The Expression of Macrophage and Neutrophil Elastases in Rat Periradicular Lesions

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Abstract

Macrophage elastase and neutrophil elastase are involved in tissue destruction in periradicular lesions. The purpose of this study was to examine these elastases immunohistochemically during development of periradicular lesions induced in rat mandibular first molar by pulpal exposure for 7, 14, 21, 28, and 42 days. Histologically, periapical inflammation developed from 7 to 21 days and then subsided after 28 days. The area of these lesions gradually increased from 7 to 28 days and subsequently decreased at 42 days. Macrophage elastase was first detected at 7 days and predominantly shown from 14 to 28 days, whereas neutrophil elastase gradually increased from 14 to 28 days. Macrophage elastase was significantly greater than neutrophil elastase from 7 to 21 days. These results suggest that macrophage elastase was enhanced from an early stage during the development of these lesions and that neutrophil elastase was related to the expansion of periapical tissue destruction including bone resorption. *(J Endod 2008;34:1072–1076)*

Key Words

Elastase, macrophage, neutrophil, periradicular lesion, rat

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A periradicular lesion evoked by bacterial infection consists of periapical inflammation with destruction of the periodontal ligament and resorption of the alveolar bone and root (1). The periodontal ligament is a dense connective tissue localized between the cementum and the alveolar bone and supports the tooth. The destruction of the apical periodontal ligament is initiated by degradation of the extracellular matrix (ECM). ECM is composed mainly of collagen fibers, and elastic-system fibers including oxytalan fibers and elastin-containing fibers (ie, elastic and elaunin fibers) are ubiquitous components of this ligament (2). Elastic-system fibers have been investigated in the rat molar periodontal ligament including its apical portion (3). The enzymes of ECM degradation are both serine proteinases and matrix metalloproteinases (MMPs).

MMPs are a family of zinc-dependent endopeptidases that possess the ability to degrade all components of the ECMs and play important roles in many physiologic and pathological processes (4, 5). Especially, MMP-2 (geratinase A), -7 (matrilysin), -9 (geratinase B), and -12 (macrophage metalloelastase) have elastinolytic properties (6). MMP-12 was discovered in thioglycolate-stimulated mouse peritoneal macrophages (7). Murine MMP-12 complementary DNA was cloned (8), and, subsequently, human and rat homologues of MMP-12 complementary DNA were reported (9–11). MMP-12 has the ability to degrade ECM components by attacking elastin (12) and other basement membrane components such as fibronectin or collagen type V but not gelatins (13, 14). Collagen type IV or laminin is also degraded by murine or human MMP-12 (13, 14) but not by rat MMP-12 (11). Macrophages with those proteolytic activities are able to penetrate basement membranes and invade several tissues (14). Various roles of MMP-12 in disease models have been revealed to date, such as the development of lung emphysema (15), prevention of tumor growth through angiostatin generation (16, 17), and degeneration of the aortic medium in abdominal aortic aneurysm (18). By using the complementary DNA microarray analysis, MMP-12 in rat periradicular lesion was upregulated compared with healthy control (19). But, to date, few reports have been published with respect to the role of MMP-12 in periodontal and periradicular tissues.

Neutrophil elastase, which is involved in the degradation of elastin, is one of the serine proteinases and it has strongly degradative action toward several proteins. Secreted cathepsin G and proteinase 3 also have an elastinolytic property in serine proteinase (20). Polymorphonuclear leukocytes (or neutrophils) contain an abundance of lysosomal granules filled with proteolytic enzymes and other components. Neutrophil granules can be differentiated into specific and azurophilic (or primary) granules, the latter containing the serine proteinases such as elastase and cathepsin G as well as other enzymes (21). Neutrophil elastase secreted by activated neutrophils can bind to and degrade ECM. This protease has wide substrate specificity and has strong competency in degrading a variety of the proteins including elastin (22). There is a possibility that it evokes the severe tissue destruction seen in pulpitis (23) and periradicular lesions (24). In human periapical disease, neutrophil elastase levels of periapical exudates associated with clinical symptoms (25) and there may be a possibility that it serves as a diagnostic marker (26).

The purpose of the present study was to examine the involvement of macrophage elastase and neutrophil elastase in the development of periradicular lesions in rats.

Material and Methods

The Induction of Periradicular Lesions

Twenty-four male Wistar rats, weighing about 250 g, were used in the present study. Periradicular lesions were induced as described previously (27, 28). In brief, all animals were anesthetized, and the pulpal tissues in the left and right mandibular first molars were exposed so that perforation of the furcation would be avoided. The exposed areas were left open to the oral environment until the animals were killed.

Tissue Preparation and Immunohistochemistry

Animals were sacrificed at 7, 14, 21, 28, and 42 days (each period, n = 4) after the pulpal exposure. Animals without the exposure were used as the control (day 0, n = 4). At the time of sacrifice, the mandibles of all animals were removed and fixed in periodate-lysine-paraformaldehyde solution for 24 hours at 4°C. The mandibles were decalcified by being soaked in 5% EDTA solution until complete decalcification (approximately 60 days) at 4°C. Then, these were embedded in OCT compound (Miles Scientific, Naperville, IL) and sectioned serially at 5 μm in the mesiodistal plane.

The presence of macrophage elastase and neutrophil elastase in the periapical tissue were examined immunohistochemically. The primary polyclonal antibodies reactive with macrophage elastase (sc-8839) and neutrophil elastase (sc-9521) were obtained from Santa Cruz Biotechnologies (Santa Cruz, CA). Normal goat serum was used as the negative control. The sections were stained by use of ABC Staining System (sc-2018, Santa Cruz Biotechnologies). The slides were exposed to hydrogen peroxide in deionized phosphate-buffered saline (PBS) for 10 minutes to quench the endogenous peroxidase activity and subsequently reacted with 1.5% goat serum diluted in PBS for an hour to block nonspecific antibody binding. These slides were incubated with each primary antibody for 12 hours, followed by biotinylated antibody for 30 minutes. Then, they were incubated with avidin-biotinylated peroxidase complex for 30 minutes. For the final chromogenic reaction, the slides were exposed to the substrate solution consisting of diaminobenzidine-tetrahydrochloride and hydrogen peroxide and were counterstained with hematoxylin. The periapical tissue of the mesial root of the mandibular first molar was examined for both antigen-positive cells.

Histometry

Quantitative analysis was performed on 4 serial sections from each molar. The area of the periradicular lesion was measured histometrically as described previously (26, 27). The area of the lesion was the

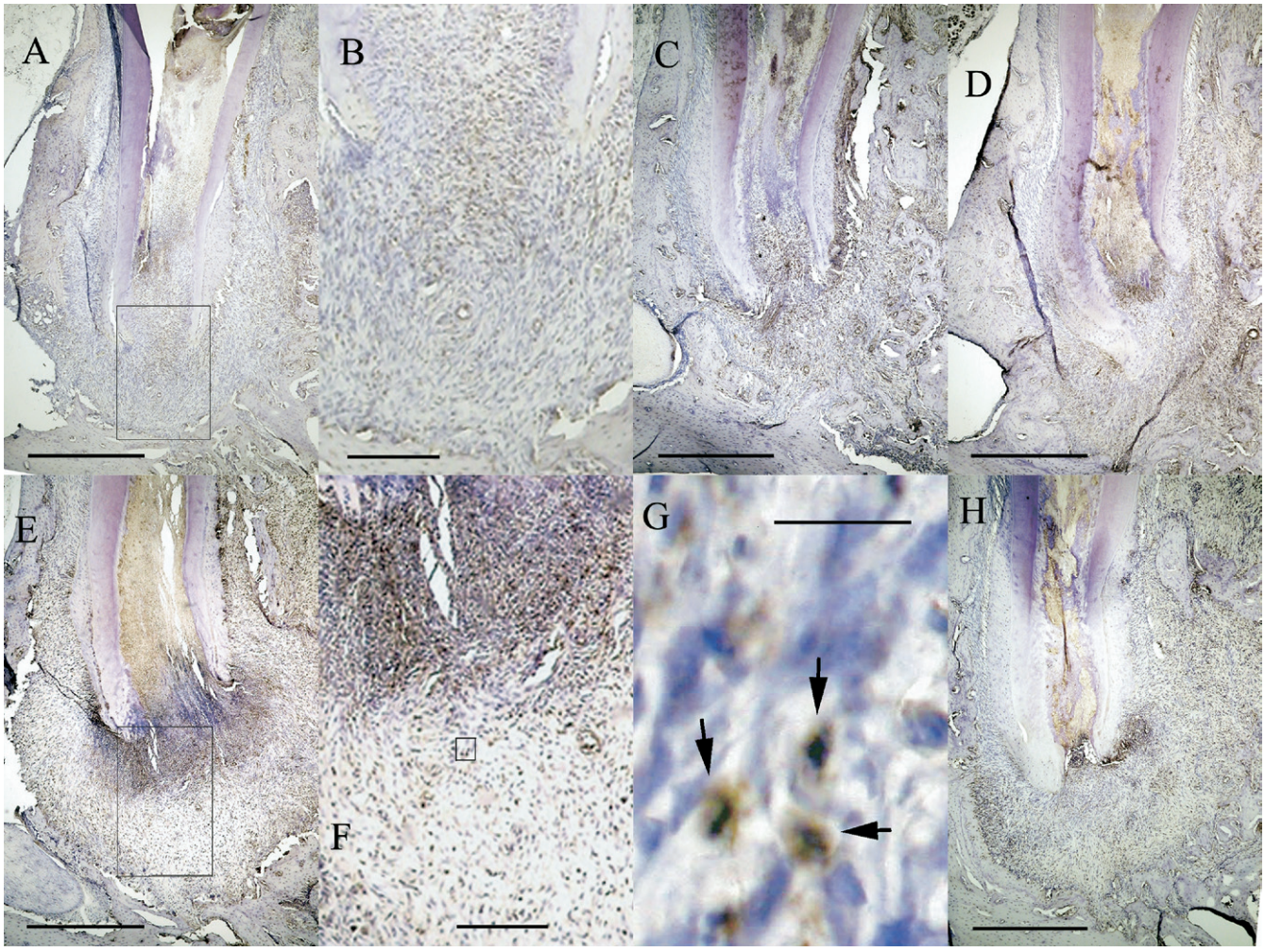

Figure 1. Immunohistochemical staining for macrophage elastase. (*A*) In the mandibular first molar at 7 days after the exposure, some elastase-expressing cells detect in the residual pulpal tissue and around the root apex. *B* is a high-magnification view of cropped area in *A*. These cells gradually increase at (*C*) 14 and (*D*) 21 days. (*E*) By 28 days, these cells increase in number and are observed in and around the abscess. *F* is a high-magnification view of cropped area in *E*. *G* is a cropped area in *F*. The arrows indicate macrophage elastase-positive cells. These cells are round-shaped monocytes in high magnification. By 42 days, these cells decrease in number, but some still remain in the abscess (*H*). *A*, *C*, *D*, *E*, and *H* scale bars = 1.0 mm. *B* and *F* scale bars = 0.25 mm. *G* scale bar = 0.025 mm.