH), and the images were transferred to the ImageJ program (Image Processing and Analysis in Java; National Institutes of Mental Health, Bethesda, MD). The number of positive and negative cells in each component per photographed microscopic field was determined, permitting calculation of the percentage of positive cells with nuclear or cytoplasmic/membrane staining in relation to the total number of cells counted.

The results were analyzed using the IBM SPSS Statistics 20.0 program (IBM SPSS Inc, Armonk, NY). After the verification of normality and variance of the data, the nonparametric Mann-Whitney and Kruskal-Wallis tests were applied. The Spearman correlation test was used to evaluate possible correlations among the expression of the different galectins. In view of the small number of cases exhibiting nuclear staining for galectins-3 and -7 in connective tissue cells and for galectins-1 and -3 in epithelial cells, only descriptive statistics were used to analyze these data. A level of significance of 5% ($P < .05$) was adopted for all tests.

Results

Morphologic Analysis

Analysis of the inflammatory infiltrate classified all cases (100%) of PGs as grade III. For RCs, 16 (80%) cases were classified as grade III, 2 (10%) as grade II, and 2 (10%) as grade I. In contrast, 12 (60%) of the RRC cases were classified as grade I, 6 (30%) as grade III, and 2 (10%) as grade II.

Evaluation of the epithelial lining of the cystic lesions revealed 10 (50%) cases of RCs with an atrophic epithelium and 10 (50%) with a hyperplastic epithelium. In RRCs, 13 (65%) cases exhibited the atrophic pattern and 7 (35%) the hyperplastic pattern.

TABLE 1. Specificity, Clone, Manufacturer, Dilution, Antigen Retrieval, and Incubation of Primary Antibodies

Specificity	Clone	Manufacturer	Dilution	Antigen retrieval	Incubation
Galectin-1	(E-2)	Santa Cruz Biotechnology, Dallas, TX	1:2500	Citrate, pH = 6.0, steamer, 60 min	60 min
Galectin-3	(H-160)	Santa Cruz Biotechnology	1:1000	Citrate, pH = 6.0, steamer, 60 min	60 min
Galectin-7	(H-8)	Santa Cruz Biotechnology	1:2000	No treatment	60 min