The Expression of Interferon Regulatory Factor 8 in Human Periapical Lesions

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Abstract

Introduction: Interferon regulatory factor 8 (IRF8) is a critical transcription factor in innate immune responses that regulates the development and function of myeloid cells. Human periapical lesions are caused by endodontic microbial infections. However, the presence of IRF8 in human periapical lesions remains elusive. This study aims to explore the expression of IRF8 in human periapical lesions and the possible association of IRF8 with macrophages, nuclear factor kappa B (NF-κB) signaling, and the autophagy process. **Methods:** Thirty-nine human periapical tissues, including healthy control tissues (n = 15), radicular cysts (RCs, n = 11), and periapical granulomas (PG, n = 13), were examined. Tissues were fixed in paraformaldehyde and analyzed. The inflammatory infiltrates of lesions were evaluated by hematoxylin-eosin, and the expression of IRF8 was analyzed by immunohistochemistry. Double immunofluorescence assessment was performed to colocalize IRF8 with CD68, NF-κB p65, and LC3B. **Results:** The expression of IRF8 was significantly higher in RCs and PGs than in the healthy control group, but no significant difference was found between RCs and PGs. There were significantly more IRF8-CD68 double-positive cells in RCs and PGs than in the healthy control group, but no significant difference was observed between RCs and PGs. Double-labeling analysis of IRF8 with NF-κB and LC3B indicated that IRF8 expression is associated with NF-κB signaling and the autophagy process during periapical lesions. **Conclusions:** IRF8 could be observed and might possibly be involved in macrophages in the development of periapical lesions. *(J Endod 2018;■:1–7)*

Key Words

Autophagy, interferon regulatory factor 8, macrophage, nuclear factor kappa B signaling, periapical lesions

Significance

The expression of IRF8 is observed and might possibly be involved in macrophages in the development of periapical lesions. Our findings provide new clues to the mechanisms of periapical diseases and the treatment of the disease by targeting IRF8.

Periapical lesions originate from root canal infection and ultimately result in periradicular bone inflammation and destruction. Most of the periapical lesions are periapical granulomas (PGs) and radicular cysts (RCs); both are transitional entities of the same pathological lesions (1). The immune response of hosts plays a critical role in the inflammatory progression of periapical lesions (2). These inflammatory cells produce proinflammatory and anti-inflammatory cytokines, such as interferon gamma (IFN-γ), transforming growth factor beta, and receptor activator of nuclear factor kappa B ligand (3). IFN-γ mediates macrophage activation through up-regulating interleukin 1 and tumor necrosis factor alpha expression (2, 4). Macrophages are primary cells in pathogen-induced or tissue damage–induced inflammation (5). Large numbers of macrophages that infiltrate inflamed tissues participate in killing pathogens, promoting tissue repair as well as producing inflammatory mediators (6). However, there is still no valid treatment to diminish inflammation in periapical disease.

Interferon regulatory factor 8 (IRF8) is a transcription factor of the IRF family that is induced by interferon (IFN-α/β and IFN-γ) in an innate response against infections (7). IRF8 binds to specific DNA sequences when it forms heterodimers with partner transcription factors, acting either as an activator or a repressor (8). This transcription factor is activated by IFN-γ in macrophages and stimulates genes essential for a host response (9). Studies showed that IRF8 plays a vital role in the formation and function of macrophages, including differentiation, maturity, and activation (10, 11). During infection, IRF8 triggers antimicrobial defenses in myeloid cells and transmits proinflammatory signals to amplify early immune responses (12). Once expressed, IRF8 either triggers or inhibits the transcription of downstream target genes depending on their interacting partners (8). Nonetheless, the presence of IRF8 in human periapical lesions remains unknown. Thus, we hypothesized that IRF8 expression might be involved in the inflammatory progression of periapical lesions.

Previous studies found that the stimulation of Toll-like receptors by pathogens leads to the cooperation of IRFs with nuclear factor kappa B (NF-κB) (13, 14). NF-κB is a transcription factor that plays a critical role in numerous essential biological processes, including cell survival and inflammation (15). Autophagy is a highly regulated cellular process that is activated by a variety of stress signals, such as starvation and inflammation (16). Notably, IRF8 coordinates with NF-κB to regulate inducible

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nitric oxide synthase expression in myeloid cells (17). Additionally, IRF8 is also an autophagy master regulator that acts in macrophages in response to diverse stresses (18). Autophagy is a highly regulated cellular process that is activated by a variety of stress signals, such as starvation and inflammation (19). LC3B, a reliable autophagy factor and frequently used autophagic biomarker, was found to have an important role in periapical lesions (20). Therefore, this study aimed to determine the expression of IRF8 in periapical lesions and to explore the association of IRF8 with macrophages, the NF-κB pathway, and the autophagy process.

Materials and Methods

Sample Collection

The study was approved by the Ethics Committee of the School and Hospital of Stomatology of Wuhan University, Wuhan, China, as required by the Declaration of Helsinki. Before sample collection, written consent was obtained from each patient after the nature of the procedures and the possible discomforts and risks were fully explained. The patients ranged in age from 20–58 years old (22 men and 17 women). No systemic disease was observed in any of the patients, and they had not taken antibiotics within 6 months. Periapical tissues were obtained from 24 patients with symptoms of periapical disease diagnosed by clinical examination including periapical radiography, panoramic pantomography, or cone-beam computed tomographic imaging before surgery and histologic examination after surgery at the Department of Oral and Maxillofacial Surgery, School and Hospital of Stomatology, Wuhan University. RCs were defined as follows: lesions located on the periapical region of dead teeth, fluid or semisolid content discovered in a cavity during surgery, or histologic evidence of stratified nonkeratinizing squamous epithelium completely or partially lining the oral cavity (Fig. 1*B*). PGs consisted of granulomatous tissue with a large number of infiltrating inflammatory cells but no observation of epithelial cells (Fig. 1*C*). A total of 11 tissues of human RCs and 13 tissues of human PGs were collected during surgery. The control group, which did not show any inflammation, consisted of dental pulp and gingival tissues obtained from 15 patients with a surgical indication for the extraction of impacted healthy third molars. These samples were fixed in 4% buffered paraformaldehyde for 24 hours. Then, the tissues were embedded in paraffin, and the blocks were cut into histologic sections (approximately 4 μm in thickness).

Histologic Examination

Four-micrometer-thick histologic sections from each tissue sample were stained with hematoxylin-eosin to select specimens. RCs were identified based on the granulation tissue with with a cavity lined by stratified squamous epithelium. PGs were identified based on the granulation tissue without a cavity lined by epithelium. Endothelial cells were characterized by their spindle-shaped morphology, mononuclear inflammatory cells were identified as cells with a large single nucleus, and polymorphonuclear leukocytes were identified by their multilobed nuclei.

Immunohistochemical Staining

Immunohistochemical staining was performed using the Ultra-Sensitive SP (Rabbit) IHC Kit (Maixin, Fuzhou, China) according to the manufacturer's instructions as previously described (20). Briefly, all sections followed the experimental procedure; they were deparaffinized in xylene, rehydrated, and treated with gastric enzymes for antigen retrieval. Then, the sections were treated with 3% H_2O_2 for 20 minutes to remove endogenous peroxidase activity. Nonspecific antibody binding was blocked by incubating with a blocking goat serum at 37°C for 30 minutes and primary antibodies against rabbit anti-IRF8 (dilution 1:500; ABclonal, Woburn, MA) at 4°C overnight. Biotinylated goat antirabbit immunoglobulin G was applied as a secondary antibody for 15 minutes at 37°C. The sections were exposed to streptavidin-peroxidase conjugate for 10 minutes at 37°C and visualized by the application of diaminobenzidine solution (Zhongshan, Beijing, China) for 1 minute. Negative controls were obtained by the omission of primary antibodies, which were substituted with nonimmune bovine serum. Finally, the sections were lightly counterstained with hematoxylin, mounted, and analyzed by light microscopy (Olympus, Tokyo, Japan).

Immunofluorescence Double Staining

Immunofluorescent double staining of IRF8-CD68, IRF8-NF-κB p65, and IRF8-LC3B was performed in this study. First, the sections were deparaffinized, rehydrated, and incubated with pepsin (ZSGB-BIO, Beijing, China) to retrieve antigens. After rinsing with phosphate-buffered saline, the sections were preincubated with normal goat serum (Maixin, Fuzhou, China) for 60 minutes and incubated with rabbit anti-IRF8 antibody (dilution 1:500 [A5798, ABclonal]), mouse anti-CD68 monoclonal antibody (dilution 1:200 [ZM-0060, Zhongshan]), mouse anti–NF-κB p65 monoclonal antibody (dilution 1:200; Santa Cruz Biotechnology, Santa Cruz, CA), and mouse anti-LC3B (dilution 1:500; Sigma-Aldrich, St Louis, MO) at 4°C overnight. Negative controls were obtained by the omission of primary antibodies, which were substituted with nonimmune bovine serum. Then, the sections were rinsed with phosphate-buffered saline and subsequently incubated with goat antirabbit Alexa Fluor 549–conjugated polyclonal antibody (dilution 1:200; Jackson ImmunoResearch Laboratories, Inc, West Grove, PA) and goat antimouse Alexa Fluor 488–conjugated polyclonal antibody (dilution 1:200, Jackson ImmunoResearch Laboratories, Inc) for 60 minutes at 37°C in the dark. After being thoroughly washed, the sections were incubated with 4′,6-diamidino-2-phenylindole (ZSGB-BIO) to stain the nuclei before fluorescent microscopic analysis (Leica, Nussloch, Germany). In each experiment, the sections were stained at the same time and observed immediately under a fluorescence microscope for analysis.

Morphometric Analysis

The immunopositive cells were quantified by Image-Pro Plus version 6.0 (Media Cybernetics, Inc, Bethesda, MD) with images of the histologic sections captured with a microscope. All of the signals were located in the cell membrane or cytoplasm. Overlapping of the red and green fluorescence and/or blue in the same field showed as yellow or white. Each specimen was randomly divided into 5 fields and analyzed at $\times$400 magnification (0.0714 mm^2/field), and the expression values of IRF8-positive and double labeling–positive cells in the periapical tissues were counted in each field. The density of positive cells was expressed as the number of cells per square millimeter.

Statistical Analysis

Data were analyzed using SPSS 20.0 (IBM Corp, Armonk, NY). After analysis for normality and data variance, the Kruskal-Wallis test was applied to compare the groups. $P < .05$ was considered to be significantly different.