Original Paper

IL-1α and TNF-α Expression in Rat Periapical Lesions and Dental Pulp after Unilateral Sympathectomy

Athanasia Bletsa   Karin Johanne Heyeraas   Sivakami Rethnam Haug
Ellen Berggreen

Department of Physiology, Faculty of Medicine, University of Bergen, Bergen, Norway

Abstract

Objectives: Apical periodontitis is an inflammatory disease characterized by bone resorption, and sympathetic nerves are known to modulate bone resorption and bone remodeling. Higher numbers of osteoclasts and larger periapical lesions have been observed after sympathectomy in rats, but the mechanisms underlying the inhibitory effect of sympathetic nerves on osteoclasts are unknown. This study aimed to test the hypothesis that sympathetic nerves inhibit the production of the bone-resorbing pro-inflammatory cytokines IL-1α and TNF-α in rat periapical lesions. *Methods:* Rats were unilaterally sympathectomized and apical lesions were induced by exposing the dental pulp of molar teeth to the oral microflora. We quantified the cytokines IL-1α and TNF-α by enzyme-linked immunosorbent assay, and immunohistochemical analysis was done for qualitative localization. Pulp from intact incisor teeth was tested as a control. *Results:* We showed that IL-1α was increased, but not TNF-α, in the periapical lesions on the sympathectomized side. Both IL-1α and TNF-α were expressed in unexposed pulp. TNF-α was significantly decreased in the denervated incisor pulp, whereas the level of IL-1α remained unchanged. *Conclusions:* This study suggests that sympathetic nerves have an inhibitory effect on IL-1α in periapical lesions and a stimulatory effect on TNF-α in the intact rat pulp.

Copyright © 2004 S. Karger AG, Basel

Introduction

Bacterial infection of the dental pulp results in inflammation and pulpal destruction. If untreated, the inflammation will proceed and involve inflammation with bone destruction at the periapical area (periapical lesions) with subsequent sprouting of sensory [1] and sympathetic fibers [2]. This condition is known as apical periodontitis. Sympathetic nerves are known to modulate bone resorption and bone remodeling. Regional sympathectomy increases osteoclast-mediated bone destruction [3, 4], indicating that sympathetic nerves may inhibit bone resorption. Furthermore, chemical sympathectomy is found to increase both the osteoclast surface and number and, thus, to increase remodeling of bone [5–7].

Interleukin-1α (IL-1α) and tumor necrosis factor-α (TNF-α) are pro-inflammatory cytokines with osteoclastic activity linked to the progression of inflammatory dis-

Athanasia Bletsa
Institutt for Biomedisin, Seksjon for fysiologi
Jonas Lies vei 91
NO–5009 Bergen (Norway)
Tel. +47 55 58 6337, Fax +47 55 58 6410, E-Mail Nancy.Bletsa@fys.uib.no

eases with bone destruction such as rheumatoid arthritis, osteomyelitis, marginal and apical periodontitis [8–10]. There is growing evidence that sympathetic nerves can directly affect cytokine production. In lipopolysaccharide-activated macrophages it has been shown that catecholamines have a suppressive effect on the secretion of TNF-α [11, 12]. Chemical sympathetic denervation enhances the synthesis of IL-1β and IL-6 in rats, suggesting a tonic inhibitory control of the sympathetic nerves on these inflammatory cytokines [13]. Several other studies also support that sympathetic nerves have anti-inflammatory effects [14–16].

We have recently reported that rats subjected to unilateral sympathectomy and pulp exposure exhibited more ED1-immunoreactive (IR) osteoclasts and larger periapical lesions in the sympathectomized side compared with the non-sympathectomized side [2]. The mechanism behind this inhibitory effect of the sympathetic nerves on osteoclasts is unknown. Our hypothesis is that sympathetic nerves inhibit the production of cytokines that have a proliferative and stimulatory effect on osteoclasts.

The aim of the present study was therefore to examine if levels of the pro-inflammatory cytokines IL-1α and TNF-α in periapical lesions were affected by sympathectomy. For this purpose, rats were unilaterally sympathectomized and periapical lesions were induced by exposing the dental pulp of molar teeth to the indigenous oral microflora. Unexposed rat incisor pulp was tested as a negative control for the above cytokines. Immunohistochemical analysis was performed to localize the areas of IL-1α and TNF-α expression in the rat jaws and to confirm the success of the surgical sympathectomy. In addition, sensory nerves were immunolabelled for calcitonin-gene-related peptide (CGRP) since we have previously shown an upregulation of substance P- and CGRP-IR neurons in the trigeminal ganglion after sympathectomy [17], but until now it is unknown if an increased transport of these neuropeptides to the peripheral target area takes place.

We show that IL-1α is increased in periapical lesions on the denervated side, indicating an inhibitory effect of the sympathetic nerves on IL-1α production in inflamed tissue. For TNF-α, no differences were observed between the two sides. Surprisingly, both IL-1α and TNF-α were expressed in unexposed dental pulp. TNF-α was significantly decreased on the denervated side, indicating that sympathetic nerves may have a stimulatory effect on this cytokine in intact rat incisor pulp.

Table 1. Distribution of animals used

Group	Procedures			n
	identified right SCG	removed right SCG	pulp exposures 1st and 2nd maxillary molars	
Experimental	yes	yes	yes	20
Sham	yes	no	yes	6
Control	no	no	no	3

Materials and Methods

Preparation of Animals (table 1)

A total of 29 adult male Sprague-Dawley (Ntac: SD) rats weighing 290–350 g were used. The animals were housed in polycarbonate cages and kept on a 12-hour light-dark cycle, with food (standard rodent pellets) and water available ad libitum. Animals were allowed to acclimate to laboratory conditions for a minimum of 7 days before experimental manipulation. All the procedures were carried out with the approval and under the supervision of the Norwegian Experimental Animal Board.

On day 0, 26 animals were anesthetized subcutaneously with (1:1) Hypnorm® (Janssen Pharmaceutica, Beerse, Belgium) and Dormicum® (Hoffmann-La Roche, Basel Switzerland) each diluted with an equal volume of sterile water, to a total of 2.7 ml/kg body weight. The resulting mixture contains 2.5 mg/ml fluanisone, 0.079 mg/ml fentanyl citrate and 1.25 mg/ml midazolam. Unilateral (right) surgical removal of the superior cervical ganglion (SCG) was performed in 20 experimental animals under a stereomicroscope. Briefly, a vertical incision was made on the ventral surface of the neck adjacent to the midline. The SCG was localized at the bifurcation area of the common carotid artery, dorsally and removed with microscissors. The skin incision was closed with sutures. Sympathectomy was confirmed by observation of Claude Bernard-Horner's syndrome on the operated side (ptosis of the eyelid and constriction of the pupil) and by immunohistochemistry. Six animals were subjected to sham operation where the right SCG was identified but not removed. Sympathectomized and sham-operated rats were mounted on a jaw-retraction board. Pulp exposures were performed bilaterally under a stereomicroscope on the first and second maxillary molar, with 006 round burs to the depth of the diameter of the burs, avoiding furcal perforation. The exposed pulps were left open to the oral environment for 3 weeks in order to develop periapical inflammation (fig. 1). All the animals exhibited normal feeding habits and gained weight during the experimental period. Three rats were used as unoperated controls.

On day 21, all except 3 sympathectomized rats were killed by an overdose of Mebumal® (50 mg/ml pentobarbital; Svaneapoteket, Bergen, Norway). In the sympathectomized (n = 17) and sham-operated (n = 6) rats, the right and left maxillary jaws and the pulp of the incisor teeth were immediately removed. In the unoperated controls (n = 3), only pulps of the incisor teeth were removed. The collected tissues were kept in RNAlater™ (Ambion, Cambridgeshire, UK) at –20°C until further processing (ELISA). The last 3 sympathectomized rats were used for immunohistochemistry. Under deep anesthe-
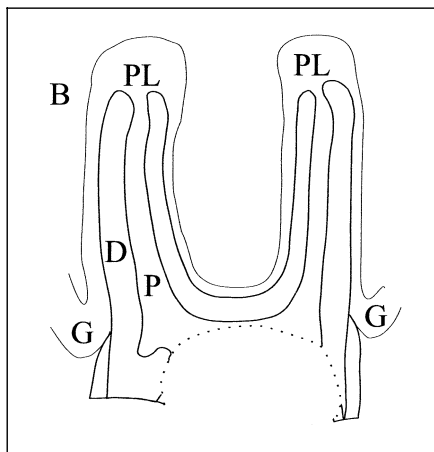
Fig. 1. Schematic diagram of periapical lesions in rat molar. By drilling cavities (dotted area) and exposing the pulp to the oral environment, we induced inflammation with bone destruction at the periapical area. G = Gingiva; D = dentin; P = pulp; PL = periapical lesion; B = bone.

Table 2. TNF-α levels in periapical lesions from unilateral (right) sympathectomized and sham-operated rats

Group	TNF-α, pg/mg tissue	
	right side	left side
Experimental (n = 12)	1.11±0.18	1.37±0.17
Sham-operated (n = 6)	0.56±0.16	1.03±0.38

Data are presented as means ± SEM. n = Number of pairs.

sia, these animals were transcardiacally perfused with heparinized saline followed by 4% paraformaldehyde with 0.2% picric acid in 0.1 M phosphate-buffered saline, pH 7.4. The maxillary jaws were removed and postfixed in the above fixative solution overnight. The tissues were demineralized in Kristensen's decalcification solution (equal volumes of 8 N formic acid and 1 M sodium formate) at 4°C. After demineralization, the jaws were saturated in 30% sucrose in phosphate-buffered saline and stored at –80°C until sectioning.

Sample Preparation for ELISA

The jaws were treated on ice under the microscope. Gingiva and oral mucosa were dissected free of the samples. The buccal and lingual plates of bone overlying the roots of the molars were removed and the periapical lesions were scraped off with a small dental curette. The collected tissues were weighed and immediately frozen and stored at –80°C until extract preparation.

The frozen tissues were cut with microscissors and ground in sterile Eppendorf tubes with precooled instruments. The tissue fragments were dissolved in known volumes of lysis buffer (300–500 μl) consisting of 100 μg/ml bovine serum albumin (fraction V; Sigma, Steinheim, Germany), 100 μg/ml Zwittergent-12 (Boehringer-Mannheim, Indianapolis, Ind., USA), 50 μg/ml Garamycin (Schering-Plough, Brussels, Belgium), 10 mM Hepes buffer (Life Technologies, Paisley, Scotland, UK), 1 μg/ml aprotinin (Sigma), 1 μg/ml leupeptin (Sigma), 0.1 μM EDTA (Sigma), in RPMI-1640 (Sigma) [18]. The mixture was incubated on ice for 15–30 min. The digestate was sonicated for 20–30 s. Debris was removed by centrifugation. The supernatant was then collected and aliquoted into smaller volumes that were either used immediately for ELISA or stored at –80°C. The levels of IL-1α and TNF-α in the tissues were assayed in duplicate with commercially available ELISA kits according to the manufacturer's instructions (BioSource International Cytoscreen™ Rat IL-1α and TNF-α, Camarillo, Calif., USA). The concentration of cytokines present in the samples was calculated with reference to a stan-

dard curve. Results were expressed as picograms of cytokine per milligram of tissue, calculated from the known weight of the tissue sample and the volume of lysis buffer in which the sample was homogenized.

Statistical Analysis

Data are presented as means ± SEM. Differences between the two sides were tested with paired t test, and a p value of less than 0.05 was considered as statistically significant.

Immunohistochemistry

Serial 40-μm sections of the jaws were made in a freezing slide microtome. Immunoreaction was performed on free-floating sections in tissue culture wells. Alternate serial sections were incubated for 72 h in rat IL-1α (1:400 dilution; Endogen, Cambridge, Mass., USA), TNF-α (1:300 dilution; Endogen), CGRP (1:6,000 dilution; Diagnostika, Falkenberg, Sweden) and neuropeptide Y (NPY; 1:4,000 dilution; Diagnostika) polyclonal antibodies raised in rabbits at 4°C. Omission of the primary antibody tested the specificity of the immune reaction. Antigen-antibody complexes were detected by the avidin-biotin peroxidase (ABC) method, using a commercially available kit (Vectastain ABC kit; Vector Laboratories, Burlingame, Calif., USA) and visualized by 3,3'-diaminobenzidine (Sigma) in the presence of 0.2% $(NH_4)_2Ni(SO_4)_26H_2O$ to enhance the immunostaining. Finally, the sections were mounted on gelatin-coated slides and counterstained with methylene blue/azure II in 1% sodium borate and distilled water. They were then dehydrated in graded alcohol series, cleared in xylene and coverslipped with Eukitt (Kindler, Freiburg, Germany). The sections were evaluated using a Leitz photomicroscope.

Results

Detection of Cytokine Proteins with ELISA

Periapical Lesions. IL-1α and TNF-α were both present in the tissues from the periapical area of the exposed 1st and 2nd maxillary molars. Periapical lesions from the sympathectomized side exhibited a significant increase in IL-1α when compared to the contralateral side (p < 0.006; fig. 2). No such difference was seen in the sham-operated

Fig. 2. IL-1α levels in periapical lesions from unilateral (right) sympathectomized and sham-operated rats. Data are presented as means ± SEM, n = number of pairs. ** p < 0.006 vs. the contralateral side of the same group.
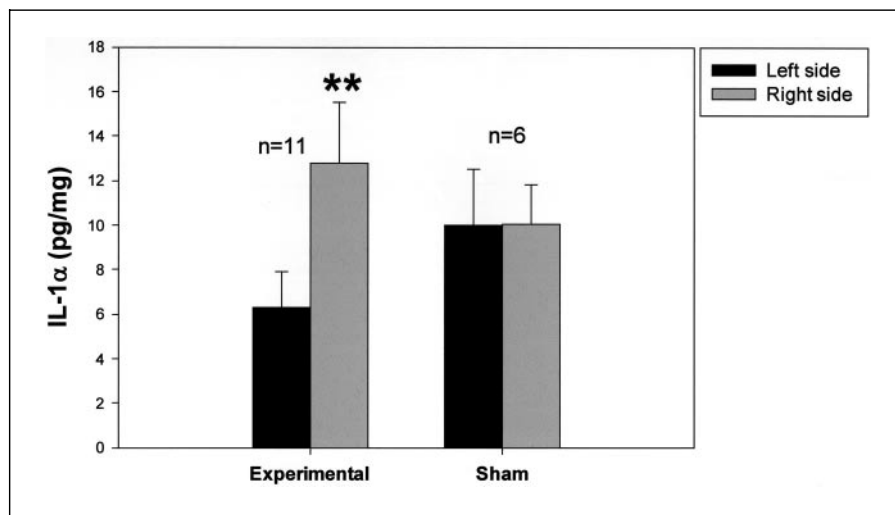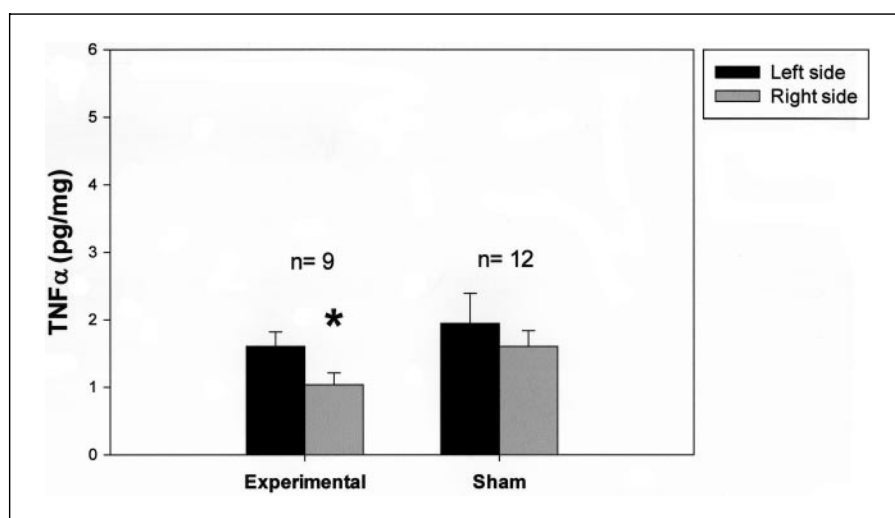

Fig. 3. TNF-α levels in normal incisor pulp from unilaterally (right) sympathectomized and sham-operated rats. Data are presented as means ± SEM, n = number of pairs. * p < 0.05 vs. the contralateral side of the same group.

rats (fig. 2). Low levels of TNF-α were present in the periapical lesions. There was no difference between right and left sides in either the sympathectomized or the sham-operated rats (table 2).

Incisor Pulps. Both IL-1α and TNF-α were detected with ELISA in the incisor pulps of all rats (sympathectomized, sham-operated and unoperated controls). There was, however, no significant difference in the IL-1α content in the pulps when right and left sides were compared in all three groups. In the sympathectomized rats, the pulps showed significantly decreased levels of TNF-α in the right (sympathectomized) side compared to the left side (p < 0.05), whereas in the sham-operated rats no significant difference in TNF-α levels between right and left sides was found (p = 0.4; fig. 3). The control rats exhibited

no difference between the two sides with respect to TNF-α (p = 0.29).

Immunohistochemistry

Neuropeptides. In the sympathectomized animals, no NPY-IR fibers were seen in the right jaws as demonstrated in figure 4A for the dental pulp, confirming the success of the unilateral sympathectomy. On the contralateral side, numerous NPY-IR fibers were mainly localized in the pulp of the intact third molar (fig. 4B) as well as in areas of vital root pulp of the exposed 1st and 2nd molars. These fibers appeared dense and beaded (fig. 4C) and they were numerous compared to normal pulps of control rats (data not shown), suggesting an NPY increase contralateral to the sympathectomized side.
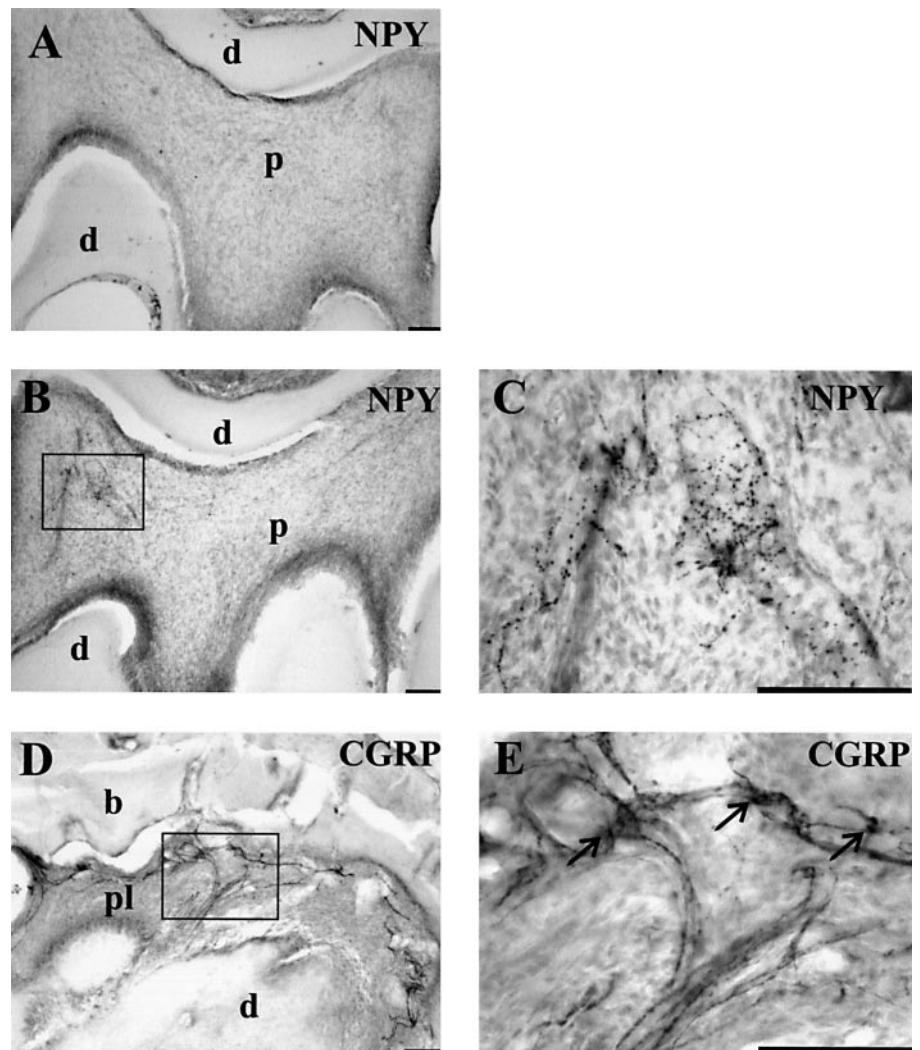
Pro-Inflammatory Cytokines after
Sympathectomy

Fig. 4. Immunohistochemical staining for NPY (**A–C**) in the pulp and CGRP (**D**, **E**) in the periapical area. **A** Complete loss of NPY staining in the pulp of a maxillary 3rd molar in the sympathectomized side. **B** NPY-IR in a maxillary 3rd molar contralateral to the sympathectomized side. **C** Higher magnification of the boxed area in **B** showing beaded, densely stained fibers. **D** Sprouting of CGRP-IR fibers in periapical lesions on the sympathectomized side. **E** Higher magnification of the boxed area in **D** shows CGRP-IR fibers with knob-like and beaded appearance (arrows). d = Dentin; p = pulp; pl = periapical lesion; b = bone. Bar = 100 µm.

No visible differences in the staining of CGRP-IR fibers were seen between the two sides. These fibers showed sprouting in the periapical areas (fig. 4D, E) and in vital apical pulps of the exposed 1st and 2nd molars, indicating periapical and pulpal inflammation.

Cytokines. In intact teeth (incisors and 3rd molars), the odontoblast layer of the pulp was intensely stained for IL-1α (fig. 5A) but not for TNF-α. Scarce TNF-α staining appeared in the pulp body (fig. 5B). During the 21-day experimental period, not all the exposed 1st and 2nd molars developed periapical lesions. Some exposed roots exhibited coronally a zone of pulp necrosis with inflamed pulp right underneath, followed by healthy pulp apically and were surrounded by normal periodontal ligament and bone structure. In the inflamed zone underneath the pulpal exposure (fig. 5C, D), the odontoblast layer seemed to lack the IL-1α-staining capacity. IL-1α-expressing cells appeared in the pulp body in this zone. The odontoblast layer kept the IL-1α staining capacity more apically, where the pulp appeared normal (fig. 5C, E).

IL-1α- and TNF-α-expressing cells were also localized in periapical lesions, root resorption lacunae and gingiva. At the exposed molars, IL-1α-expressing cells were seen in abscesses surrounding the apical foramen or between roots (fig. 6A). TNF-α staining was mainly localized in the periphery of periapical lesions (fig. 6B). Areas of resorption along the inflamed roots with cells IR for both IL-1α and TNF-α were commonly observed (fig. 6C, D). These cells had multinucleated appearance in high magnification. In addition, the interdental papillae showed cells with cytoplasmic staining for IL-1α and TNF-α (fig. 6E, F), revealing inflammation in the gingiva. No visible dif-
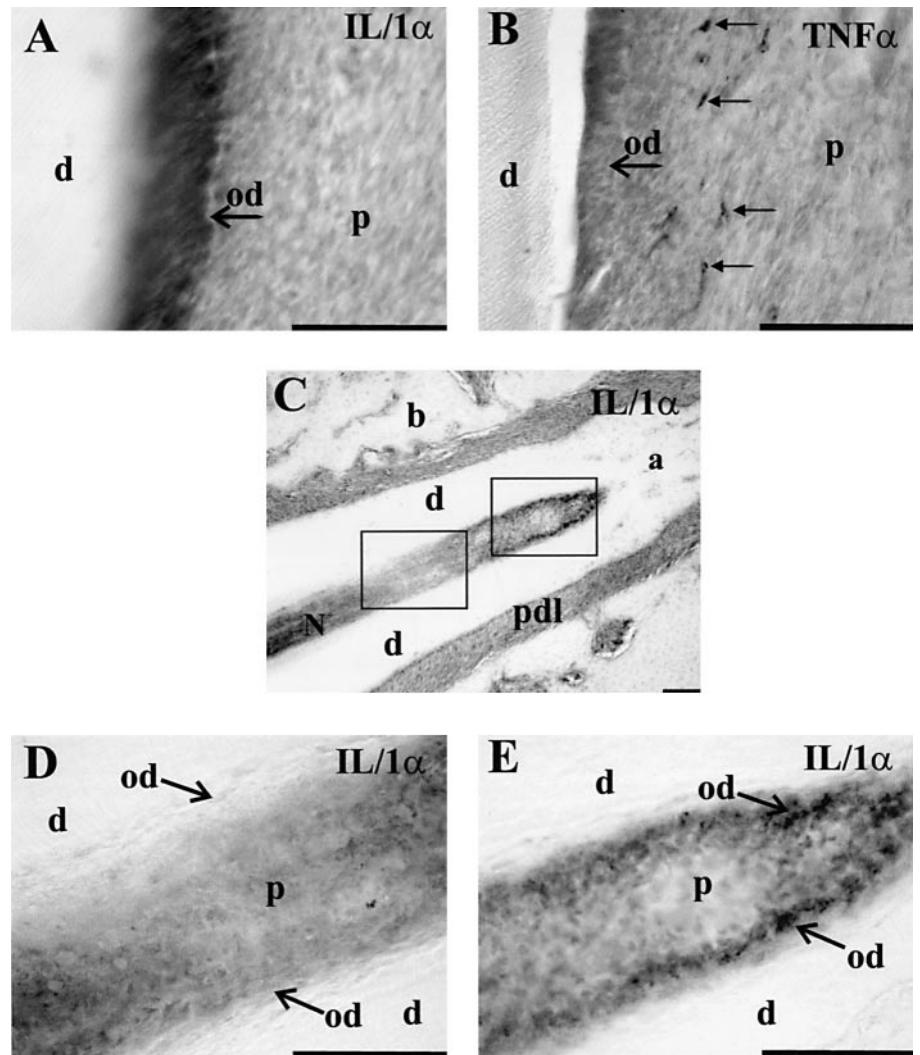
Fig. 5. Immunohistochemical micrographs from intact (**A**, **B**) and exposed (**C–E**) pulp for IL-1α- or TNF-α-IR cells. **A** Intact pulp from a maxillary 3rd molar showing distinct staining of the odontoblast layer for IL-1α (arrow). **B** The odontoblasts do not stain positively for TNF-α (large arrow), but scarcely cells in the pulp body do (small arrows). **C** Low magnification of a root from an exposed molar that did not develop periapical lesion. The zone of necrotic pulp (N) is followed by a zone of inflamed pulp (left box) and a zone of normal pulp apically (right box). **D** Higher magnification of the left boxed area in **C** showing lack of IL-1α staining at the odontoblast layer (arrows). **E** Higher magnification of the right boxed area in **C** showing IL-1α-IR odontoblasts (arrows). N = Necrotic pulp; p = pulp; d = dentin; od = odontoblasts; b = bone; a = apex; pdl = periodontal ligament. Bar = 100 μm.

ferences were observed between sympathectomized and contralateral sides with respect to IL-1α and TNF-α immunoreactivity.

All the negative controls showed lack of specific immunostaining.

Discussion

Our data indicate that the IL-1α levels significantly increase in periapical lesions in response to sympathectomy, suggesting that sympathetic nerves inhibit the synthesis of the pro-inflammatory cytokine IL-1α in inflamed tissue. This suppressed production of IL-1α in periapical lesions in the non-sympathectomized side may be explained by a direct inhibiting effect of sympathetic neuro-transmitters on cytokine-producing cells. Chemical denervation of peripheral sympathetic nerves has been shown to enhance the production of peripheral IL-1β and IL-6, suggesting a tonic inhibitory control of sympathetic nerves on these inflammatory cytokines [13]. In contrast, the present TNF-α expression in periapical lesions appeared unaffected by sympathectomy. Although both IL-1α and TNF-α are pro-inflammatory cytokines produced by similar cell populations, our findings indicate that sympathetic nerves exhibit a selective inhibition on IL-1α production. This is in line with other studies that reported no effect of chemical sympathectomy in TNF-α serum levels whereas IL-1 was increased after administration of lipopolysaccharides in rats [13]. Another explanation for the present finding of increased IL-1α production, but not TNF-α, may be the different localization of IL-1α- and
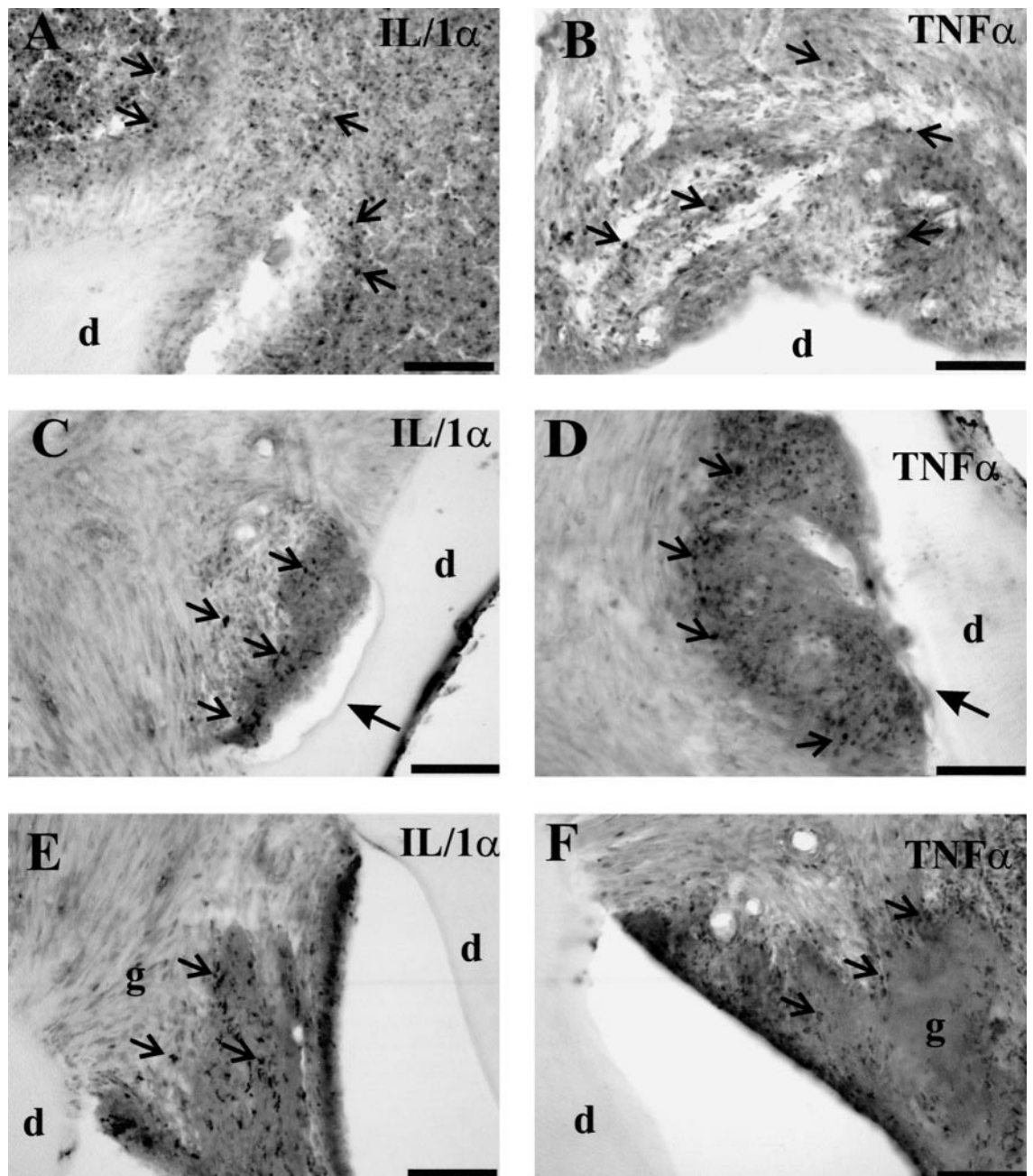
Fig. 6. Immunohistochemical micrographs from lesions between exposed roots (**A**, **B**), resorption lacunae (**C**, **D**) and gingiva (**E**, **F**) stained for IL-1α or TNF-α. **A** Abscess between the exposed roots of a 1st maxillary molar in the sympathectomized side. Numerous IL-1α-positive cells are scattered in the lesion (arrows). **B** Area corresponding to **A** from a different molar (left side) showing staining for TNF-α at the periphery of the abscess (arrows). **C** Big arrow points to resorption lacunae along the root of an exposed molar on the sympathectomized side. The dentin appears resorbed and the inflammatory tissue that infiltrates the area exhibits IL-1α-stained cells (small arrows). **D** Resorption lacunae corresponding to **C** (big arrow) from the contralateral side with cells stained for TNF-α (small arrows). **E** Cells (arrows) with cytoplasmic staining for IL-1α in the interdental papilla (gingiva between 1st and 2nd maxillary molar) from the sympathectomized side. **F** Area corresponding to **E** from the contralateral side showing TNF-α-IR cells (arrows). d = Dentin; g = gingiva. Bar = 100 μm.

TNF-α-producing cells in periapical lesions. By immunohistochemical staining we observed that the cells producing TNF-α were located at the periphery of the inflamed periapical lesions in contrast to IL-1α staining that was scattered in the lesions (fig. 6A, B). This observation is in agreement with previous findings from immunohistochemical analyses of human periapical granulomas that showed IL-1β-positive cells located in active exudation and TNF-α-positive cells scattered more at the periphery of the lesion [19]. It seems possible that remnants of tissue in the periphery of the lesion might be left in the jaws upon collection and, therefore, differences in TNF-α production remained undetectable in our study. It is also possible that sympathectomy could have affected the TNF-α expression earlier, before collection of tissue for ELISA measurements took place, suggesting a different time frame for the effect of sympathectomy on the two mentioned cytokines.

Interactions between sensory and sympathetic nerves have been described. It is shown that selective denervation of one type of fibers results in a marked sprouting response of the other type of fibers [20]. We have observed an upregulation of neuron IR for substance P and CGRP in the trigeminal ganglion after sympathectomy [17], but it is unknown if an increased transport to the peripheral target area takes place. Sensory neuropeptides have a chemotactic effect on neutrophils, macrophages and T lymphocytes [21–23], and stimulate cytokine production [24]. An upregulation of the sensory neuropeptides on the sympathectomized side might therefore influence the level of IL-1α and TNF-α in the periapical lesions. Sprouting of sensory nerves was observed around the periapical lesions (fig. 4D, E) bilaterally. However, we were not able to quantify differences in the number/distribution of CGRP-IR fibers between sympathectomized and contralateral sides with the present immunohistochemical technique.

The immunohistochemical analysis revealed IL-1α staining of the odontoblast layer that disappeared underneath necrotic pulp tissue. We speculate that IL-1α production might be downregulated by factors derived from the necrotic tissue, or that the odontoblasts underwent pathological changes and lost their capacity to express IL-1α. We observed strong IL-1α immunoreactivity of the odontoblast layer in intact teeth (both molars and incisors) confirmed also by ELISA measurements. This is an important finding because normal pulp tissue has previously been used as a negative control for polymerase chain reaction measurements of this cytokine [25]. The odontoblast layer is the outermost layer of cells in the den-

tal pulp and represents the first line of defense against bacterial challenges. Since these cells were shown to be IL-1α positive, they might be significant in the initiation and perpetuation of inflammation in the dental pulp. Sympathectomy had no effect on the levels of IL-1α in the incisor pulp. This might be due to the fact that sympathetic fibers are not normally found in the odontoblast layer and dentin, but are mainly associated with larger blood vessels in the root pulp and deeper parts of the pulp proper [26–28].

In this study, ELISA measurements exhibited significantly lower levels of TNF-α in incisor pulp from the sympathectomized side compared to the non-sympathectomized side indicating that sympathetic nerves may have a stimulatory effect on this cytokine in the dental pulp. This observation contradicts earlier findings that showed decreased TNF-α production after sympathectomy [29, 30]. However, numerous studies have described contradictory effects of neurotransmitters in immunomodulation [31]. Immunohistochemical analysis showed that TNF-α-positive cells were scattered in the pulp body, in close proximity to sympathetic nerves. The different localization of the IL-1α- and TNF-α-producing cells in the intact pulp may explain the diversity in the effect of sympathectomy on cytokine expression.

In summary, the periodontal diseases are inflammatory conditions mediated by host-microbial interactions and characterized by bone resorption. Several risk factors have been identified for the progression of these diseases, and physical or psychological stress seems to be one of them [32]. Under stress the sympathetic nervous system is activated. The precise mechanism(s) by which the sympathetic system might alter the progression of periodontitis is unclear. The present study provides evidence that the sympathetic nerves do affect the production of bone-resorptive cytokines in oral tissues providing a basis for different treatment strategies in the area of periodontal diseases. Further investigation is needed.

Acknowledgments

References

1 Kimberly CL, Byers MR: Inflammation of rat molar pulp and periodontium causes increased calcitonin gene-related peptide and axonal sprouting. Anat Rec 1988;222:289–300.

2 Haug SR, Heyeraas KJ: Effects of sympathectomy on experimentally induced pulpal inflammation and periapical lesions in rats. Neuroscience 2003;120:827–836.

3 Sandhu HS, Herskovits MS, Singh IJ: Effect of surgical sympathectomy on bone remodeling at rat incisor and molar root sockets. Anat Rec 1987;219:32–38.

4 Ladizesky MG, Cutrera RA, Boggio V, Mautalen C, Cardinali DP: Effect of unilateral superior cervical ganglionectomy on bone mineral content and density of rat's mandible. J Auton Nerv Syst 2000;78:113–116.

5 Hill EL, Turner R, Elde R: Effects of neonatal sympathectomy and capsaicin treatment on bone remodeling in rats. Neuroscience 1991; 44:747–755.

6 Sherman BE, Chole RA: Effect of pharmacological sympathectomy on osteoclastic activity in the gerbilline auditory bulla in vivo. Ann Otol Rhinol Laryngol 1999;108:1078–1087.

7 Sherman BE, Chole RA: Effect of chemical sympathectomy with 6-hydroxydopamine on osteoclast activity in the gerbilline middle ear bulla. Otol Neurotol 2001;22:237–241.

8 Offenbacher S: Mediators of bone resorption in periodontal disease. School of Dental Medicine. Boston, Harvard University, 1981.

9 Wang CY, Stashenko P: The role of interleukin-1 alpha in the pathogenesis of periapical bone destruction in a rat model system. Oral Microbiol Immunol 1993;8:50–56.

10 Stashenko P, Jandinski JJ, Fujiyoshi P, Rynar J, Socransky SS: Tissue levels of bone resorptive cytokines in periodontal disease. J Periodontol 1991;62:504–509.

11 Hu XX, Goldmuntz EA, Brosnan CF: The effect of norepinephrine on endotoxin-mediated macrophage activation. J Neuroimmunol 1991;31:35–42.

12 Spengler RN, Chensue SW, Giacherio DA, Blenk N, Kunkel SL: Endogenous norepinephrine regulates tumor necrosis factor-alpha production from macrophages in vitro. J Immunol 1994;152:3024–3031.

13 De Luigi A, Terreni L, Sironi M, De Simoni MG: The sympathetic nervous system tonically inhibits peripheral interleukin-1beta and interleukin-6 induction by central lipopolysaccharide. Neuroscience 1998;83:1245–1250.

14 Levine JD, Dardick SJ, Roizen MF, Helms C, Basbaum AI: Contribution of sensory afferents and sympathetic efferents to joint injury in experimental arthritis. J Neurosci 1986;6: 3423–3429.

15 Benzon HT, Chomka CM, Brunner EA: Treatment of reflex sympathetic dystrophy with regional intravenous reserpine. Anesth Analg 1980;59:500–502.

16 Hannington-Kiff JG: Relief of Sudeck's atrophy by regional intravenous guanethidine. Lancet 1977;i:1132–1133.

17 Haug SR, Berggreen E, Heyeraas KJ: The effect of unilateral sympathectomy and cavity preparation on peptidergic nerves and immune cells in rat dental pulp. Exp Neurol 2001;169:182–190.

18 Kawashima N, Stashenko P: Expression of bone-resorptive and regulatory cytokines in murine periapical inflammation. Arch Oral Biol 1999;44:55–66.

19 Artese L, Piattelli A, Quaranta M, Colasante A, Musani P: Immunoreactivity for interleukin 1-beta and tumor necrosis factor-alpha and ultrastructural features of monocytes/macrophages in periapical granulomas. J Endod 1991;17: 483–487.

20 Terenghi G, Zhang SQ, Unger WG, Polak JM: Morphological changes of sensory CGRP-immunoreactive and sympathetic nerves in peripheral tissues following chronic denervation. Histochemistry 1986;86:89–95.

21 Marasco WA, Showell HJ, Becker EL: Substance P binds to the formylpeptide chemotaxis receptor on the rabbit neutrophil. Biochem Biophys Res Commun 1981;99:1065–1072.

22 Roch-Arveiller M, Regoli D, Chanaud B, Lenoir M, Muntaner O, Stralzko S, Giroud JP: Tachykinins: Effects on motility and metabolism of rat polymorphonuclear leucocytes. Pharmacology 1986;33:266–273.

23 Foster CA, Mandak B, Kromer E, Rot A: Calcitonin gene-related peptide is chemotactic for human T lymphocytes. Ann NY Acad Sci 1992;657:397–404.

24 Lotz M, Vaughan JH, Carson DA: Effect of neuropeptides on production of inflammatory cytokines by human monocytes. Science 1988; 241:1218–1221.

25 Wang CY, Tani-Ishii N, Stashenko P: Bone-resorptive cytokine gene expression in periapical lesions in the rat. Oral Microbiol Immunol 1997;12:65–71.

26 Fristad I, Heyeraas KJ, Kvinnsland I: Nerve fibres and cells immunoreactive to neurochemical markers in developing rat molars and supporting tissues. Arch Oral Biol 1994;39:633–646.

27 Zhang JQ, Nagata K, Iijima T: Scanning electron microscopy and immunohistochemical observations of the vascular nerve plexuses in the dental pulp of rat incisor. Anat Rec 1998; 251:214–220.

28 Wakisaka S, Ichikawa H, Akai M: Distribution and origins of peptide- and catecholamine-containing nerve fibres in the feline dental pulp and effects of cavity preparation on these nerve fibres. J Osaka Univ Dent Sch 1986;26:17–28.

29 Molina PE, Abumrad NN: Central sympathetic modulation of tissue cytokine response to hemorrhage. Neuroimmunomodulation 1999; 6:193–200.

30 Tiegs G, Bang R, Neuhuber WL: Requirement of peptidergic sensory innervation for disease activity in murine models of immune hepatitis and protection by beta-adrenergic stimulation. J Neuroimmunol 1999;96:131–143.

31 Felten DL, Felten SY, Bellinger DL, Carlson SL, Ackerman KD, Madden KS, Olschowki JA, Livnat S: Noradrenergic sympathetic neural interactions with the immune system: Structure and function. Immunol Rev 1987;100: 225–260.

32 Genco RJ, Ho AW, Grossi SG, Dunford RG, Tedesco LA: Relationship of stress, distress and inadequate coping behaviors to periodontal disease. J Periodontol 1999;70:711–723.