Histochem Cell Biol (2001) 116:57–62
DOI 10.1007/s004180100298

ORIGINAL PAPER

**Mutsumi Miyauchi · Sunao Sato · Shoji Kitagawa
Masae Hiraoka · Yasusei Kudo · Ikuko Ogawa
Ming Zhao · Takashi Takata**

Cytokine expression in rat molar gingival periodontal tissues after topical application of lipopolysaccharide

Accepted: 28 May 2001 / Published online: 5 July 2001

Abstract It is well known that proinflammatory cytokines produced by host cells play an important role in periodontal tissue destruction. However, the localization of the cytokines in in vivo periodontal tissues during development of periodontal disease has not been determined. Immunohistochemical expression of proinflammatory cytokines including IL-1α, IL-1β, and TNF-α was examined at 1 and 3 h, and 1, 2, 3, and 7 days after topical application of lipopolysaccharide (LPS; 5 mg/ml in physiological saline) from *E. coli* into the rat molar gingival sulcus. In the normal periodontal tissues, a small number of cytokine-positive epithelial cells were seen in the junctional epithelium (JE), oral sulcular and oral gingival epithelium, in addition to macrophages infiltrating in the subjunctional epithelial area and osteoblasts lining the alveolar bone surface. Epithelial remnants of Malassez existing throughout periodontal ligament were intensely positive for IL-1β but negative for the other two cytokines. At 3 h after the LPS treatment, almost all cells in the JE were strongly positive for the cytokines examined. In addition, several cytokine-positive cells, including neutrophils, macrophages, and fibroblasts, were seen in the subjunctional epithelial connective tissue. At day 2, expression of the cytokines in the JE gradually decreased, while cytokine-positive cells in the connective tissue increased in number. Positive staining of the cytokines was seen in osteoclasts and preosteoclasts which appeared along the alveolar bone margin in this period. The number of cytokine-positive cells decreased by day 7. These findings indicate that, in addition to macrophages, neutrophils, and fibroblasts, the JE cells are a potent source of TNF-α, IL-1α, and IL-1β reacting to LPS application, and suggest that JE cells may play an important role in the first line of defense against LPS challenge, and the proinflammatory cytokines transiently produced by various host cells may be involved in the initiation of inflammation and subsequent periodontal tissue destruction.

Keywords Lipopolysaccharide · Cytokine · Periodontitis · Animal studies

M. Miyauchi (✉) · S. Sato · S. Kitagawa · M. Hiraoka · Y. Kudo
T. Takata
Department of Oral Pathology,
Hiroshima University Faculty of Dentistry, 1-2-3, Kasumi,
Minami-ku, Hiroshima 734-8553, Japan
e-mail: mmiya@hiroshima-u.ac.jp
Tel.: +81-82-2575633, Fax: +81-82-2575619

I. Ogawa
Clinical Laboratory,
Hiroshima University Faculty of Dentistry, 1-2-3,
Kasumi, Minami-ku, Hiroshima 734-8553, Japan

M. Zhao
Department of Periodontics/Prevention/Geriatrics,
The University of Michigan School of Dentistry,
1011 N. University Avenue, Ann Arbor, MI 48109-1078, USA

Introduction

Lipopolysaccharide (LPS), a major component of the outer membrane of gram-negative bacteria, induces the synthesis of cytokines such as interleukin-1 (IL-1; Lindemann et al. 1988; Garrison and Nichols 1989; McFarllane et al. 1990) and tumor necrosis factor (TNF; Lindemann et al. 1988; McFarllane et al. 1990) from macrophages, fibroblasts, and endothelial cells. These cytokines appear to play a central role in the pathogenesis of inflammatory periodontal diseases. Since these cytokines cause various tissue responses, such as activation of macrophages, induction of prostaglandin and/or collagenase from host cells and resorption of bone by osteoclasts, the cytokines derived from the various host cells in response to LPS is believed to act not only for host defense but also for periodontal tissue breakdown in plaque-associated periodontitis (Page 1991; Howell 1996; Okada and Murakami 1998).

In previous studies, we have used an experimental model in which initial periodontal tissue destruction is provoked by topical application of LPS from *Escherichia coli* into the rat gingival sulcus (Ijuhin 1988; Ijuhin et al. 1992; Takata et al. 1997; Miyauchi et al. 1998). In-

filtration of polymorphonuclear leukocytes and macrophages, vascular dilatation, and inflammatory edema in the subepithelial gingival connective tissue (Ijuhin 1988), enhancement of collagen phagocytosis by periodontal ligament fibroblasts (Ijuhin et al. 1992), and stimulation of osteoclastic bone resorption (Ijuhin 1988) were observed in this animal model. Furthermore, we reported transient accumulation of exudative macrophages in the subjunctional epithelial area with the maximum level at day 2 and suggested that cytokines released from transiently accumulated exudative macrophages may cause various histological changes in the periodontal tissue, especially the increase of osteoclasts after 3 days (Miyauchi et al. 1998). Recently, it has been accepted that periodontal disease progresses by recurrent acute episodes (Goodson et al. 1982; Socransky et al. 1984). Although the typical B-cell lesion observed in human marginal periodontitis did not develop in our animal model, we believe that this animal model permits the elucidation of the possible pathway linking plaque-associated bacteria with the periodontal tissue destruction during an acute inflammatory episode.

The aim of this study was to demonstrate immunohistochemically the dynamic changes of in vivo expression of IL-1α, IL-1β, and TNF-α in rat periodontal tissues after topical application of LPS into the gingival sulcus. Roles of the cytokines in periodontal tissue destruction are discussed.

Materials and methods

A total of 21, 7-week-old (about 190 g), male Wistar strain rats were used in this study. They were divided into seven groups of three rats each. Under intraperitoneal anesthesia with 20% ethyl carbamate (100 mg/100 g body weight), a rat was fixed on his back on an experimental stand. A cotton roll (2 mm in diameter and 1 cm in length) saturated with 5 mg/ml LPS from *E. coli* (Sigma Chemical, St. Louis, Mo., USA) in sterile physiological saline (Otsuka Med., Tokyo, Japan) was placed on the occlusal plane of the right and left upper molars for 1 h. The cotton roll was changed every 20 min. Three rats each were killed at 1, or 3 h, or 1, 2, 3, or 7 days after the LPS treatment by an overdose of ethyl ether. The remaining three rats were used as an untreated control group. The experimental protocol was approved by the animal care committee of Hiroshima University.

Tissue samples were resected en bloc from the right and left upper molar regions, fixed for 8 h in a periodic-lysine paraformaldehyde solution (4% paraformaldehyde, 0.01 M $NaIO_4$, 0.075 M lysine in 0.05 M phosphate buffer, pH 7.4) at 4°C, and washed serially in graded phosphate buffers containing 5–20% sucrose (Watanabe et al. 1983). The specimens were cut into two slices which included the first or second molar, respectively, at the buccopalatal plane parallel to each distopalatal root. They were then decalcified in a 10% ethylenediaminetetraacetate solution in a 1 mM phosphate-buffered saline (PBS; pH 7.4) for 5 days at 4°C. The decalcified tissue blocks were embedded in OCT compound (Tissue Tec; Miles Scientific, Naperville, Ill., USA). Serial frozen sections (8 μm thick) parallel to the long axis of the tooth, including root apex, were cut and collected on glass slides.

The following immunostaining was carried out using a Dako-LSB2 kit (Dako, Carpinteria, Calif., USA). After washing in PBS each section was incubated with a normal goat serum for 30 min at 4°C and then with polyclonal antibodies to cytokines for 24 h at 4°C in a humid atmosphere. Antibodies to TNF-α, IL-1α, and IL-1β (Genzyme Diagnostics, Cambridge, Mass., USA) were diluted in 1 mM PBS containing 5% normal rat serum to 1/2000, 1/2000, and 1/50, respectively. The polyclonal antibodies used in this study and their specificity (Pelletier et al. 1993; Martin et al. 1995; Teti et al. 1995) are listed in Table 1. After rinsing with PBS, the sections were incubated with a biotinylated rabbit anti-mouse IgG serum containing 5% normal rat serum for 30 min. The sections were rinsed in PBS and immersed in 0.3% hydrogen peroxide in PBS to block the endogenous peroxidase activity for 1 h. The sections were rinsed with PBS, incubated with the peroxidase-conjugated streptavidin for 30 min, and rinsed with PBS again. The color was developed with 0.025% 3,3′-diaminobenzidine tetrahydrochloride in TRIS-HCl buffer plus hydrogen peroxide (Kyowa Medics, Tokyo, Japan). The specimens were counterstained with Mayer's hematoxylin, dehydrated, and then mounted.

Staining specificity was ascertained by: (1) omission of primary antisera and (2) substitution of primary antisera with non-immunized normal rabbit serum.

Results

Untreated control gingival and periodontal tissues

TNF-α-positive cells were detected in the coronal half of junctional epithelium (JE). The number of positive cells and intensity of the immunoreactivity increased in an apicocoronal direction and were maximal in the superficial layer, which faced the tooth surface and gingival sulcus (Fig. 1A). Weakly positive reactions for TNF-α were partially seen in oral gingival epithelium (OGE) and oral sulcular epithelium (OSE). In the gingival connective tissue, a small number of macrophage-like cells stained positively for TNF-α (Fig. 1A). Osteoblasts lining the outer surface of alveolar bone crest were weakly stained for TNF-α (Fig. 1B). Positive reactions for IL-1α and IL-1β were seen in a small number of cells of OGE, OSE, and the coronal half of the JE (Fig. 2A) and in osteoblasts (Fig. 2B). Especially, the superficial layer of the coronal half of the JE was strongly positive for IL-1α and IL-1β (Fig. 2A). In addition, IL-1α- and IL-1β-positive macrophages

Table 1 Data of primary antibodies used

Antibodies	Antigen	Species specificity	Source
Anti-human TNF-α (IP-300)	Recombinant human TNF-α	Human and rat TNF-α (Martin et al. 1995)	Genzyme Diagnostics
Anti-mouse IL-1α (IP-110)	Recombinant mouse IL-1α	Mouse and rat IL-1α (Teti et al. 1995)	Genzyme Diagnostics
Anti-human IL-1β (LP-712)	Recombinant human IL-1β	Human, mouse and canine IL-1β (Pelletier et al. 1993)	Genzyme Diagnostics

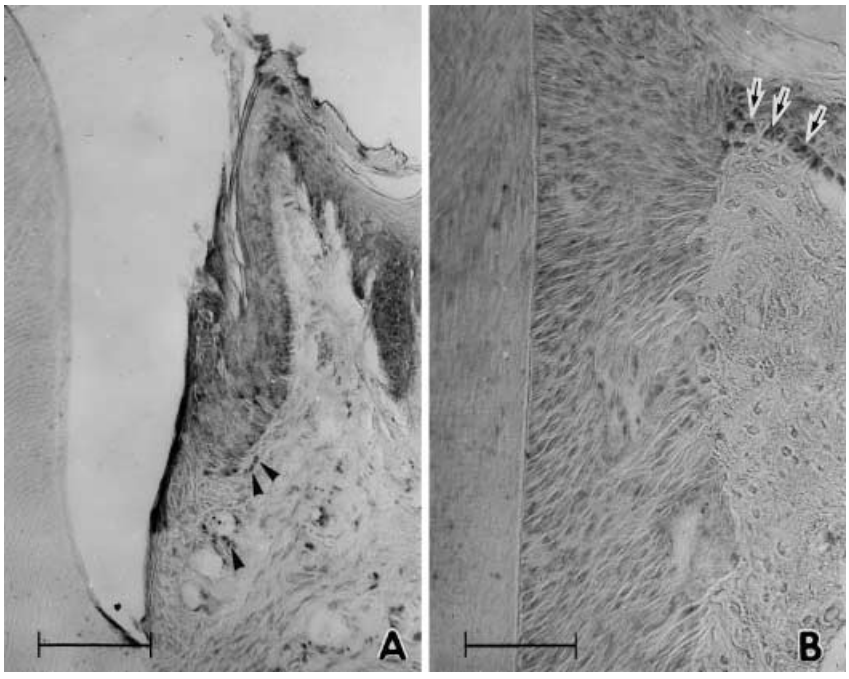

Fig. 1 Immunohistochemical staining of TNF-α in the gingival tissue (**A**) and periodontal ligament (**B**) of an untreated control rat. **A** Positive staining is seen in the junctional epithelial cells facing the tooth surface. *Arrowheads* show weakly stained macrophage-like cells in the subepithelial connective tissue. **B** *Arrows* show positively stained osteoblasts lining the outer surface of alveolar bone crest. *Scale bars* 0.1 mm (×150, SAB method)

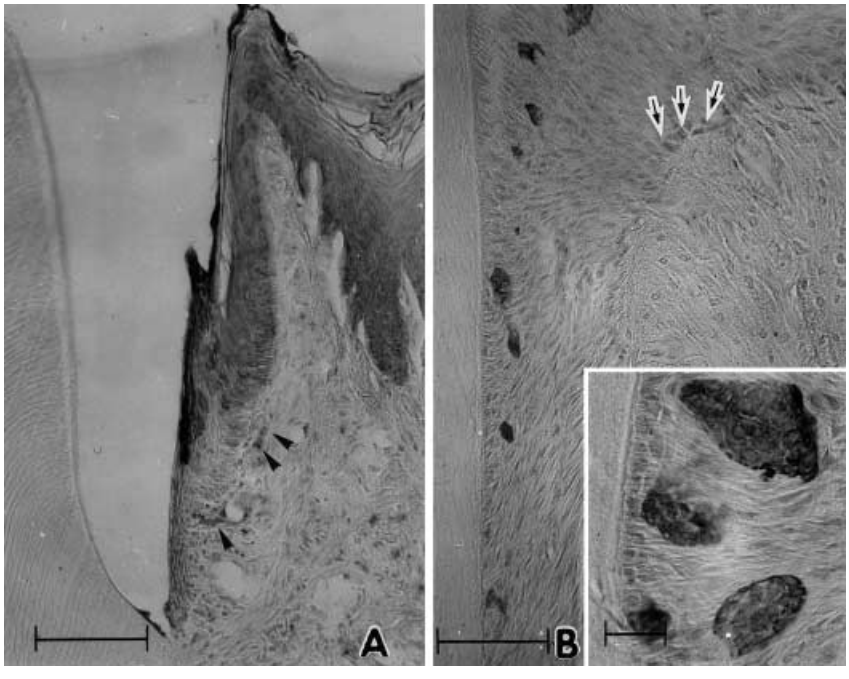

Fig. 2 Immunohistochemical staining of IL-1β in gingival tissue (**A**) and periodontal ligament (**B**) of untreated control rat. **A** Cells of junctional epithelium were strongly positive in its coronal half. IL-1β-positive macrophages (*arrowheads*) were seen in the subjunctional epithelial area. **B** Osteoblasts (*arrows*) were slightly positive for IL-1β. Epithelial remnants of Malassez show intense staining of IL-1β (*inset*). *Scale bars* **A**, **B** 0.1 mm; *inset* 0.03 mm (**A**, **B** ×150; *inset* ×300, SAB method)

(Fig. 2A) were occasionally seen in the subjunctional epithelial area. Epithelial remnants of Malassez were unreactive for TNF-α (Fig. 1A) and IL-1α, but they showed intensive staining for IL-1β (Fig. 2B).

LPS applied gingival and periodontal tissues

LPS enhanced TNF-α expression in gingival tissue at 1 h. In the gingival epithelium, many epithelial cells were strongly positive for TNF-α with a peak at 3 h (Fig. 3A). Especially in JE, the superficial layer was consistently positive for TNF-α and numerous TNF-α-positive cells were observed in deeper layers. TNF-α-positive cells were also detected in OSE and OGE located near the gingival sulcus. In this period, neutrophils infiltrated into the JE and its subepithelial area, and macrophages and most of the fibroblasts in the connective tissue subjacent to the JE were also positive for TNF-α (Fig. 3A). The expression pattern of IL-1α (Fig. 3B) and IL-1β (Fig. 3C) in the gingival tissue was similar to that of TNF-α. The staining intensity of IL-1α and IL-1β in

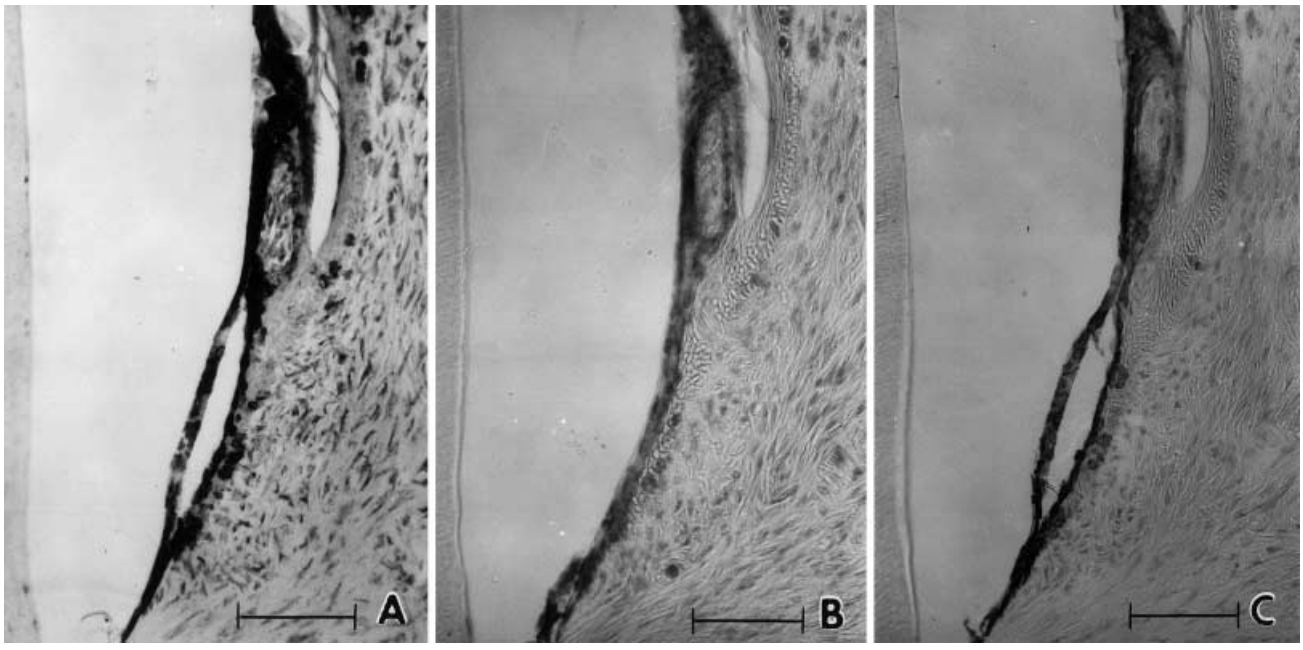

Fig. 3 Immunohistochemical staining of TNF-α (**A**), IL-1α (**B**), and IL-1β (**C**) in the gingival tissue at 3 h after topical application of lipopolysaccharide (LPS). Almost all cells in junctional epithelium are strongly positive for TNF-α, IL-1α, and IL-1β. Cytokine-positive macrophages and fibroblasts are also seen in the subjunctional epithelial area. The staining intensity for TNF-α in the subjunctional epithelial area is stronger than those of others. *Scale bars* 0.1 mm (×150, SAB method)

the gingival fibroblasts was remarkably weak compared to that of TNF-α.

After 1 day, intensive staining for TNF-α, IL-1α, and IL-1β in the JE was still observed and the area of gingival connective tissue with cytokine-positive cells spread in a more apical direction.

Two days after the LPS application, the expression of TNF-α (Fig. 4A), IL-1α, and IL-1β in the JE was gradually reduced especially in the apical part of the JE. In the subjunctional epithelial area, however, the TNF-α-expressed macrophages and gingival fibroblasts was increased. The expression of TNF-α in macrophages and gingival fibroblasts was enhanced. TNF-α-positive fibroblasts and osteoblasts were seen not only in the crestal area, but also in deeper regions of the periodontal ligament (Fig. 4B). TNF-α was also expressed in osteoclasts and preosteoclasts that increased along the alveolar bone margin in this period. Such expression of proinflammatory cytokines in the periodontal tissues was maintained until 3 days after LPS application and then declined.

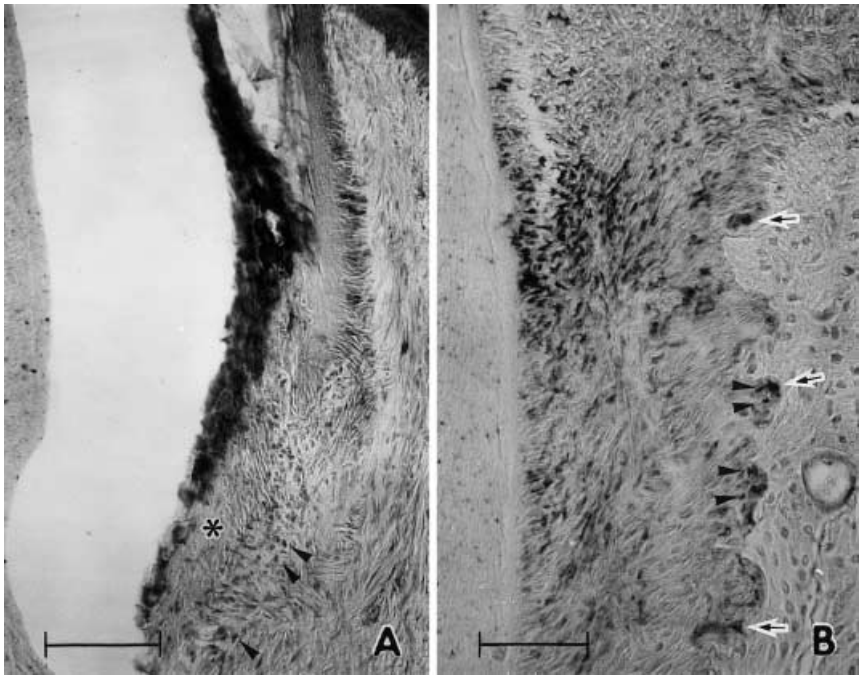

Fig. 4 Immunohistochemical staining of TNF-α in the gingival tissue (**A**) and periodontal ligament (**B**) at 2 days after topical application of LPS. **A** The expression of TNF-α in junctional epithelium, especially in its apical part (*asterisk*) was gradually reduced. In the subjunctional epithelial area, numbers of macrophages showing TNF-α positivity are seen (*arrowheads*). **B** Notice numerous TNF-α-positive fibroblasts in periodontal ligament. Positive osteoclasts (*arrows*) and preosteoclasts (*arrowheads*) show TNF-α. *Scale bars* 0.1 mm (×150, SAB method)

At 7 days, the number of the cytokine-positive infiltrating and resident cells in the periodontal tissues was decreased to a level similar to that of untreated controls.

Intense staining in epithelial remnants of Malassez was constantly observed throughout the experimental period.

No immunoreactivity was observed in the negative control specimens, which were treated with PBS or were substituted by non-immunized normal rabbit serum for each antibody.

Discussion

In the present study, we used LPS from *E. coli* to provoke initial periodontal tissue destruction. It is well known that biological activities of LPS are different among bacterial species, but it is evident that LPS from *Actinobacillus actinomycetemcomitance*, which is one of principal periodontal pathogens, is similar to *E. coli* LPS and shares numerous biological activities on the host cells with *E. coli* LPS. To avoid the periodontal tissue destruction caused by the application method itself, we used a cotton roll applicator saturated with the LPS solution.

We could successfully demonstrate the immunohistochemical localization of proinflammatory cytokines in rat periodontal tissue using commercially available antibodies. We have preliminarily examined the crossreactivity of these antibodies and detected positively stained monocytes and macrophages in the rat spleen and bone marrow tissues (data not shown).

In the normal periodontal tissues, we observed that a small number of macrophages, osteoblasts on the alveolar bone surface, and scattered gingival epithelial cells were weakly stained for TNF-α, IL-1α, and IL-1β. In addition, the coronal half of the JE, especially in superficial layers, was strongly positive for these cytokines. Using ELISA of gingival tissue extracts, Stashenko et al. (1991) reported that these cytokines were detected in clinically healthy gingival tissues, although their concentrations were relatively lower than those in inflamed gingiva. Immunohistochemically, IL-1α-, IL-1β-, and TNF-α-positive cells seemed to be macrophages present in the lamina propria of normal human gingival tissue (Jandinski et al. 1991; Stashenko et al. 1991). The coronal half of the JE, which intensively expressed cytokines, and cytokine-positive macrophages observed in a normal condition may play a role in the first defense against continuously invading irritants from gingival sulcus by their cytokine production. It has been reported that keratinocytes could constitutively produce proinflammatory cytokines, such as TNF-α, IL-1α, and IL-1β in vitro and these cytokines stimulated proliferation of keratinocytes via an autocrine pathway (Kupper et al. 1986; Dower et al. 1990; Hillmann et al. 1995). In the bone tissue, it is known that TNF-α and IL-1 are also produced by osteoblasts and stimulate proliferation of osteoblasts in vitro (Frost et al. 1997). Therefore, the small amount of these cytokines consistently produced in gingival epithelium and osteoblasts may also have an important role in the homeostasis of the periodontal tissues by regulating the proliferation of keratinocytes and osteoblasts.

Interestingly, in both of the untreated control animals and the LPS-treated experimental animals, the epithelial remnants of Malassez existing throughout the periodontal ligament showed intensive expression of IL-1. IL-1β may exert some critical effects in functions of this epithelium. Since it is known that IL-1β is involved in bone remodeling (Stashenko et al. 1987; Nguyen et al. 1991), IL-1β constitutively produced by the epithelial remnants may have a role in keeping the width of the periodontal ligament space.

After application of LPS, not only fibroblasts, neutrophils, and macrophages in the gingival connective tissue, but also almost all the JE cells expressed TNF-α, IL-1α, and IL-1β. The cytokine-positive epithelial cells were also increased in OSE and OGE near the gingival sulcus. It is reported that colonic epithelial cells continuously exposed to pathogenic bacteria produced TNF-α in response to bacterial invasion, and the TNF-α could activate the inflammatory response in the intestinal mucosa by secondarily upregulated IL-8 and MCP-1 production from the epithelial cells (Jung et al. 1995). In vitro studies demonstrated that oral keratinocytes produced IL-1β, TNF-α, IL-6 GM-CSF (Yamamoto et al. 1994), and IL-8 (Tonetti 1997). Moreover, TNF-α induced dose-dependent expression of IL-8 in cultured gingival keratinocytes (Tonetti 1997). IL-8 and MCP-1 are prototypic members of the C-X-C and C-C families of chemokines and act as potent chemoattractants and activators of neutrophils and monocytes in vivo and in vitro (Oppenheim et al. 1991; Fitzgerald and Kreutzer 1995). In this animal model, we previously reported that LPS caused infiltration of neutrophils into the JE and subjunctional epithelial areas (Ijuhin 1988) and transient accumulation of exudative macrophages in the subjunctional epithelial area with a peak at 2 days after the LPS application (Miyauchi et al. 1998). The present study shows that the JE cells may respond rapidly to LPS challenge and produce excessive amounts of TNF-α and IL-1. These cytokines may subsequently enhance chemokine production by the JE cells themselves. Thus, in addition to the infiltrating and resident cells in the gingival connective tissue, the JE cells may activate the recruitment of inflammatory cells by their cytokine production and may be responsible for initiation of periodontal inflammation.

Two days after LPS application, periodontal ligament fibroblasts were positively stained for TNF-α and IL-1. Both cytokines have numerous overlapping functions including mediation of inflammation, initiation of tissue destruction via inducing collagenase production by fibroblasts, and osteoclastic bone resorption (Page 1991; Howell 1996; Okada and Murakami 1998). Moreover, IL-1 and TNF-α are known to stimulate IL-6 production and bone resorption synergistically (Passeri et al. 1994). These proinflammatory cytokines secreted by fibroblasts in the periodontal ligament may play an important role in bone resorption and collagen degradation after LPS challenge.

In summary, constitutive cells as well as inflammatory cells in the periodontal tissues produced proinflammatory cytokines, TNF-α, IL-1α, and IL-1β at the LPS challenge. Especially, JE cells rapidly reacted to LPS stimulation and produced proinflammatory cytokines. These findings suggested that JE cells may play an important role in the first line of defense against LPS challenge and the proinflammatory cytokines transiently produced by various host cells may be involved in the initiation of inflammation and subsequent periodontal tissue destruction. However, there is a possibility that the positively stained cells include receptor-possessing cells showing internalization of a cytokine-receptor complex. Further studies using an in situ hybridization technique are required to clarify the cytokine-producing cells in periodontal tissues during LPS-induced periodontitis.

Acknowledgements This work was supported in part by a Grant-in Aid for Scientific Research (A) (number 10307054) and (C) (number 10671702) from the Ministry of Education, Science, Sports and Culture, Japan

References

Dower SK, Qwarnstrom EE, Page RC, Blanton RA, Kupper TS, Raines E, Ross R, Sims JE (1990) Biology of the interleukin-1 receptor. J Invest Dermatol 94:68–73

Fitzgerald JE, Kreutzer DL (1995) Localization of interleukin-8 in human gingival tissue. Oral Microbiol Immunol 10:297–303

Frost A, Jonsson KB, Nilsson O, Ljunggren O (1997) Inflammatory cytokines regulate proliferation of cultured human osteoblasts. Acta Orthop Scand 68:91–96

Garrison SW, Nichols FC (1989) LPS-elicited secretory responses in monocytes: altered release of PGE2 IL-1β in patients with adult periodontitis. J Periodontal Res 24:88–95

Goodson JM, Tanner ACR, Haffajee AD, Sornberger GC, Socransky SS (1982) Patterns of progression and regression of advanced destructive periodontal disease. J Clin Periodontol 9: 472–481

Hillmann G, Hillmann B, Geurtsen W (1995) Immunohistochemical determination of interleukin-β in inflamed human gingival epithelium. Archs Oral Biol 40:353–359

Howell GL (1996) Cytokine networks in destructive periodontal disease. Oral Dis 1:266–270

Ijuhin N (1988) Light and electron microscopic studies of experimentally induced pathologic changes in the rat periodontal tissue. Adv Dent Res 2:209–214

Ijuhin N, Miyauchi M, Ito H, Takata T, Ogawa I, Nikai H (1992) Enhanced collagen phagocytosis by rat molar periodontal fibroblasts after topical application of lipopolysaccharide: ultrastructural observations and morphometric analysis. J Periodontal Res 27:167–175

Jandinski JJ, Stashenko P, Feder LS, Leung CC, Peros WJ, Rynar JE, Deasy MJ (1991) Localization of interleukin 1β in human periodontal tissue. J Periodontol 62:36–43

Jung HC, Eckmann L, Yang S-K, Panja A, Fierer J, Morzycka-Wroblewska E, Kagnoff MF (1995) A distinct array of proinflammatory cytokines is expressed in colon epithelial cells in response to bacterial invasion. J Clin Invest 95:55–65

Kupper TS, Ballard DW, Chua AO, McGuire JS, Flood PM, Horowitz MC, Langdon R, Loghtfoot L, Gubler U (1986) Human keratinocytes contain mRNA indistinguishable from monocyte interleukin 1 mRNA. J Exp Med 164:2095–2100

Lindemann RA, Economou JS, Rothermel H (1988) Production of interleukin-1 and tumor necrosis factor by human peripheral monocytes activated by periodontal bacteria and extracted lipopolysaccharides. J Dent Res 67:1131–1135

Martin A, Molina A, Bricio T, Mampaso F (1995) Passive dual immunization against tumor necrosis factor-alpha (TNF-α) and IL-β maximally ameliorates acute aminonucleoside nephrosis. Clin Exp Immunol 99:283–288

McFarllane CG, Reynolds JJ, Meikle MC (1990) The release of interleukin-1β, tumor necrosis factor-α and interferon-γ by cultured peripheral blood mononuclear cells from patients with periodontitis. J Periodontal Res 25:207–214

Miyauchi M, Takata T, Ito H, Ogawa I, Kudo Y, Takekoshi T, Nikai H (1998) Distribution of macrophage lineage cells in rat gingival tissue after topical application of lipopolysaccharide: an immunohistochemical study using monoclonal antibodies: OX6, ED1 and ED2. J Periodontal Res 33:345–351

Nguyen L, Dewhis FE, Hauschka PV, Stashenko P (1991) Interleukin-1β stimulates bone resorption and inhibits bone formation in vivo. Lymphokine Cytokine Res 10:15–21

Okada H, Murakami S (1998) Cytokine expression in periodontal health and disease. Crit Rev Oral Biol Med 9:248–266

Oppenheim J, Zachariae C, Mukaida N, Matsushima K (1991) Properties of the novel pro-inflammatory supergene "interkrine" cytokine family. Annu Rev Immunol 9:617–648

Page CP (1991) The role of inflammatory mediators in the pathogenesis of periodontal disease. J Periodontal Res 26:230–242

Passeri G, Girasole G, Manolagas SC (1994) Endogenous production of tumor necrosis factor by primary culture of murine calvarial cells: influence on IL-6 production and osteoclast development. Bone Miner 24:109–126

Pelletier JP, Faure MP, DiBattista JA, Wilhelm S, Visco D, Martel-Pelletier J (1993) Coordinate synthesis of stromelysin, interleukin-1 and oncogene protein in experimental osteoarthritis. Am J Pathol 142:95

Socransky SS, Haffajee JM, Goodson JM, Lindhe J (1984) New concepts of destructive periodontal disease. J Clin Periodontol 11:21–32

Stashenko P, Dewhirst FE, Rooney ML, Desjardins LA, Heeley JD (1987) Interleukin 1β is a potent inhibitor of bone formation in vitro. J Bone Miner Res 2:559–565

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

Takata T, Miyauchi M, Ogawa I, Ito H, Kobayashi J, Nikai H (1997) Reactive change in proliferative activity of the junctional epithelium after topical application of lipopolysaccharide. J Periodontol 68:531–535

Teti G, Mancuso G, Tomasello F (1995) Cytokine appearance effects of anti-tumor necrosis factor alpha antibodies in a neonatal rat model of group B streptococcal infection. Infect Immun 61:227

Tonetti MS (1997) Molecular factors associated with compartmentalization of gingival immune responses and transepithelial neutrophil migration. J Periodontal Res 32:104–109

Watanabe S, Sato Y, Kodama T, Shimosato Y (1983) Immunohistochemical study with monoclonal antibodies on immune response in human lung cancers. Cancer Res 43:5883–5889

Yamamoto T, Osaki T, Yoneda K, Ueta E (1994) Cytokine release by keratinocytes and mononuclear infiltrates during oral lichen planus. J Oral Pathol Med 23:309–313