Immunoexpression of Interleukin 17 in Apical Periodontitis Lesions

Natasha C. Ajuz, MSc, Henrique Antunes, MSc,*† Thais A. Mendonça, PhD,† Fábio R. Pires, PhD,* José F. Siqueira, Jr, PhD,* and Luciana Armada, PhD**

Abstract

Introduction: Interleukin (IL)-17 expression has been detected in apical periodontitis lesions, but its role in the disease process remains unclear. The present study compared the expression of IL-17 in periradicular cysts and granulomas and evaluated the association of this cytokine with clinical and radiographic findings. **Methods:** Apical periodontitis lesions (18 cysts and 20 granulomas) were obtained from 38 patients subjected to periradicular surgery. Some clinical, radiographic, and cone-beam computed tomographic features were recorded. Silanized slides containing paraffin sections were used for the immunohistochemical reactions using anti–IL-17 antibody. Image analysis was performed using an optical microscope, and each sample was divided into 5 high-power fields, which were evaluated for the expression of IL-17 in the epithelium and connective tissues. Results were evaluated for correlations with the lesion size and the occurrence of symptoms and sinus tract. **Results:** Expression of IL-17 was significantly higher in cysts than in granulomas (P = .02). Among the periradicular cysts, a thin epithelium showed significantly increased labeling for IL-17 when compared with a hyperplastic epithelium (P = .003). IL-17 expression was usually associated with focal accumulations of polymorphonuclear leukocytes. No association of IL-17 expression with symptoms, sinus tract, or lesion size was observed (P > .05). **Conclusions:** The present study reinforces the notion that IL-17 may take part in the pathogenesis of apical periodontitis lesions. A role in the exacerbation of chronic inflammation and cyst formation is suspected. Further studies are required to shed light on the specific functions of IL-17 in periradicular inflammatory processes. *(J Endod 2014;40:1400–1403)*

Key Words

Apical periodontitis, immunohistochemistry, interleukin 17, periradicular cyst, periradicular granuloma

Apical periodontitis is an inflammatory disease of microbial origin primarily caused by bacterial infection of the root canal system (1). Approximately 500 bacterial species and phylotypes belonging to 100 genera and 9 phyla have been detected in different types of endodontic infections (2). In response to bacterial infection of the root canal, the host mobilizes the immune defense to the periradicular tissue areas near the portal of exit of bacteria. Host defense mechanisms are represented by cells and molecules that, in most cases, are effective in eliminating bacteria that leave the root canal but cannot eliminate the source of infection located intraradicularly (3).

The role of T lymphocytes in the pathogenesis of apical periodontitis has been widely studied (4–6). After sensitization by antigen-presenting cells, T lymphocytes can release proinflammatory cytokines such as interleukin (IL)-1β, IL-6, IL-11, IL-17, and tumor necrosis factor alpha, which play a crucial role in the process of the formation of apical periodontitis by stimulating up-regulation of receptor activator nuclear factor kappa B ligand and decrease of osteoprotegerin, with increased osteoclastogenesis (7). As a consequence of chronic inflammation established at the periradicular tissues, a granuloma and a cyst may develop. Studies comparing the immunologic features of these 2 conditions have shown differences in the cellular infiltrates and cytokine expression patterns (8–11). Even so, several aspects related to the origin and development of periradicular cysts remain unknown.

In addition to T_H1 and T_H2 cells, T_H17 cells comprise another subset of specialized effector T cells, which are characterized by their secretion of IL-17 and their association with inflammatory responses dominated by polymorphonuclear neutrophils (PMNs). T_H17 cells may be important mediators of tissue damage in immune-mediated inflammatory diseases and seem to play a dominant role in exacerbating inflammation (12). These cells do not produce either interferon gamma or IL-4. In fact, their differentiation from naive $CD4^+$ T cells is inhibited by the presence of either interferon gamma or IL-4, indicating that T_H17 cells are a unique subset distinct from T_H1 and T_H2 cells. A study has suggested a role for IL-17 in the pathogenesis of apical periodontitis (13). By stimulating the production of IL-8/CXCL8, IL-17 may play a role in exacerbating inflammation in apical periodontitis lesions (8, 14, 15). The ability of IL-17 to regulate the production of matrix metalloproteinases may contribute to periradicular tissue destruction (16). This cytokine may also be related to bone resorption (17–19).

Although IL-17 expression has been recently detected in apical periodontitis lesions in humans (6, 8, 11, 20, 21), its role in the disease process remains unclear. In the present study, we intended to compare IL-17 expression in periradicular cysts and granulomas and to evaluate the association of this cytokine with some clinical and radiographic findings.

From the *Department of Endodontics and Immunohistochemistry Laboratory, Faculty of Dentistry, Estácio de Sá University, Rio de Janeiro, RJ, Brazil; and †Department of Endodontics, Faculty of Dentistry, Grande Rio University, Duque de, Caxias, RJ, Brazil.

Address requests for reprints to Prof Luciana Armada, Postgraduate Program in Dentistry, Estácio de, Sá University, Av Alfredo Baltazar de Oliveira, 580 Recreio dos Bandeirantes, CEP 22790-710, Rio de Janeiro/RJ, Brazil. E-mail address: luadias@hotmail.com

0099-2399/$ - see front matter

http://dx.doi.org/10.1016/j.joen.2014.03.024

TABLE 1. Distribution of Demographic Parameters

Parameters	Cysts (*n*) (%)	Granulomas (*n*) (%)
Sex		
Male	7 (39)	10 (50)
Female	11 (61)	10 (50)
Location		
Maxilla	14 (78)	14 (70)
Mandible	4 (22)	6 (30)
Region		
Anterior	12 (67)	13 (65)
Posterior	6 (33)	7 (35)

Materials and Methods

Biopsy specimens obtained by periradicular surgery from 104 patients who had been referred for surgery at the Clinic of Endodontics, Faculty of Dentistry, Grande Rio University, Duque de Caxias, RJ, Brazil, were initially included in this study. Apical periodontitis lesions were obtained by curettage, fixed in 10% buffered formalin for 48 hours, and then processed for histologic analysis. The histologic diagnosis was performed by 2 experienced observers. Of these cases, 38 were selected for immunohistochemical analysis based on dental and medical history, physical examination and laboratory tests, radiographic and cone-beam computed tomographic (CBCT) analyses, and histologic diagnosis (cyst or granuloma). From the analysis of the clinical records of patients involved in the study, it was possible to obtain data on symptoms, sinus tracts, and lesion size. Exclusion criteria included patients with immunologic and immunosuppressive diseases such as diabetes mellitus, human immunodeficiency virus infection, and autoimmune diseases; patients with insufficient radiographic and CBCT analysis or clinical and treatment data records; and cases in which the surgical specimen was insufficient for adequate histologic analysis. The participants were informed about the nature of the study and signed an informed consent form. The study protocol was approved by the Ethics Committee of the Estácio de Sá University.

Immunohistochemistry

For immunohistochemical analysis, 3-μm tissue sections were mounted on 3-aminopropyltriethoxy silane–coated glass slides (Sigma Chemical Co, St Louis, MO), deparaffinized with xylene, and rehydrated in graded alcohol. Slides were then immersed in citrate buffer (pH = 6.0) for 2 cycles of 12 minutes in a microwave for antigen retrieval. Afterward, the slides were submitted to 5 consecutive 5-minute baths in 6% hydrogen peroxide and left in 1 × phosphate-buffered saline (PBS) (pH = 7.4). The slides were incubated with primary rabbit polyclonal antibody anti–IL-17 (H-300; Santa Cruz Biotechnology, Santa Cruz, CA) and diluted 1:200 in PBS/bovine serum albumin at 4°C overnight in a humidified chamber. After this period, the slides were washed in PBS, and the sections were treated with a conjugated secondary antibody labeled with streptavidin-biotin (Dako, Glostrup, Denmark). Peroxidase activity was visualized by immersing tissue sections in 3,3′-diaminobenzidine (Dako) followed by counterstaining with Mayer hematoxylin. IL-17 expression in the tissue sections was independently analyzed by 2 experienced observers (L.A. and F.R.P.) using light microscopy. Negative controls consisted of reactions using PBS/bovine serum albumin with no primary antibody.

Each specimen was divided into 5 fields and analyzed under high-power view (400×), and the expression values were obtained from the amount of positive cells in each field. Each observed area was categorized according to the following scores: 0, negative/focal, if there were no positive cells or less than 5% of the cells were positively stained; 1, mild to moderate, if >5% to 50% of the cells were positively stained; and 2, strong, if more than 50% of the cells were positive. The mean score for the whole specimen was calculated, and IL-17 expression was

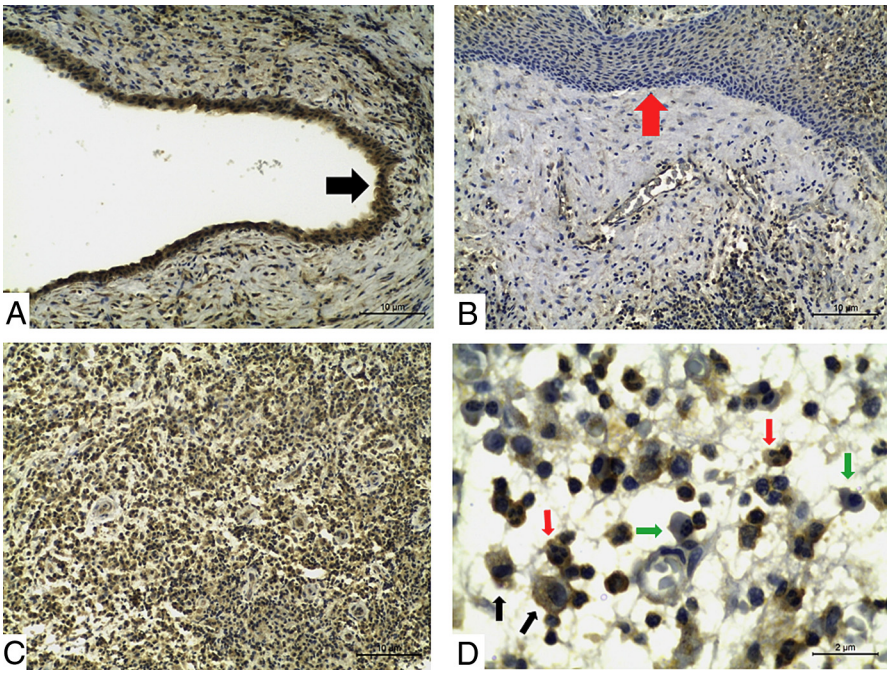

Figure 1. IL-17 expression in apical periodontitis specimens. (*A*) Strong IL-17 expression in a thin lining epithelium (*black arrow*) (hematoxylin-eosin [H&E], 200×). (*B*) A hyperplastic lining epithelium showing negative/focal IL17 expression (H&E, 200×). (*C*) Intense chronic and acute inflammatory infiltrate in the connective tissue (H&E, 200×). (*D*) Detail of the inflammatory cells showing IL-17 expression in macrophages (*black arrows*) and PMNs (*red arrows*) and the absence of expression in plasma cells (*green arrows*) (H&E, 1,000×).