Role of IL-2 and Helper T-Lymphocytes in Limiting Periapical Pathosis

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Abstract

The purpose of this study was to examine the role of IL-2 and helper T-lymphocytes in the development of periradicular lesions in rats. In control animals, periradicular lesions developed within 28 days following pulpal infection. Immunologically, some anti IL-2 and anti CD4-reactive helper T-lymphocytes infiltrated the periapical tissue at 14 days, and their numbers increased at 28 days. In experimental animals, tacrolimus (FK506) was injected every day to inhibit the IL-2 production by helper T-cells. Histologically, the pulpal necrosis and periradicular inflammation in tacrolimus-treated rats were more severe than those in the control rats. Furthermore, the areas of pulpal necrosis and periradicular lesion in the immunosuppressed rat were significantly greater than those in the normal ones. The numbers of IL-2- and CD4-positive cells in the lesion of the experimental rats were statistically lower than those of the control ones. These results show that the decrease in IL-2 might have promoted the development of periradicular lesions. *(J Endod 2006;32:24–29)*

Key Words

CD4, interleukin 2, periradicular lesion, rat, tacrolimus

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Cytokines are intercellular regulatory proteins that mediate various immunologic biological functions, and it is clear that the development of periradicular lesions is associated with the actions of cytokines (1, 2). In the inflammatory lesion, interleukin 2 (IL-2) plays a role in the pathogenesis or progression of inflammatory disease (3, 4, 5). IL-2 is a potent immunostimulant produced and secreted principally by helper T-cells, and it stimulates the clonal expansion of these cells (6). The formation of periradicular lesions involves tissue changes that include inflammatory cell infiltration, necrosis, abscess, and the resorption of root and alveolar bone as the result of an immunological response to continuous bacterial stimulation from the root canal. Actually, IL-2 is detected in both human inflamed pulp (7, 8) and periapical granulation tissue (9), but such investigations have not revealed the relation between IL-2 and endodontic inflammation (8, 9). However, Kim and Lim (10) demonstrated that the IL-2 concentration was increased in rat pulpal inflammation. We also have supposed that IL-2 may activate a multitude of the cytokines that orchestrate the cell-mediated immune response in periradicular lesion.

Helper T-lymphocytes were shown to be regulated by IL-2 and to associate with the immune response (6). In human periapical lesions, including granuloma, cyst, and scar, helper T-lymphocytes were found to be the predominant mononuclear cell type (11, 12). In rat periradicular lesions after experimentally produced pulpal exposure, the number of T lymphocytes was greater than that of B lymphocytes (13); and helper T-cells were predominant in the expansion phase of the lesions, whereas suppressor T-cells predominated in the chronic phase (14, 15). On the basis of these studies, it appeared that T lymphocytes and their subpopulations play an important role in the pathogenesis of the periradicular lesion and that helper T-cells are responsible for the development of this lesion (13–15). We have assumed that IL-2, which stimulates clonal expansion of the helper T-cells, may play an important role in periradicular inflammation. Therefore, in the present study, we looked immunohistochemically for the presence of IL-2 and helper T-lymphocytes (CD4 antigen+) during the development of periradicular lesions induced experimentally by pulpal exposure in rats. Moreover, we decided to examine the difference in periapical inflammation between normal and tacrolimus-treated rats, in which both IL-2 production and helper T-cell number are reduced in the latter. Tacrolimus, which is designated as FK506 (Fujisawa Pharmaceutical, Osaka, Japan), has been shown to be an effective and safe immunosuppressive agent for the prevention of graft-versus-host disease after transplantation (16, 17), and to cause untoward side effects at a low incidence (18). Its action may be based on the ability of this drug to inhibit the IL-2 production that stimulates the clonal expansion of helper T-cells (19). So we chose tacrolimus to determine if inhibition of IL-2 production would affect the tissue changes during the development of the periradicular lesion.

Materials and Methods

Induction of Periradicular Lesions

Twenty-four specific pathogen-free male Wistar rats, weighing about 240 g, were divided equally into the control and experimental groups. All animals were anesthetized with diethyl ether, and then the pulpal tissue in the left mandibular first molar was exposed with a #1/2 round bar to a depth equal to the diameter of the bar, so that furcal perforation would be avoided. The exposed tooth was left open to the oral environment throughout the experiment (20, 21).

TABLE 1. Body weight and blood findings

	Days	Control Group	Experimental Group
		Mean ± SD	Mean ± SD
Body weight (gm.)	14	344.7 ± 14.8	294.0 ± 17.6**
	28	382.4 ± 12.7	324.9 ± 19.0**
Number of erythrocytes (cells/mm^3)	14	7942.0 ± 270.8	8513.3 ± 366.9
	28	8236.0 ± 241.7	8741.7 ± 195.2
Number of total leukocytes (cells/mm^3)	14	18040.0 ± 978.8	15366.7 ± 1576.9*
	28	19420.0 ± 2679.0	13683.0 ± 1722.1*
Number of neutrophils (cells/mm^3)	14	1858.0 ± 859.9	2345.7 ± 824.1
	28	2246.8 ± 966.3	1864.5 ± 927.5
Number of lymphocytes (cells/mm^3)	14	15467.0 ± 1597.7	12212.0 ± 1188.0**
	28	16304.0 ± 2484.4	11498.0 ± 2315.7*

*$p < 005$, ** $p < 0.01$ vs. control.

Statistical analysis was performed using Mann-Whitney analysis. There were significant differences between control and experimental groups for body weights at 14 and 28 days (both $p < 0.01$), for total leukocytes at 14 and 28 days (both $p < 0.05$), and for lymphocytes at 14 days ($p < 0.01$) and 28 days ($p < 0.01$).

Immunosuppression with Tacrolimus

Tacrolimus (0.32 mg/kg) was given by intramuscular injection to the experimental group (16, 22, 23), whereas only saline (the vehicle) was injected in the control group. These injections were performed 1 day before the pulpal exposure and daily during the entire experimental period.

Tissue Preparation for Histology and Immunohistochemistry

Six rats from each group were sacrificed at 14 and 28 days after the pulpal exposure. Before the sacrifice, body weight was measured, and peripheral blood was taken to count the numbers of erythrocytes, total leukocytes, neutrophils, and lymphocytes. After the sacrifice, the left mandible of each animal was fixed in periodate-lysine-paraformaldehyde fixative, decalcified in solution of 0.5M EDTA, embedded in paraffin, and sectioned serially at 5 μm in a mesiodistal plane. The sections were then stained with hematoxylin and eosin. The pulpal and periradicular tissues of the mesial root of the mandibular first molar were investigated histologically.

The presence of IL-2 and CD4 in the periapical area was examined by immuno-staining with polyclonal antibodies. CD4 is a membrane glycoprotein that contains 4 extracellular immunoglobulin-like domains expressed on helper T-lymphocytes. Goat polyclonal antibodies reactive with IL-2 (sc-1255) and CD4 (sc-1140) were obtained from Santa Cruz Biotechnologies (Santa Cruz, CA). Cells reactive with either antibody were detected by use of an ABC staining system (sc-2023: Santa Cruz Biotechnologies). For the final chromogenic reaction, the slides were exposed to a freshly prepared substrate solution consisting of diaminobenzidine-tetrahydrochloride and hydrogen peroxide. All sections were counterstained with methyl green. The periradicular tissue on these slides was then examined immediately under a light microscope.

Histometry

Quantitative analysis was performed on four serial sections from each animal. We chose sections that included the site of pulp exposure and the root canal foramen, and the sections were therefore made in the central portion of the periradicular lesions. The areas of pulpal necrosis, apical abscess, and periradicular lesion were measured histometrically as described previously (20, 21). The area of pulpal necrosis was that with no cellular tissue in the root canal under the furcation of the root, the area of apical abscess was the abscess adjacent to the root apex, and the area of the periradicular lesion was the periodontal ligament between the root apex and the alveolar bone in the mesial root. The cells reactive with the anti-IL-2 and anti-CD4 in the periapical portion were counted in sections immunohistochemically examined. An area 0.6 mm-square of periapical portion surrounding the root apex of the mesial root was examined, and the number of antibody-reactive cells per unit area (cells/mm^2) was calculated.

Statistical Analysis and Animal Protocol

Results of all measurements were presented as mean values ± SD. The average values were determined for each animal, and the average values were calculated for each group. Statistical significance was determined by the Mann-Whitney *U* test.

The principles of laboratory animal care according to animal protocol institutionally approved by the ethics committee of School of Dentistry, Aichi-Gakuin University were followed.

Results

Body Weight and Blood Findings

The body weights of both control and experimental groups increased during the experiment. At 14 and 28 days, the mean weight for the experimental group was statistically (both $p < 0.01$) less than that for the control one. As to the blood findings, the numbers of erythrocytes and neutrophils in the control and experimental groups did not show any changes. However, the number of total leukocytes in the experimental group was significantly less than that in the control one at 14 and 28 days (both $p < 0.05$), and the number of lymphocytes in the experimental group was statistically less than that in the control one at 14 ($p < 0.01$) and 28 ($p < 0.05$) days (Table 1).

Histological Results

In the control group at 14 days (Fig. 1*A*), most of the pulpal tissue was necrotic and severe inflammation was observed in the residual pulpal tissue. Slight inflammation in the apical periodontal ligament and alveolar bone resorption were also noted. By 28 days (Fig. 1*B*), the pulpal tissue had become completely necrotic, and an abscess had formed around the root apex. Signs of severe inflammation in the apical periodontal ligament and resorptions of alveolar bone and apical root were observed.

In the experimental group at 14 days (Fig. 1*C*), the pulpal tissue was completely necrotic, and a small abscess was observed around the root apex. Moderate inflammation in the apical periodontal ligament and resorption of alveolar bone and apical root were found. The pulpal necrosis, inflammation in the periodontal ligament, and bone resorption were severer than those in the control group. At 28 days (Fig. 1*D*), the abscess was still present around the root apex. Evidence of severe inflammation in the apical periodontal ligament and resorption of alve-