Immunoexpression of Transforming Growth Factor Beta and Interferon Gamma in Radicular and Dentigerous Cysts

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Abstract

Introduction: The aim of this study was to evaluate and compare the immunohistochemical expression of transforming growing factor beta (TGF-β) and interferon gamma (IFN-γ) between radicular cysts (RCs) and dentigerous cysts (DCs). **Methods:** Twenty RCs and DCs were selected for analysis of the immunoexpression of TGF-β and IFN-γ in the epithelium and capsule. **Results:** The cell reactivity of TGF-β and IFN-γ in the lining epithelium and capsule of RCs showed no significant differences when compared with DCs ($P > .05$). There was a tendency of a higher expression of TGF-β in the capsule of DCs. **Conclusions:** Our results showed the presence of TGF-β and IFN-γ in RCs and DCs, supporting the hypothesis that both participate in the development of these lesions, where IFN-γ usually plays a role in bone resorption, which is counterbalanced by the osteoprotective activity performed by TGF-β. *(J Endod 2014;40:1293–1297)*

Key Words

Dentigerous cyst, immunohistochemistry, interferon gamma, radicular cyst, transforming growth factors

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Odontogenic cysts are one of the most common osseous-destructive lesions in the jaws. Traditionally, they are classified as developmental cysts, including dentigerous cysts (DCs), and inflammatory cysts, such as radicular cysts (RCs). The origin of inflammatory cysts is thought to be from an inflammatory process, whereas the origin of developmental cysts remains unknown; however, it does not seem to be related to an inflammatory process (1–3).

The precise biological mechanisms of the development and enlargement of jaw cysts have not been completely enlightened. Disturbances in the number and activity of osteoclastic cells cause most of the bone diseases, resulting in improper bone resorption, which exceeds the compensatory capacity of osteoblasts (1, 4). The regulation of osteoclast biology and bone metabolism implies various mediators, including receptor activator of nuclear factor kappa-B (RANK), receptor activator of nuclear factor kappa-B ligand (RANKL), and osteoprotegerin (OPG), (1, 5, 6) and their complex interrelationships with cytokines and growth factors, such as interferon-gamma (IFN-γ) and transforming growth factor-beta (TGF-β), respectively (7–9).

IFN-γ has been implicated as an inductor of osteoclastogenesis by inducing the synthesis of RANKL (9–11). On the other hand, IFN-γ may suppress osteoclast differentiation induced by RANKL, inhibiting bone resorption (12, 13). Therefore, the final effect of IFN-γ on bone tissue depends on the imbalance between its osteoprotective and osteoresorption activity, which under circumstances of inflammatory and infectious diseases, is in favor of bone resorption (9, 11).

TGF-β has also been associated with bone resorption, representing an indispensable factor in RANKL-induced osteoclastogenesis (7, 14). However, TGF-β may act as a stimulator of bone formation through a favorable effect in osteoblastogenesis (15–17). Recent studies have shown the participation of IFN-γ and TGF-β in inflammatory periapical lesions (8, 10, 18–22). The purpose of this study was to evaluate and compare the immunohistochemical expression of IFN-γ and TGF-β between DCs and RCs.

Materials and Methods

The original hematoxylin-eosin–stained slides and formalin-fixed paraffin-embedded specimen blocks of all RCs and DCs diagnosed between January 2000 and October 2009 were retrieved from the files of the Oral Pathology Service at Federal University of Rio Grande do Norte (UFRN), Natal, Brazil, from the Department of Histopathology. The hematoxylin-eosin slides were reviewed to confirm the diagnosis. The tissues were classified as cysts whenever a partial or total epithelium lining was present. The diagnosis of cysts was based mainly on radiographic and histopathologic examination. DCs showing intense inflammation and cysts with inadequate tissue samples were excluded. A total of 20 RCs and 20 DCs were selected for the study. The clinical and radiographic information, including age, sex, and anatomic site, were obtained from biopsy forms submitted by the clinicians.

Immunohistochemistry

For the immunohistochemical reactions, the tissue section samples were deparaffinized with xylene, rehydrated in graded alcohols, and washed in deionized water and phosphate-buffered saline (PBS). Samples were then incubated with 3% hydrogen

peroxide and immersed in pepsin buffer at 37°C, with a pH level of 1.8 for 60 minutes. Sections were then blocked by incubation with 3% normal goat serum at room temperature for 20 minutes, and slides were incubated at 4°C overnight in a humidified chamber with the following primary rabbit polyclonal antibodies: anti–IFN-γ (SC-8308; Santa Cruz Biotechnology, Santa Cruz, CA) diluted 1:200 and anti–TGF-β1 (SC-146, Santa Cruz Biotechnology) diluted 1:700. The tissue sections were then washed twice in PBS and treated with an immunoperoxidase-based kit (ADVANCETM HRP; Dako, Carpinteria, CA) at room temperature in order to bind the primary antibodies. Peroxidase activity was visualized by immersing tissue sections in diaminobenzidine (Liquid DAB + Substrate, Dako), resulting in a brown reaction product. Finally, tissue sections were counterstained with Mayer's hematoxylin and coverslipped. Positive controls were sections of breast cancer for TGF-β and central giant cell lesions for INF-γ and negative controls; samples were treated as described previously, except that the primary antibody was replaced by a solution of bovine serum albumin in PBS.

Cell Counting

The immunoexpression of TGF-β and INF-γ was evaluated in lining epithelium and fibrous capsule. The epithelial immunoexpression was quantitatively evaluated by 2 observers using ×400 magnification and classified according to the following scores: 0: no staining (<10% of positive immunostaining cells), 1: weak (11%–25%), 2: moderate (26%–75%), and 3: strong (>76%). In the fibrous capsule, the analysis was quantitative, and the number of positive cells was counted in 10 representative and consecutive microscopic high-power fields (1000×) over totally counted cells irrespective of the cell type. Digital images were loaded on the software IMAGE J (National Institutes of Health, Bethesda, MD) to count the number of immunostained cells. The results are expressed as the mean percentage of observations per field, with the following modifications. Based on this mean percentage, a score was determined for each case, taking into account the standard scoring system used for the lining epithelium: 0: no staining (<10% immunostained cells), 1: weak (11%–25% of cells), 2: moderate (26%–75% of cells), and 3: strong (more than 76% of cells) (1). These counting procedures were performed for the 2 biomarkers in both lesions.

Statistical Analysis

Comparative analysis of data was performed using the nonparametric Mann-Whitney *U* test. Statistical significance was set at $P \leq .05$.

Results

Clinical, Radiographic, and Histologic Findings

This study analyzed 20 cases of RCs and 20 cases of DCs, with mean ages of 32.5 ± 13.67 and 24.79 ± 12.35 years, respectively. Female preponderance was found in RC cases and male preponderance in DCs. The most common location for RCs was the anterior maxilla, and for DCs it was the posterior mandible. Regarding the radiographic aspect, all samples were described as well-circumscribed unilocular radiolucency. Histologic appearance of the cysts revealed the presence of a hyperplastic epithelium and an inflammatory infiltrate, which was moderate to intense in most RCs. DCs showed an atrophic epithelium, hemorrhagic areas, and scarce infiltrate in most of the cases.

Quantitative Analysis of Lining Epithelium

Immunohistochemical reactivity for TGF-β and INF-γ was detected in the nuclei and cytoplasm of epithelial cells. TGF-β appears positive in the suprabasal and basal epithelial cells in RCs (Fig. 1*A*) and DCs (Fig. 1*B*). TGF-β revealed a predominance of score 3 (>75% positive cells) in RCs (75%) (Fig. 2) and DCs (85%) (Fig. 3), with no significant differences between the groups ($P = .620$).

INF-γ exhibited positivity in the basal and suprabasal epithelial cells in RCs (Fig. 1*C*) and DCs (Fig. 1*D*). The analyses of the immunoreactivity of INF-γ according to the percentage of the scores revealed a predominance of score 3 (>75% positive cells) in RCs (85%) (Fig. 2) and DCs (75%) (Fig. 3), with no significant differences between groups ($P = .565$).

Quantitative Analysis of Fibrous Capsule

With regard to reactivity for TGF-β and INF-γ in the stromal cells, the presence of positive fibroblasts, polymorphonuclear neutrophils, plasmocytes, lymphocytes, and macrophage cells were observed. The immunoreactivity was predominantly in the cytoplasm.

Figure 3 summarizes the quantitative analysis of lesions immunostained for TGF-β and INF-γ in the fibrous capsule. TGF-β revealed a predominance of score 3 (>75% positive cells) in RCs (80%) (Fig. 2) and DCs (95%) (Fig. 3), with no significant differences between groups ($P = .429$). The analyses of the immunoreactivity of INF-γ according to the percentage of the scores revealed a predominance of score 3 (>75% positive cells) in RCs (75%) (Fig. 2) and DCs (60%) (Fig. 3), with no significant differences between groups ($P = .429$).

Discussion

Cyst formation is believed to be related to the release of cytokines and growth factors leading to the activation of the proliferation of epithelial remnants (23). In the present study, we have examined the immunoexpression to IFN-γ and TGF-β in RCs and DCs. Although presenting as pathological entities of a different nature, the presence of these molecules may act in a direct or indirect way favoring the enlargement of the cysts. Irrespective of the stimulus for the development of injury, the presence of a Th1 or Th2 immune response may lead to changes in TGF-β and IFN-gamma immunoexpression through costimulation with proinflammatory cytokines. For RCs, the presence of inflammation promotes the release of proresorptive factors. In DC development, there may be indirect stimuli associated with the activation of other factors such as the vascular endothelial growth factor (VEGF), which may act as substitute for macrophage colony-stimulating factor (M-CSF) in the support of osteoclastic bone resorption (1, 24). Furthermore, as suggested by our team in previous studies (1, 24), an increased expression of factors such as RANKL and VEGF may induce the differentiation and survival of osteoclasts. In this way, by the frequent presence of hemorrhagic areas in the capsule of DCs, we can assume that the increased immunoreactivity of VEGF in this entity may contribute to bone resorption and cystic expansion because VEGF acts as an important molecule for the recruitment of osteoclasts.

The immunoinflammatory reaction in periapical lesions causes the activation of a Th1 immune response was characterized by the production of proinflammatory cytokines such as IFN-γ, tumor necrosis factor alpha (TNF-α), and interleukin (IL)-1, IL-2, and IL-12, and the Th2 response was characterized by synthesis of anti-inflammatory cytokines such as IL-4, IL-5, IL-6, IL-10, and IL-13. In addition to these cytokines, there is also the presence of growth factors such as TGF-β. Th1 and Th2 have opposite activities; in the context of periapical lesions, it is believed that a Th1 immune response with its major cytokine IFN-γ is associated with progression and the process of bone resorption, whereas the immunosuppressive action of TGF-β and Th2 cytokines acts in the modulation of the immune-inflammatory response and the repair of damaged bone tissue (8, 9, 25–28).