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Tumor Necrosis Factor Identified in Periapical Tissue Exudates of Teeth with Apical Periodontitis

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Root canal samples, taken from periapical tissue exudates during routine root canal treatment procedures, were processed for identification of tumor necrosis factor using a mouse anti-human monoclonal antibody and enzyme-linked immunosorbent assay. Detectable levels of tumor necrosis factor were identified in periapical tissue exudates in chronic apical periodontitis.

Tumor necrosis factor (TNF), a cytokine initially identified as a cause of hemorrhagic necrosis in certain tumors, was later shown to be the same molecule as Cachectin, a serum product earlier known as a mediator of wasting in chronic disease (1–4). TNF, a 17-kD polypeptide, is known to have a wide variety of hormone-like effects on cells (1, 3, 4) and appears to be a major mediator of the inflammatory process (1). Produced in great abundance, TNF may fulfill protective functions such as contributing to tissue repair and host defense against tumors and pathogens, but it also capable of causing chronic wasting syndrome, anorexia and ensuing weight loss, cachexia and lethal Gram-negative (endotoxin-induced) shock (1–3). In addition to these diverse bioactivities, TNF is the only molecule, other than interleukin 1, that is presently known to have osteoclast-activating function (1). TNF induces the release of calcium from bone in vitro and may play a significant role in a variety of inflammatory disease states involving bone resorption (4). Methods to detect normal and minute elevations of TNF have been described (5–9). Elevated levels of circulatory TNF have been detected in the bloodstream of human volunteers after endotoxin administration (5), in sera of children suffering from Gram-negative sepsis (7), in serum of patients with systemic lupus erythematous (8), and in synovial exudates of patients with rheumatic arthritis (9).

TNF is almost exclusively produced by macrophages in response to the bacterial endotoxin lipopolysaccharide (LPS) (1, 4).

Lipopolysaccharide-producing bacteria play a major causative role in the pathogenesis of apical periodontitis (10, 11). The necrotic pulp is insufficient by itself to cause apical periodontitis, and only when infected with certain anaerobic Gram-negative species will apical periodontitis and resorption of periapical bone ensue (10, 11).

The inflammatory tissue present in chronic apical periodontitis is populated predominantly by macrophages (12). One can expect, therefore, that detectable levels of TNF may be present in periapical tissues of teeth with apical periodontitis.

Reports on the role of TNF in apical periodontitis, indeed its presence in normal and inflamed pulpal and periapical tissues, are lacking in the literature.

The purpose of this study was, therefore, to determine whether elevated levels of TNF were present in periapical tissue exudates of teeth associated with apical periodontitis.

MATERIALS AND METHODS

Samples were taken via the root canals from periapical exudates during routine treatment of teeth with apical periodontitis (Table 1). The teeth selected for this study were clinically asymptomatic, but had necrotic pulps. Radiographically, they had distinctly observable root canals and periapical radiolucent areas (Table 1). The samples were taken when the pulp chamber was initially opened and root canal treatment was to immediately follow. For control purposes, samples were also taken from five teeth that had no clinical or radiographic evidence of pulpal and periapical pathosis but needed routine endodontic treatment following exposure of a vital pulp. In this group, the samples were taken from tissue fluids in the root canals after pulp extirpation procedure.

The teeth were isolated with rubber dam, their surface was scrubbed with a 30% solution of hydrogen peroxide, and the operating field and teeth were disinfected with a 5% solution of iodine tincture. Access cavities were prepared and working lengths established 1 to 2 mm short of the radiographic apex. At this point of the treatment, root canal samples were taken in the following manner: in teeth with apical periodontitis, immediately after establishment of the working length, but before any instrumentation or irrigation procedures were performed, a paper cone, previously soaked in 10 ng of purified human serum albumin in phosphate-buffered saline (PBS) and air dried, was inserted into the root canal and extended apically as close as possible to the established working length. If the paper cone withdrawn from the canal was dry, the tooth was excluded from the study. The sample taking continued until the pulp space was dry. Each paper cone was placed in a 2.5-ml sterile plastic centrifuge tube, and the tubes were placed in crushed ice. In control teeth, after establishment of the working length and pulpectomy, tissue fluids were

TABLE 1. Total TNF recovered from samples taken from root canals of teeth with apical periodontitis

Tooth	Radiolucent Area (mm)	TNF (ng/ml)
2.2	2 × 2	4900
2.3	3 × 5	1400
1.6	2 × 3	250
1.5	2 × 2	153
2.4	3 × 4	150
1.6	3 × 3	124

collected from the root canal in the manner described above. Routine treatment of the patient was then continued. Except for the collection of paper cone samples, at no time was treatment of the patients different from the routine, and results of study had no effect on the patients' treatment.

After completion of a treatment visit, the samples were transported to the laboratory and maintained at 4°C until they were analyzed for the presence of TNF.

An enzyme-linked immunosorbent assay method previously described (5) was modified and used to detect TNF in the samples. Mouse anti-human TNF monoclonal antibody was used to extract TNF from the paper cones in the following manner: each single paper cone was placed on the bottom of a sample well. PBS containing 0.05% Tween 20 and 2% fetal calf serum (100 μl) was added to each well, and paper cones were incubated in the plate for 1 h at 37°C. The paper cones were then removed and plates were processed. A 96-well plate (Dynatech Immulon I) plate was coated with a 1/1000 dilution of monoclonal antibody to TNF in 0.1 *M* sodium carbonate-sodium-sodium bicarbonate buffer at pH 9.6. The plates were incubated overnight at room temperature, emptied, and carbonate buffer (pH 9.6) with 2% fetal calf serum (FCS) was added and incubated at room temperature for 4 h or at 4°C until used. The plates were then washed four times with 0.01 *M* PBS with 0.05% Tween 20 (PBST) with a Bioptek automated microplate washer, TNF stock solution at a concentration of 0.75 mg per ml was diluted in the PBST to a concentration of 1500 pM and serial dilutions used in the plates for a standard curve of 1500 pM to 2 pM.

Each paper cone sample was laid in the wells with 100 μl of PBST-2% FCS and incubated for 1 h at 37°C. The paper cones were removed and the plates were washed four times with PBST. Then rabbit anti-TNF serum diluted 1/100 in PBS-2% FCS was added to each well and incubated for 1 h at 37°C followed by washing four times with PBST. A 1/5000 dilution of horseradish peroxidase-labeled goat anti-rabbit IgG (Organon Tekinka, West Chester, PA) in PBST-2% FCS was added to each well, incubated for 1 h at 37°C, and washed four times with PBST in the washer. Then 150 μl of 2,2'-azinobis(3-ethyl benzthiazoline sulfonic) at a concentration of 44 mg per ml in water were added to 10 ml of 100 mM critic acid-sodium citrate buffer (pH 4.0); 40 μl of 3% hydrogen peroxide were also added to the 2,2-azinobis(3-ethyl benzthiazoline sulfonic-citrate buffer. One-hundred microliters of this solution were also added to each well and color was allowed to develop. The plates were read at 414 nm on a Titertek Multisskan MCC/340, and data were analyzed by Skansoft from Linbro on a Basic Time BT/XT. Recombinant TNF was used as a standard for the construction of calibration curves.

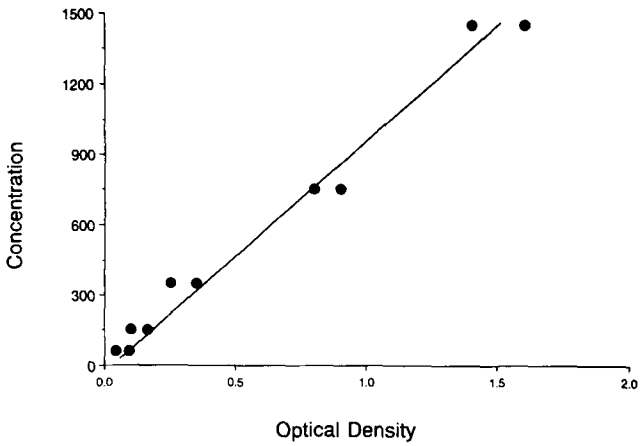

FIG 1. An example of reproducibility of TNF detection using enzyme-linked immunosorbent assay. Standard curve of optical density, read at 414 nm, is linear (r = ±0.966).

RESULTS

The TNF assays were reproducible and sensitive to 20 pM TNF (340 pg per ml) and the standard curve was linear from 1500 pM to 20 pM (Fig. 1).

TNF was detected in all periapical tissue exudate samples taken from the apical periodontitis group (Table 1). No TNF was detected in the control teeth.

DISCUSSION

Minute levels of TNF (<7 to 40 pg per ml) have been reported in sera of normal subjects (5, 8). In the present study, TNF, if at all present in tissue exudates of the control group, was not high enough to be detected with the method used. The TNF levels detected in periapical tissue exudates of the apical periodontitis group, therefore, may be correlated with the presence of apical periodontitis. TNF is an important bioactive material and its elevated levels have farreaching systemic consequences (3). Host responses to microorganisms residing in the dental pulp space (10, 11) play a major role in the breakdown of periapical bone. TNF is one of the two mediators known to cause bone resorption. The detection of relatively high levels of TNF in this preliminary study, therefore, warrants further investigations to elucidate the role this important mediator may play in oral infections.

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