Immunohistochemical Localization of IL-17 in Induced Rat Periapical Lesions

Haofei Xiong, DDS, PhD,‡ Lili Wei, DDS, PhD,*‡ and Bin Peng, DDS, PhD†*

Abstract

Interleukin (IL)-17 is a member of a novel family of proinflammatory cytokines produced almost exclusively by a newly recognized subclass of activated T cells called "Th17" cells. From an endodontic perspective, IL-17 potently regulates cells of the innate immune system, serving as an important bridging molecule between the adaptive and innate immune systems. The purpose of this study was to investigate the immunohistochemical localization of IL-17 during the development of periapical lesions in rats. Periapical lesions developed within 28 days after mandibular first molar pulp exposure in Sprague-Dawley rats. The animals were randomly sacrificed at 0, 7, 14, 21, and 28 days after pulpal exposure. The jaws that contained the first molar were obtained and routinely prepared for histologic analysis, immunohistochemistry, and enzyme histochemistry. From day 0 to day 28, the number of IL-17–positive cells and neutrophils ascended and peaked on day 28. Osteoclast numbers substantially multiplied from day 0 to day 14 and then gradually decreased from day 14 to day 28. In addition, the osteoclast decrease contrasted with the increased number of IL-17–positive cells and neutrophils. These findings showed that IL-17 could be observed and might possibly be involved in the inflammatory response and bone resorption of periapical tissues as well as associated with periapical lesion pathogenesis. *(J Endod 2009; 35:216–220)*

Key Words

Interleukin-17, neutrophils, osteoclast, periapical lesions

From the *School and Hospital of Stomatology and †Department of Conservative Dentistry and Endodontics, Wuhan University, Wuhan, China.‡Haofei Xiong and Lili Wei contributed equally to this article.

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Address requests for reprints to Dr Bin Peng, Key Lab for Oral Biomedical Engineering of Ministry of Education, School and Hospital of Stomatology, Wuhan University, 237 Luoyu Road, Wuhan 430079, China. E-mail address: phs301@vip.163.com.

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Periapical lesions, which form as a result of a root canal infection, are characterized by a host response to continuous antigenic stimulation in the infected canals and periapical bone destruction (1, 2). The host response is complex; it involves both the recruitment of different inflammatory cells and the participation of an extensive network of immunologic mechanisms, including cytokine production. Studies have shown that a number of proinflammatory cytokines are synthesized in response to bacteria and their products, which induce and maintain an inflammatory response (3, 4). Therefore, it is clear that periapical lesion development is associated with the actions of these cytokines.

Originally cloned from a rodent T-cell hybridoma library screen, interleukin (IL)-17A (often called simply IL-17) is the founding member of a novel cytokine family of cytokines termed IL-17A through IL-17F (5, 6). IL-17 is produced almost exclusively by activated T cells and is a definitive of a new class of effector T-helper cell subsets called "Th17" that is distinct from Th1 or Th2 (7–9). Although produced primarily by T cells, IL-17 is clearly a proinflammatory cytokine with potent effects on numerous cells of the innate immune system, particularly the granulocyte lineage, and is thus considered an important bridging molecule between the adaptive and innate immune systems (6, 10). The proinflammatory functions of IL-17 have been examined in many contexts. In vitro, IL-17 has been shown to activate fibroblast, epithelial cells, endothelial cells, and osteoblasts to produce proinflammatory cytokines such as IL-6, IL-8, granulocyte colony-stimulating factor, and matrix metalloproteinases (11). IL-17 plays an essential, nonredundant role in neutrophil activation, maturation, and homeostasis (12). It could also promote bone resorption by stimulating osteoblasts to produce the receptor activator of nuclear factor-kappa B (RANKL) that affects the activity and formation of osteoclasts (13). In humans, IL-17 has been associated with the pathology of numerous autoimmune and inflammatory diseases, such as rheumatoid arthritis (RA), systemic lupus erythematosus, multiple sclerosis, psoriasis, and allograft rejection (9). Furthermore, the presence of IL-17 has also been documented in periodontitis patients, suggesting that this cytokine might mediate inflammation in periodontal diseases (14, 15).

To our knowledge, there have been few reports on IL-17 expression in periapical lesions. Therefore, we hypothesize that IL-17 might be involved in the pathogenesis of periradicular lesions, particularly the role it might play in both inflammatory response and alveolar bone destruction. The aim of this study was to examine the presence of IL-17 and discuss its relationship with inflammatory response and bone resorption at different stages in rat periapical lesions.

Materials and Methods

Induction of Periapical Lesions and Sample Preparation

Thirty-five Sprague-Dawley rats, weighing about 220 g, were purchased from the Experimental Animal Center of Wuhan University. The rats were randomly divided into five groups. All rats were anesthetized with a ketamine (0.5 mL/kg) intramuscular administration. The pulps of the lower first molars were exposed with a #1/2 round bur to a depth equal to the bur diameter so that furcal perforation would be avoided. The exposed teeth were left open to the oral environment throughout the experiment. Seven rats in each group were sacrificed at 0, 7, 14, 21, and 28 days after lesion induction. Day 0 samples served as the negative control group. Experimental procedures followed the guiding principles of the Animal Care and Use Committee in the School of Stomatology, Wuhan University.

Histologic Analysis

The jaws were removed, defleshed, and fixed in 4% paraformaldehyde for 48 hours. Then, the specimens were transferred to a 10% EDTA solution for demineralization for 4 weeks at 4°C. After that, the samples were dehydrated in an ethanol series solution and embedded in paraffin. Next, 4-μm thick frontal serial sections prepared in a mesiodistal direction were cut. One from every four sections was observed under a light microscope. The sections, which included the distal root of the first mandibular molars and exhibited a patent root canal apex representing the central portion of root canal, were selected for histologic, immunohistochemical, and enzyme histochemical analysis.

The number of neutrophils that infiltrated the inflammatory tissues was also analyzed. The counting area definition was centered at a fixed distance from the distal root apical foramina throughout the selected section of each different specimen. For morphometric analysis, the number of neutrophils was counted by using specimens stained with hematoxylin and eosin. On average, we counted the number of neutrophils at 10 different sites under a high power field (hpf) at a magnification of ×400 in each specimen. Within these designated areas, the dark-stained cells with a multilobed, horseshoe-shaped nucleus were deemed neutrophils (16, 17). Observations were verified by an oral pathologist. The number of neutrophils was then calculated.

Enzyme Histochemistry (Tartrate-resistant Acid Phosphatase Assay)

Tartrate-resistant acid phosphatase (TRAP) is usually a histochemical marker specifically for osteoclasts. TRAP-positive cells ranged in color from dark red to purple. A TRAP kit (Sigma, St Louis, MO) was used to detect TRAP activity and identify osteoclasts. Sections that contained the periapical region were selected and subjected to TRAP activity examination. Procedures were performed as previously described (18, 19). Briefly, after the sections had been rehydrated and washed, they were incubated in a Naphthol AS-BI phosphoric acid solution (Sigma, St. Louis, MO) and Fast Garnet GBC for 1 hour at 37°C. After incubation, the sections were then stained with hematoxylin. Some sections were incubated in a substrate-free medium to serve as TRAP activity controls. Quantitative analysis of TRAP-positive osteoclast numbers was determined by counting multinucleated TRAP-positive cells under a microscopic hpf at a magnification of ×400 in five randomly fields that had direct contact with alveolar bone in the periapical regions. The technician who performed this task was not aware of the original specimen.

Immunohistochemistry

Immunohistochemistry was performed by the streptavidin-biotin complex method. Rabbit polyclonal antibodies against human origin IL-17 (Santa Cruz Biotechnology, CA, sc 7927) at a 1:100 dilution were used as the primary antibody. The sections were washed and stained using the streptavidin-biotin complex kit (Boshide, Wuhan, China) according to the manufacturer's manual. The procedures were as follows: after the sections were rehydrated, endogenous peroxidase activity was blocked with 3% hydrogen peroxide for 10 minutes at room temperature. After being washed in distilled water, the sections were first incubated with 5% BSA for 20 minutes at room temperature and then with the primary antibody for 24 hours at 4°C. Next, the sections were washed with phosphate-buffered saline and then incubated with streptavidin-biotin complex for 20 minutes. Finally, the sections were developed with 3, 3′-diaminobenzidine (Boshide, Wuhan, China) and confirmed under a microscope. Counter staining was performed by hematoxylin. Regarding the negative controls, the nonimmune goat serum was used instead of the primary antibody. Next, the cells reactive with the anti–IL-17 in the periapical portion were counted. The vicinity was defined as the area centered at a fixed distance from the distal root apical foramina throughout the selected section of each different specimen. The areas that were subjected to cell counting were strictly defined by the research design. Under a microscopic hpf at a magnification of ×400, a technician randomly picked up five visual fields in the area and counted cells according to the single blind principle. The number of antibody-reactive cells per hpf (cells/hpf) was then calculated.

Statistical Analysis

All measurement results were presented as mean values ± standard deviation. Average values were determined for each specimen and then were calculated for each group. Data were analyzed with SPSS13.0 software (SPSS Inc, Chicago, IL). Statistical differences in each group were subjected to a one-way analysis of variance test ($\alpha = 0.05$). Pair-wise multiple comparisons were performed in cases in which the analysis of variance test showed significant differences.

Results

Histologic Analysis

The periapical region was intact, and neither inflammation nor bone resorption could be observed in the negative controls on day 0. On day 7, a slight infiltration of inflammation cells could be observed. Then small areas of periapical alveolar bone resorption occurred. On day 14, lots of inflammation cells in the periapical tissues and alveolar bone resorption were observed. On day 21, a small abscess was seen around the root apex with inflammatory cell infiltration, and alveolar bone resorption was found. On day 28, the apical abscess enlarged, and alveolar bone resorption was still found.

The neutrophils in the negative controls were seldom observed on day 0. After the rats were induced with lesions, neutrophils could be readily seen in the inflamed periapical regions on day 7 and subsequently increased on day 14 (Fig. 1*A*). On day 21, the number of neutrophils ascended, especially in the abscess tissues. Climax was reached on day 28 (Fig. 1*B*). From 1 week to 4 weeks after pulpal exposure, there was a significant difference in the number of neutrophils in each group compared with the other time group (Table 1).

Enzyme Histochemical Observation

No multinucleated osteoclasts were observed in the normal uninflamed periapical areas. On day 7, a small amount of osteoclasts could be seen in the periapical tissues. From day 7 to day 14, the number of osteoclasts increased and climaxed on day 14 (Fig. 1*C*). Then, on day 21, osteoclast numbers decreased dramatically. Few osteoclasts could be seen on day 28 (Fig. 1*D*). The cell counts and their corresponding standard deviation values from day 7 to day 28 are depicted in Table 1.

Immunohistochemical Finding

It was observed that IL-17–positive cells were present in all phases of the induced rat periapical lesions, and the number of IL-17-positive cells increased during the lesion expansion periods. The lymphocyte was the predominant IL-17–positive cell type. Some IL-17–positive cells were found 7 days after pulpal exposure. Subsequently, a significant increase in cell number was detected at 14 days (Fig. 1*E*). Then, on day 21, the number of IL-17–positive cells was still rising and then peaked at 28 days (Fig. 1*F*). The cell counts and their corresponding standard deviation values from day 7 to day 28 are also depicted in Table 1.