Expression of Inflammatory Cytokines and Chemokines in Replanted Permanent Teeth with External Root Resorption

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Abstract

Introduction: The progressive forms of inflammatory external root resorption (IERR) and replacement external root resorption (RERR) are serious complications and the main causes of tooth loss after replantation. This study aimed to investigate the expression pattern of inflammatory molecules in extracted human teeth presenting with external root resorption (ERR) after replantation. **Methods:** Root fragments from 22 teeth showing IERR and 20 teeth with RERR were triturated using a homogenizer to extract inflammatory molecules. Interleukin-1β (IL-1β), IL-1Ra, transforming growth factor beta, IL-8/CXCL8, CCL2, CCL3, and CCL5 were measured using double-ligand enzyme-linked immunosorbent assay, and IL-2, IL-4, IL-6, IL-10, tumor necrosis factor alpha, interferon gamma, and IL-17A detection was performed using the multiplex Th1/Th2/Th17 Cytometric Bead Array kit (BD Biosciences, San Jose, CA). Cytokine and chemokine concentrations were compared in the RERR and IERR groups corrected by patients' age at the moment of extraction, survival time after replantation, and index of ERR, adopting a generalized estimation equation model. **Results:** The IERR group showed higher levels of tumor necrosis factor alpha than the RERR group, even after correction for the index of ERR ($P < .05$). IL-1Ra levels were higher in the IERR group for moderate cases but higher in the RERR group for severe cases ($P < .05$). IL-4 concentration became higher with the increase of patients' age in the RERR group but did not vary in the IERR group ($P < .05$). CCL2 levels decreased with the increase of the patients' age at the moment of extraction irrespective of the type or index of ERR ($P < .05$). **Conclusions:** The present results showed differences in the immunologic profile of IERR and RERR that may be relevant to understanding the biological mechanisms underlying ERR. *(J Endod 2016;■:1–7)*

Key Words

Avulsion, chemokines, cytokines, inflammatory external root resorption, replacement external root resorption, replantation

Significance

This article provided a great contribution to the understanding of the molecular mechanisms underlying external root resorption in replanted teeth. This is a first step to manipulate clastic activity, a key point to maintaining avulsed teeth replanted under unsuitable conditions.

External root resorption (ERR) represents a serious complication after replantation of avulsed teeth, and its progressive forms, replacement external root resorption (RERR) and inflammatory external root resorption (IERR), are the main causes of replanted tooth loss (1). The pathogenesis of progressive ERR involves an initial injury that allows the attachment of preosteoclasts to the root surface and their subsequent final differentiation and activation (2). Such an initial stimulus, in both types, is the mechanical damage to the periodontal ligament (PL) and the cementoblastic layer during the displacement of the tooth. Damage to the PL also results from an extended extraoral storage of the tooth in an unsuitable medium (3). However, the progression of the resorption process is dependent on additional factors that continuously stimulate recruitment, differentiation, and activation of the resorptive cells. IERR is supported by the immune response against bacteria and their by-products present inside the root canal that reach the damaged PL through dentinal tubules. Such an etiopathogenesis of IERR was suggested by experimental (4) and clinical studies showing that adequate endodontic treatment prevented or eliminated IERR (5). On the other hand, RRER results from the loss of large areas of PL in the absence of infection occurring at the expenses of stem cells derived from the alveolar side of the socket (6). This healing process leads to the incorporation of the root into the normal remodeling process of the alveolus, resulting in the gradual replacement of tooth structure by bone. The regulatory mechanisms of RERR are incompletely understood, and there is no effective treatment so far (2, 6). Cytokines play a pivotal role in modulating the cellular events involved in bone resorption and also have been suggested to participate in tooth resorption (7, 8). The imbalance between inflammatory and anti-inflammatory cytokines was implicated in the onset and

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progression of physiologic primary root resorption (9) and external apical root resorption (EARR) after orthodontic movement (10). Zhang et al (11) showed that interleukin-1 (IL-1) and, more particularly, tumor necrosis factor alpha (TNF-α) were important for the induction and progression of mechanically induced ERR in rats. Rego et al (12) showed that the impairment of the TNF-α signaling pathways decreased the severity of ERR on replanted rat molars. Chemokines also play a crucial role in regulating cell trafficking during inflammatory and homeostatic bone remodeling (13, 14). The role of chemokines in oral inflammatory diseases is well established (15) and has also been suggested to play a role in dentin matrix dissolution (15, 16). Nonetheless, there is little information regarding the specific role of these soluble factors during ERR after trauma. Therefore, the present study aimed to investigate the expression patterns of inflammatory cytokines and chemokines in teeth extracted from patients presenting with IERR and RERR after replantation.

Materials and Methods

Subjects and Teeth

The sample was composed of 40 patients, 30 males (75%) and 10 females (25%), from the dental trauma clinic at the faculty of dentistry of Federal University of Minas Gerais in Belo Horizonte, Brazil. Forty-two replanted permanent teeth referred for extraction with different stages of ERR were included. The control group comprised 5 patients with 12 mature premolars with required extraction for orthodontic reasons with good periodontal health and no radiographic evidence of periodontal bone loss. Patients who used systemic drugs that may interfere with bone metabolism; patients who underwent antibiotic, anti-inflammatory, hormonal, or other assisted drug therapy within 6 months before the study; patients with primary or secondary acute periodontitis; or females who were pregnant or breastfeeding were not included in the study. The study was performed with the informed consent of the patients and their guardians and was approved by the Committee on Ethics in Research of the Federal University of Minas Gerais.

Radiographic Assessment of Type and Extension of ERR

Periapical radiographs taken at the moment of extraction and assigned with a code were independently blindly examined by 2 investigators (J.V.B. and M.I.S.C.). Radiographic standardization followed established criteria, which were described elsewhere (17). Data regarding the presence and extension of the external root resorption were assessed using the root resorption index developed by Andersson et al (18). Bowl-shaped radiolucencies in the resorption area were classified as IERR (Fig. 1*A*). Bone structures and loss of the periodontal space in the resorption area were classified as RERR (Fig. 1*B*) according to Andreasen et al's criteria (19).

Sample Preparation

Immediately after extraction, the crowns were separated under saline irrigation. The root fragments were weighed, stored in buffer solution (0.4 mmol/L sodium chloride, 10 mmol/L NaPO4, pH = 7.4) containing protease inhibitors (0.1 mmol/L phenylmethylsulfonyl fluoride, 0.1 mmol/L benzethonium chloride, 10 mmol/L EDTA, and 0.01 mg/mL aprotinin A) and Tween 20 (VETEC, Sigma-Aldrich, Duque de Caxias, RS, Brazil) (0.05%, pH = 7.4) at a ratio of 1 mL of solution per 100 mg tissue, and triturated using a homogenizer (PowerGen Model 1000 Homogenizer; Fisher Scientific, Loughborough, UK) to allow proper extraction of inflammatory molecules. After centrifugation at 1000 rpm at 4°C, the supernatant was collected and stored at −80°C for further analysis. Control teeth were extracted and processed in the same way as in the case groups, except for the fact that they underwent a pulpectomy procedure before extraction.

Detection of Cytokines and Chemokines

The levels of the cytokines IL-1β, IL-1Ra, and transforming growth factor beta and the chemokines IL-8/CXCL8, CCL2, CCL3, and CCL5 were evaluated by double-ligand enzyme-linked immunosorbent assay using commercially available kits (R&D Systems, Minneapolis, MN) according to the manufacturer's instructions. In brief, each cytokine was detected by an anticytokine horseradish peroxidase-labeled monoclonal antibody. The o-phenylenediamine dihydrochloride (Sigma-Aldrich, St Louis, MO) peroxidase substrate kit was used to determine the amount of horseradish peroxidase bound to each well. The reaction was stopped by the addition of 1 mol/L sulfuric acid (H_2SO_4). The plates were read at 492 nm, and a standard curve was prepared for each assay. IL-2, IL-4, IL-6, IL-10, TNF-α, interferon gamma (IFN-γ), and IL-17A detection was performed using the Human Th1/Th2/Th17 Cytometric Bead Array Kit (BD Biosciences, San Jose, CA) following the manufacturer's instructions. Acquisition was performed with a FACSCanto II flow cytometer (BD Biosciences). The instrument has been checked for sensitivity and overall performance with Cytometer Setup and Tracking beads (BD Biosciences) before data acquisition. Quantitative results were generated using FCAP Array v1.0.1 software (Soft Flow Inc, Pecs, Hungary). The results were expressed as picograms of cytokine/100 mg tissue.

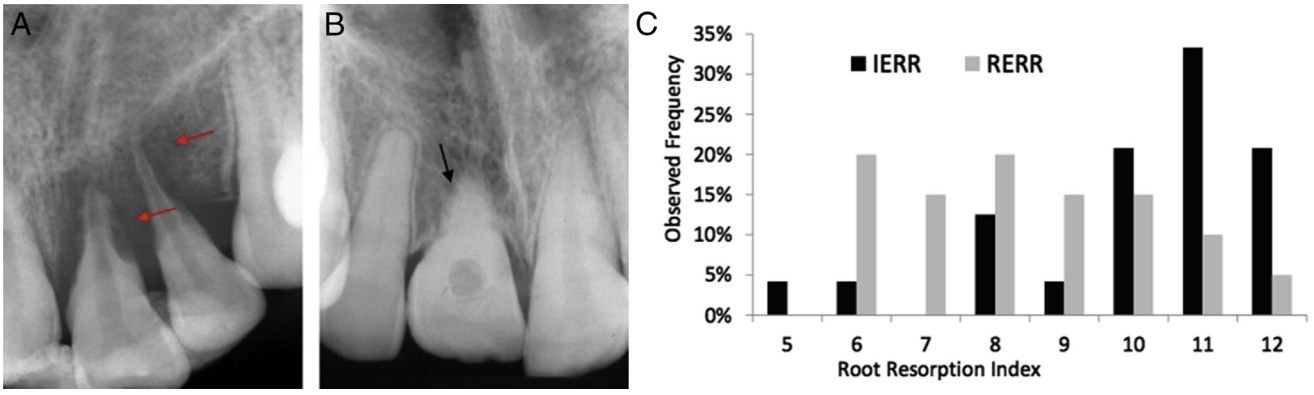

Figure 1. (*A*) The radiographic aspect of IERR: bowl-shaped radiolucency in the root surface and adjacent bone (*red arrows*). (*B*) Radiographic diagnosis of RERR: bone structures in the resorption area and loss of the periodontal space (*black arrows*). (*C*) Sample distribution according to root resorption index in the IERR (n = 22) and RERR (n = 20) groups.