Journal of Dental Sciences (2018) 13, 87–96

Available online at www.sciencedirect.com

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journal homepage: www.e-jds.com

Original Article

A histopathologic study of the controlling role of T cells on experimental periodontitis in rats

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Received 17 February 2017; Final revision received 7 May 2017
Available online 11 April 2018

KEYWORDS
T cell;
Immunization;
Periodontal destruction;
Immune complex

Abstract *Background/purpose:* The onset and progression of periodontitis involve bacterial infection and the immune response. T cells function in the immune response and reportedly induce bone resorption in inflammatory bone loss. However, the exact role of T cells in periodontal destruction remains unclear. Using our experimental model of periodontitis, we aimed to investigate the influence of T cells on periodontal destruction.
Materials and methods: Male athymic nude (Nu) and euthymic wild-type (WT) rats were divided into the immunized (I-Nu and I-WT), non-immunized (nI-Nu and nI-WT). The immunized groups were immunized intraperitoneally with lipopolysaccharide (LPS). The non-immunized groups received phosphate-buffered saline (PBS). Nothing was administered to the non-treated groups. LPS was applied to the right palatal gingival sulcus in the immunized and non-immunized groups daily for 20 days. Loss of attachment, numbers of inflammatory cells and osteoclasts, and levels of alveolar bone were investigated histopathologically and histometrically. Osteoclasts were stained with tartrate-resistant acid phosphatase. The numbers of IL-4-positive cells were evaluated immunohistologically.
Results: Loss of attachment, numbers of inflammatory cells, levels of alveolar bone, and the number of osteoclasts were significantly increased in the nI-WT group compared with the nI-Nu group. However, the parameters were significantly increased in the I-Nu group compared with the I-WT group. The number of IL-4-positive cells was greater in the I-WT group than in the I-Nu group.

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https://doi.org/10.1016/j.jds.2017.05.006

Conclusion: T cells promote inflammation in non-immunized animals; however, they regulate these processes in immunized animals.

Introduction

Periodontitis is a chronic inflammatory disease characterized by periodontal pocket formation and alveolar bone resorption. Bacterial infection and the host immune response are considered responsible for the onset and progression of periodontal destruction.[1,2] Several studies have elucidated the contribution of bacterial components to the progression of periodontitis;[3,4] however, the contribution of the immune response remains unclear.

T cells play a central role in the immune response and have been implicated in periodontal destruction by many studies. One investigation suggested that T cells suppress bone loss: periodontal bone loss in nude rats, which are congenitally deficient in T cells, is significantly increased compared with that in normal rats, but decreases when T cells from normal rats are transplanted into nude rats.[5] In contrast, other studies have suggested that T cells are activated by antigen-presenting cells and express receptor activator of nuclear factor κ-B ligand and tumor necrosis factor-alpha (TNF-alpha) induce bone resorption in inflammatory bone loss.[6,7] Previously, we showed that T cells promote alveolar bone resorption when *Escherichia coli* lipopolysaccharide (LPS) is repeatedly injected into the mouse gingiva.[8] However, the exact function of T cells in periodontal disease remains unclear.

Recently, we reported that alternate topical application of *E. coli* LPS as an antigen and its specific antibody to rat gingival sulcus induces loss of attachment and alveolar bone resorption.[9] Additionally, we reported that topical application of *E. coli* LPS into the gingival sulci of LPS-immunized rats induces loss of attachment and alveolar bone resorption when the serum level of anti-LPS immunoglobulin (Ig) G was elevated after immunization of LPS, and represents an experimental model of periodontitis.[10] This model enables elimination of the mechanical stimulation caused by ligature. Using this model, we investigated the influence of T cells on periodontal destruction in periodontitis.

Materials and methods

Animals

Male F344/NJcl (wild-type [WT]) and T cell-deficient F344/NJcl-*rnu*/*rnu* (Nu) rats were purchased from Charles River Laboratories Japan, Inc. (Tokyo, Japan) and maintained under specific pathogen-free conditions at the Biomedical Research Center, Center for Frontier Life Sciences (Nagasaki University, Nagasaki, Japan). Animal care and experimental procedures were carried out in accordance with the Guidelines for Animal Experimentation of Nagasaki University and with approval from the Institutional Animal Care and Use Committee.

Experimental design

Male WT and Nu rats were divided into two groups: an immunized (I) group comprising the I-WT and I-Nu groups; a non-immunized (nI) group, comprising the nI-WT and nI-Nu groups. Each group comprised five rats. The immunized groups received intraperitoneal injections of 0.5 mg/kg *E. coli* LPS (O111, B4; Sigma–Aldrich Corp., St. Louis, MO, USA) suspended in phosphate-buffered saline (PBS) emulsified in complete Freund's adjuvant, followed by a booster injection of LPS emulsified in incomplete Freund's adjuvant 28 days later. The non-immunized groups received intraperitoneal injections of Freund's adjuvant with PBS, followed by an injection of incomplete Freund's adjuvant 28 days later. From Day 1 after booster injection, the animals were challenged daily with a topical application of *E. coli* LPS (50 μg/μl) suspended in PBS to the palatal gingival sulcus.[10] Briefly, the animals were anesthetized with isoflurane and then *E. coli* LPS (50 μg/μl) suspended in PBS was applied using a micropipette to the palatal gingival sulcus of the right maxillary first molar (LPS side), and PBS was applied to the palatal gingival sulcus of the left maxillary first molar (PBS side). In total, 21 μl (3 μl seven times, with 5-min intervals between each application) of LPS or PBS was administrated within a 35 min time period daily for 20 days. Blood samples were collected at the first intraperitoneal injection, booster injection, and immediately after the topical application of LPS or PBS on days 5, 10, and 20. The levels of anti-LPS IgG in individual serum samples were determined by indirect enzyme-linked immunosorbent assay. All animals were sacrificed under isoflurane anesthesia 1 h after the last application of LPS or PBS.

Tissue preparation

The maxilla of each rat was removed immediately after death, fixed in 4% paraformaldehyde in PBS at 4 °C for 10 h, and decalcified with 10% ethylenediaminetetraacetic acid for 3 weeks. The first molars on both sides of the maxilla were separated and embedded in paraffin using the AMeX method.[11] Briefly, the specimens were dehydrated in acetone, cleared in methyl benzoate for 30 min and xylene for 30 min, and penetrated with paraffin at 60 °C for 2 h. The penetrated specimens were embedded into paraffin blocks, and bucco-lingually oriented serial sections (4-μm thick) at the level of the central roots of the upper first molar were obtained.