Journal of
PERIODONTAL RESEARCH

J Periodont Res 2013; 48: 733–739

JOURNAL OF PERIODONTAL RESEARCH
doi:10.1111/jre.12063

Lipopolysaccharide-induced indoleamine 2,3-dioxygenase expression in the periodontal ligament

J. S. Moon[1], N. R. Cheong[1], S. Y. Yang[1], I. S. Kim[2], H. J. Chung[2], Y. W. Jeong[3], J. C. Park[3], M. S. Kim[1], S. H. Kim[1], H. M. Ko[3]

[1]Department of Oral Anatomy, Dental Science Research Institute, School of Dentistry, 2nd stage Brain Korea, Medical Research Center for Biomineralization Disorders, Chonnam National University, Gwangju, South Korea, [2]Department of Periodontology, Dental Science Research Institute, School of Dentistry, Chonnam National University, Gwangju, South Korea and [3]Department of Microbiology, College of Medicine, Seonam University, Namwon, South Korea

Moon JS, Cheong NR, Yang SY, Kim IS, Chung HJ, Jeong YW, Park JC, Kim MS, Kim SH, Ko HM. Lipopolysaccharide-induced indoleamine 2,3-dioxygenase expression in the periodontal ligament. J Periodont Res 2013; 48: 733–739.

Background and Objective: Indoleamine 2,3-dioxygenase (IDO) is a tryptophan-oxidizing enzyme with immune-inhibitory effects. The aim of this study was to investigate the expression of IDO by lipopolysaccharide (LPS), a component of gram-negative bacteria, in human periodontal ligament (PDL) cells.

Material and Methods: Human PDL cells and gingival fibroblasts (GFs) were prepared from explants of human PDLs and from gingival tissues of clinically healthy donors, respectively. Real-time RT-PCR, western blotting and the IDO enzyme assay were performed to determine the expression of IDO following LPS treatment of cells. LPS was injected into mice tail veins to evaluate the effects of LPS *in vivo* in the maxillary first molar. Immunofluorescence staining and histological analysis were followed to localize IDO in mouse PDL.

Results: The level of expression of *IDO* mRNA in primary human PDL cells after LPS treatment was increased in a dose-dependent manner, reaching a peak 8 h after LPS treatment. The expression and activities of IDO protein were significantly increased in comparison with those of the control. In addition, the increased production of kynurenine in culture medium was observed 72 h after LPS treatment. In the immunofluorescence findings, stronger immunoreactivities were shown in PDL than in gingival tissues in the maxillae. In accordance with the immunofluorescence findings, LPS treatment induced a strong up-regulation of *IDO* mRNA in human PDL cells, whereas human GFs showed only a weak response to LPS.

Conclusion: These results clearly show that IDO was induced by LPS in primary human PDL cells, suggesting that PDL might be involved in the regulation of oral inflammatory disease.

Sun-Hun Kim, PhD, DDS, Department of Oral Anatomy, Dental Science Research Institute, School of Dentistry, Chonnam National University, Gwangju, South Korea, 500-757
Tel: +82625304822
Fax: +82625304829
e-mail: ksh@jnu.ac.kr
and
Hyun-Mi. Ko, PhD, Department of Microbiology, College of Medicine, Seonam University, Namwon, South Korea, 590-711
Tel: +82636200341
Fax: +82636200345
e-mail: gusalrh@hanmail.net

Key words: indoleamine 2,3-dioxygenase; inflammation; lipopolysaccharide; periodontal ligament

Accepted for publication January 19, 2013

Chronic bacterial infection of tooth-supporting structures causes destruction of periodontal connective tissue and bone and, in severe cases, tooth loss (1). The initiation and progression of oral inflammatory disease result from the host response to plaque bacteria such as *Porphyromonas gingivalis*, *Aggregatibacter actinomycetemcomitans* and *Prevotella intermedia* via sensory receptors such as toll-like receptors (TLRs), protease-activated receptors and nod-like receptors (2–5). Along with immune cells, TLRs are expressed in periodontal tissues, sense microbial invasion and trigger inflammatory innate immune responses, which are important for pathogen elimination (6–8). Among them, TLR2 and TLR4, which are expressed in gingival

fibroblasts (GFs) and periodontal ligament (PDL) and function as the innate sensors for cell-wall components of gram-negative bacteria, might be very important in the progression of periodontitis (8).

Recent studies showing that inflammation-induced enhancement of tryptophan catabolism could inhibit the inflammatory T-cell response suggest that chronic inflammation might be associated with a reduction in the bioavailability of tryptophan in the oral environment, which is consistently exposed to ~ 500 bacterial species of both commensal and pathogenic bacteria (9–13). Indoleamine 2,3-dioxygenase (IDO) is an enzyme that catabolizes tryptophan, an essential amino acid for mammals, along the kynurenine pathway, and shows immune-inhibitory effects and cytotoxic effects via an increase of tryptophan metabolites, such as 3-hydroxykynurenine, quinolinic acid and 3-hydroxyanthranilic acid (11,14). Tryptophan depletion and kynurenine production by IDO induce suppression of T-cell responses and immune tolerance by driving T-cell differentiation toward regulatory T cells (11,14,15). IDO is expressed in a variety of cell types, including monocytes, macrophages, dendritic cells, fibroblasts, epithelial cells and several cancer cell lines by several inflammatory cytokines and immunomodulating agents (16–19). Early studies show that interferon-gamma (IFN-γ) is a potent inducer of IDO expression, which is involved in the host defense against many intracellular pathogens, including *Toxoplasma*, *Mycobacterium* and herpes simplex virus (20–22). Tumor necrosis factor-alpha, interleukin (IL)-1 and lipopolysaccharide (LPS) also induce IDO expression alone or in combination with IFN-γ (23,24).

PDL, composed of fibroblast-like cells connecting the root cementum of the tooth to the surrounding alveolar bone, represents a multipotent population of distinct fibroblast lineages with a variety of functions, such as host defense, tissue remodeling and regeneration, and the ability to differentiate into other connective tissue cells (25–27). PDL cells were reported to express TLR2 and TLR4 in response to gram-negative bacteria (28). Recently, it was found that PDL cells stimulated with gram-negative periodontal bacteria and their LPS showed increased expression of TLR2, TLR4 and proinflammatory and anti-inflammatory cytokines (28). Furthermore, PDL cells stimulated with proinflammatory cytokines such as IL-1β, IL-17A and IFN-γ could facilitate leukocyte recruitment (29). High levels of IFN-γ, IL-1 and tumor necrosis factor-alpha were detected in the gingival tissues of chronic periodontitis (30–32), and *P. gingivalis* LPS and IFN-γ, alone or in combination, induced IDO expression in human GFs (33). Based on these findings, we hypothesized that fibroblasts in periodontal tissues could play the role of a negative immune modulator by inducing IDO expression. To our knowledge, the expression of IDO by LPS, a component of gram-negative bacteria, in PDL has not been explored. The aim of this study was to investigate the expression of IDO in human primary PDL cells and *in vivo* in periodontal tissue after LPS administration and to assess the immune-suppressive function of PDL.

Material and methods

Primary culture of PDL cells and GFs

All subjects were informed of the purpose and procedures of this study, which was approved by the Institutional Review Board at the Chonnam National University Medical School Institutional Review Board. For primary cell preparation, we obtained five tissue extractions from clinically healthy donors. Pieces of PDL tissue were harvested from the middle of tooth roots and digested with 3 mg/mL of type 1 collagenase (BioBasic, Toronto, ON, Canada) and 4 mg/mL of dispase (GIBCO BRL, Grand Island, NY, USA) for 1 h by shaking at 37°C in 5% CO_2 for single-cell release. Gingival tissue was collected from the same donors from whom PDL was harvested and was washed twice with Dulbecco's phosphate-buffered saline (GIBCO BRL) containing 2% antibiotic-antimycotic (GIBCO BRL) to remove blood clots and adherent erythrocytes. Then it was cut into fragments using a sterile scalpel and transferred to a 60-mm tissue-culture dish containing 2 mL of culture medium and incubated until the cells surrounding the tissue explants were confluent. Human PDL cells and GFs were then cultured in RPMI-1640 (GIBCO BRL) supplemented with 10% fetal bovine serum (GIBCO BRL), 2 mM L-glutamine (GIBCO BRL) and 1% antibiotic-antimycotic. Human primary cells at passages 3–6 were used in this study. Yamaji *et al.* (34) reported that human PDL exhibits high alkaline phosphatase activities. To examine if the human PDL cells and GFs used in this study were correctly prepared, we compared the alkaline phosphatase activities of the cells. Human PDL cells, but not GFs, exhibited high alkaline phosphatase activities.

Animals

Specific pathogen-free C57BL/6 mice were obtained from the Orient Bio. Institute (Seongnam, Korea) and housed in a controlled environment (25°C, 55% humidity). Mice were used at 8 wk of age. This study was conducted in accordance with the guidelines of the Chonnam National University Institutional Animal Care and Use Committee.

Real-time RT-PCR

PDL cells and GFs were challenged with LPS derived from *Escherichia coli* (O127:B8, L3024; Sigma Chemical Co., St Louis, MO, USA). Total RNA was extracted using a Trizol Reagent (GIBCO BRL). Reverse transcription was conducted using an RT system containing Moloney Murine Leukemia Virus reverse transcriptase (Promega, Madison, WI, USA) in accordance with the manufacturer's instructions. PCR was conducted on a Palm-Cycler thermocycler (Corbett Life Science, Sydney, NSW, Australia)

and the product was resolved on a 1.2% agarose gel. Real-time amplification of IDO was conducted on a Roter-Gene 3000 System (Corbett Research, Morklake, Australia) using the SYBR Green PCR Master Mix Reagent Kit (Qiagen, Valencia, CA, USA). The PCR conditions were as follows: incubation for 5 min at 95°C, followed by 30 cycles of 15 s of denaturation at 95°C, annealing for 15 s at 60°C and a 15 s extension at 72°C. The primers utilized were as follows: human IDO, 5′-CAA CAA GAG CAT TTT ATC ATA GC-3′ for the forward and 5′-TTG AAT GTC ATT TTA TTC CAA TGA-3′ for the reverse; human IFN-γ, 5′-CTA ATT AGG CAA GGC TAT GTG ATT-3′ for the forward and 5′-CAT CAA GTG AAA TAA ACA CAC AA-3′; for the reverse and beta-actin, 5′-GAT CTG GCA CCA CAC CTT CT-3′ for the forward and 5′-GGG GTG TTG AAG GTC TCA AA-3′ for the reverse. The relative levels of *IDO* mRNA were calculated using the standard curve generated from dilutions of complementary DNA. The mean cycle threshold (*C*t) values from quadruplicate measurements were employed in the calculation of gene expression, with normalization to beta-actin used as an internal control. Calculations of the relative level of gene expression were conducted using Corbett Robotics Rotorgene software (Rotorgene 6 version 6.1, Build 90 software ; Corbett Research, Mortlake, Australia).

Western blot analysis and determination of IDO activity

Cell extracts for analyzing the levels and the enzyme activity of IDO were prepared using CytoBuster Protein Extraction Reagent (Novagen, Madison, WI, USA). The extracts were electrophoresed on a 10% sodium dodecyl sulfate–polyacrylamide gel and transferred to a Protran nitrocellulose membrane (Whatman GmbH, Dassel, Germany). The membrane was blocked for 1 h by incubation at room temperature in 10 mM Tris-buffered saline/0.1% Tween 20 containing 5% skim milk, followed by overnight incubation, at 4°C with gentle shaking, with primary antibody raised against IDO (Santa Cruz Biotechnology, Santa Cruz, CA, USA). The purified mouse monoclonal primary antibody to beta-actin (Sigma) was used as the reference. The blots were washed and then incubated for 2 h at room temperature with the horseradish peroxidase-conjugated anti-rat IgG (Santa Cruz Biotechnology). The blots were washed again and developed with the horseradish peroxidase Substrate Luminol Reagent (Millipore Corporation, Billerica, MA, USA) and photographed using LAS4000 mini loaded with ImageReader LAS-4000 software (Fujifilm, Tokyo, Japan).

IDO activity was determined as described previously, with modifications (35,36). To determine the IDO activity in cell lysates, first of all cell lysates (100 μL) were incubated with 2× IDO assay buffer (100 mM phosphate-buffered saline, 40 mM ascorbate, 20 μM methylene blue, 200 μg/mL of catalase and 800 μM L-tryptophan) for 1 h at 37°C. The reaction was stopped by the addition of 30% trichloroacetic acid (40 μL) and was then incubated for an additional 30 min at 50°C. The reaction mixture was centrifuged at 13,000 *g* for 10 min at 4°C. The biological activity of IDO was evaluated by measuring the level of kynurenine in the reaction mixture and in PDL culture supernatants. The reaction mixture or culture supernatant was added to an equal volume of Ehrlich reagent (2% *p*-dimethylbenzaldehyde in glacial acetic acid) and the absorbance was read at 492 nm. A standard curve of defined kynurenine concentration (0–100 μM) permitted analysis of unknowns.

Histological analysis

Mice ($n = 3$ in each group) were injected intravenously with 2.5 mg/kg of LPS. After 1 d, the maxilla was isolated and fixed in a 4% paraformaldehyde solution overnight, then decalcified with 20% ethylenediamine tetra-acetic acid (pH 7.4) for 6 wk. The samples were then dehydrated in a graded series of ethanol and embedded in paraffin. Four-micrometer-thick sagittal sections were cut for hematoxylin and eosin and immunofluorescence staining.

Immunofluorescence staining was carried out using a TSA™ kit (Invitrogen, Carlsbad, CA, USA). Briefly, after blocking the endogenous peroxidase with 1% H_2O_2 for 1 h, the sections were reacted overnight with anti-IDO Ig (Santa Cruz Biotechnology) and subsequently with the horseradish peroxidase-conjugated secondary antibody for 1 h. Finally, the sections were incubated in a Tyramide working solution for 10 min. The reactants were imaged using an LSM confocal microscope (Carl Zeiss, Standort Gottingen–Vertrieb, Germany). The immunological specificity was checked by substituting the primary antibody with normal serum. At least three representative slides per mouse were analyzed and two observers analyzed the slides in a blinded manner.

Statistical analysis

Data were expressed as mean ± standard error. Statistical significance was assessed using the Student's *t*-test. Statistical differences with a p value of < 0.05 were considered significant. All experiments were conducted three to five times independently. Reproducible results were obtained, and representative data are shown in the figures.

Results

Increase of IDO expression by LPS in primary human PDL cells

Real-time RT-PCR was performed to investigate whether LPS induced the expression of *IDO* mRNA in human PDL cells. LPS induced the expression of *IDO* mRNA in a dose- and time-dependent manner. *IDO* mRNA showed a progressive increase in expression 4 h after LPS treatment, reaching maximal expression at 8 h, which decreased thereafter (Fig. 1A). *IDO* mRNA was significantly induced at 0.01 μg/mL of LPS and reached its maximal level at concentration of 0.1 μg/mL of LPS (Fig. 1B).

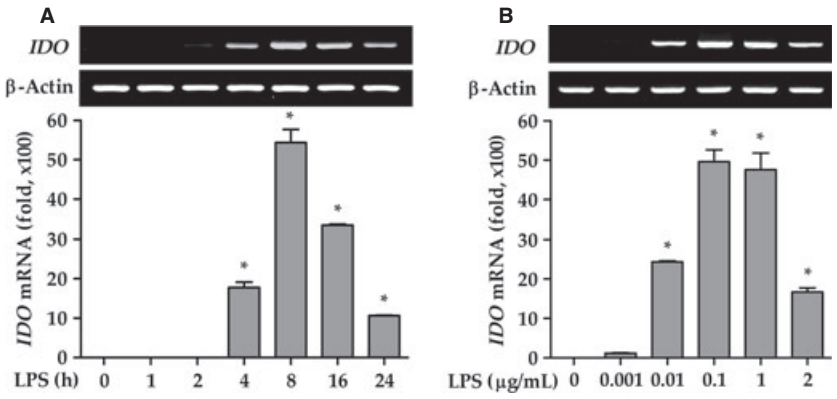

Fig. 1. Effect of lipopolysaccharide (LPS) on expression of indoleamine 2,3-dioxygenase (*IDO*) mRNA in primary human periodontal ligament (PDL) cells. Human PDL cells were treated with 0.1 μg/mL of LPS for the indicated times (A) and with various concentrations of LPS for 8 h (B). Expression of *IDO* mRNA was analyzed using real-time RT-PCR and the results are visualized on a 1.2% agarose gel. The data from a representative donor of five donors are shown and are expressed as mean ± standard error for experiments performed in triplicate. $*p < 0.01$, compared with the control group.

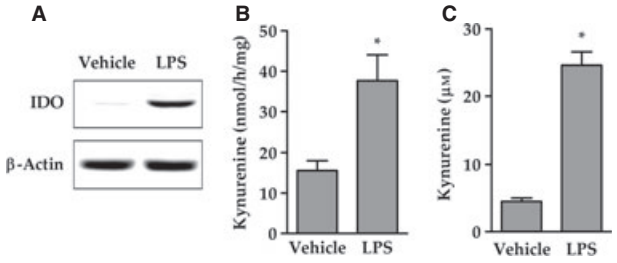

Fig. 2. Effect of lipopolysaccharide (LPS) on the expression and biological activity of indoleamine 2,3-dioxygenase (IDO) protein in human periodontal ligament (PDL) cells. Human PDL cells were treated with 0.1 μg/mL of LPS for 24 h, and the expression and biological activity of IDO protein in cell lysates were analyzed by western blotting (A) and an IDO enzyme assay (B), respectively. (C) Human PDL cells were treated with LPS for 72 h and the production of kynurenine was measured in culture supernatants. The data from a representative donor of five donors are shown and are expressed as mean ± standard error for experiments performed in triplicate. $*p < 0.01$, compared with the control group.

Therefore, this effective dose of LPS was used in our subsequent experiments. *IFN-γ* mRNA, a major inducer of IDO, could not be detected in LPS-stimulated PDL cells, even when amplified to 40 cycles (data not shown). These results suggest that LPS induced *IDO* mRNA through an IFN-γ-independent mechanism.

To evaluate whether PDL cells produce functional IDO, we determined the concentration of IDO protein and its enzymatic activity in cell lysates and culture supernatants by measuring the level of kynurenine, which is the first stable catabolite in the metabolic pathway of tryptophan. LPS induced the production of an increased level of IDO protein, as detected by western blotting (Fig. 2A). Significantly increased IDO activity was also detected in PDL cell lysates 24 h after treatment with LPS. Furthermore, a higher level of kynurenine was found in culture supernatants of the LPS-treated group than in those of the vehicle-treated group (Fig. 2B and 2C).

LPS-induced IDO expression and localization in the maxilla *in vivo* in a murine model

To identify the expression and localization of IDO in the *in-vivo* mouse model, maxillary tissue samples were obtained 24 h after LPS administration via the mouse lateral tail vein. To examine IDO expression in the maxilla, immunofluorescence staining was performed and the specimen was observed under confocal microscopy. Figure 3 shows the representative findings. A number of inflammatory cells infiltrated the PDL region and strong reactivity against IDO was observed in the PDL cells of the maxilla (Fig. 3B and 3D). By contrast, the immunoreactivity against IDO in the gingiva was relatively weak and sparse. The control sections showed no immunoreactivity (Fig. 3C). We examined the effect of LPS on IDO expression in primary human GFs and compared it with the effect in PDL cells. As shown in Fig. 4A, LPS stimulated the induction of a very low level of IDO in GFs, as seen in the *in-vivo* study. However, in human GFs, the inflammatory cytokine, IL-1β, was produced at a similar level in response to LPS as found in human PDL cells (Fig. 4B).

Discussion

IDO, which catabolizes the amino acid tryptophan, has an immunoregulatory role in infection, autoimmunity, transplantation and cancer (37). Recent findings suggest that fibroblasts, such as human gingival and dermal fibroblasts, play an important role in negative-feedback inhibition of the inflammatory T-cell response by producing IDO (38,39). It was demonstrated that PDL cells, representing an inhomogeneous and multipotent population of distinct fibroblast lineages, have a host-defense function as a result of the constitutive expression of cell-surface markers typical for antigen-presenting cells and by interacting with innate immune cells such as dendritic cells and macrophages (40,41). It has been reported that PDL cells stimulated with gram-negative periodontal bacteria and their LPS showed increased expression of proinflammatory cytokines such as IL-1β, IL-6 and tumor necrosis factor-alpha (28). Furthermore, PDL cells are involved in chronic inflammatory mechanisms by expressing a variety of cytokines that

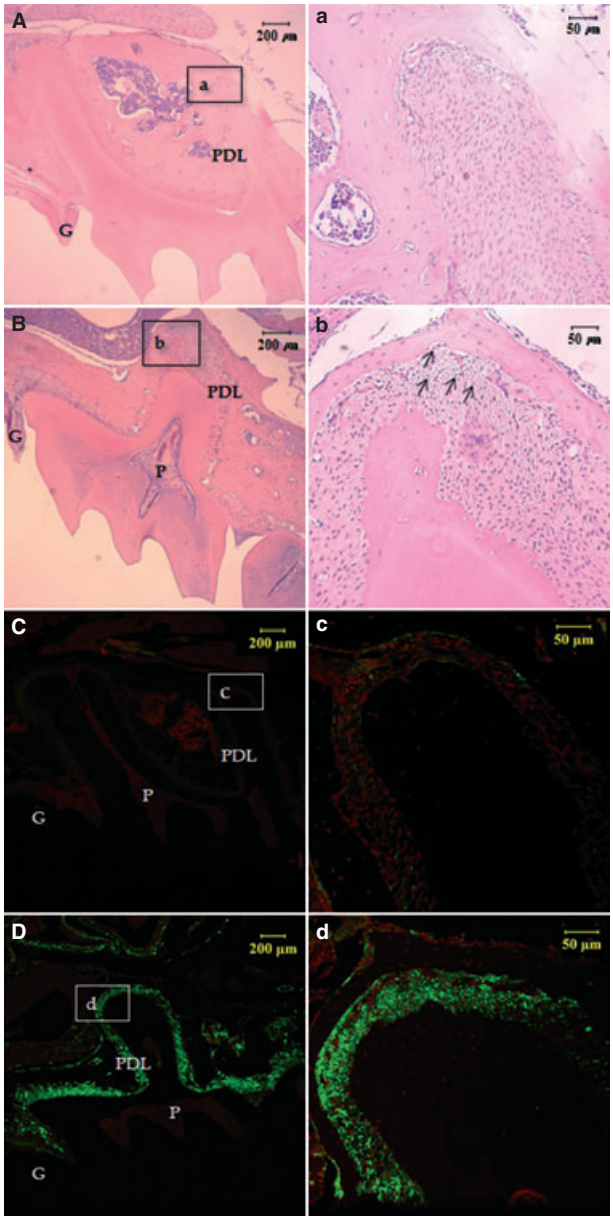

Fig. 3. Effect of lipopolysaccharide (LPS) on indoleamine 2,3-dioxygenase (IDO) expression in the mouse maxillary first-molar region. Mice were intravenously injected with 2.5 mg/kg of LPS (B, D) or vehicle (A, C). After day 1, infiltrated inflammatory cells (arrows) and morphological changes were observed by hematoxylin and eosin staining (A, B), and IDO expression was analyzed using immunofluorescence staining (C, D). The data are representative of three separate sets of experiments performed in three mice in each group of each experiment. G, gingiva; P, pulp; PDL, periodontal ligament.

regulate the T-cell-dependent immune response and by facilitating leukocyte recruitment (29).

In this study, we found that LPS induced a significant increase in expression of IDO in human primary PDL cells. A number of cells (such as epithelial cells, fibroblasts, endothelial cells and inflammatory mononuclear cells) in periodontitis gingiva, but not in healthy gingiva, express IDO (17). In human GFs, IDO expression is induced in response to ligands specific for TLRs 2, 3, 4 and 5, and is synergistically enhanced when GFs are treated with the combination of TLR ligands and IFN-γ (33). In accordance with studies of other groups, LPS induced a weak, but significant, increase of *IDO* mRNA in GFs. However, compared with GFs, a 500-fold increase in expression of *IDO* mRNA in response to LPS was observed in human PDL cells. In our *in-vivo* study, LPS, as a single agent, induced predominantly an increase of IDO in PDL, and to a lesser extent in GFs, even though CD14 and TLR4 (which are responsible for pattern recognition of LPS) were expressed strongly in GFs (7). In our unpublished data, *Staphylococcus aureus* peptidoglycan, an agonist for TLR2, was unable to induce a measurable amount of IDO expression in PDL cells even though PDL cells expressed higher levels of TLR2 than did GFs. Taken together, the response of PDL cells to LPS induced IDO expression more strongly compared with the response of GFs to TLR4.

Interestingly, our study showed that PDL cells exhibited different characteristics of IDO production compared with GFs. In accordance with our finding, many studies also described that differences between PDL cells and GFs existed with regard to protein synthesis induced by numerous microbial stimuli (42–44). Various factors could be responsible for the differences in these two cell populations. One of the factors concerns the respective functions of PDL cells and GFs. PDL cells reside between the tooth and the alveolar bone, and play an essential role not only in the maintenance of the ligament but also in processes associated with bone remodeling. GFs, on the other hand, are located supracrestally beneath the epithelium of the gums and play a role in protection against oral pathogens. Moreover, differences between these two cell populations could also be a result of the differential expression of surface receptors and of different mechanisms of intracellular signaling.

Early studies showed that IDO is involved in the IFN-γ-mediated host defense to many intracellular pathogens (20–22,45–47). Although IFN-γ has traditionally been regarded as the primary essential inducer of IDO, an IFN-γ-independent pathway of IDO expression has recently been reported (48–50). In our observations, LPS-induced *IFN-γ* mRNA was not detectable at any concentration of LPS

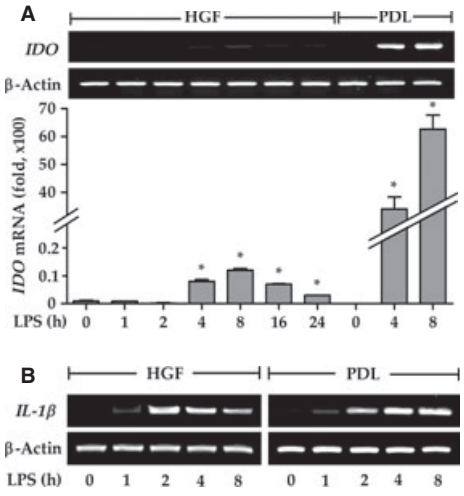

Fig. 4. Comparison of lipopolysaccharide (LPS)-induced expression of indoleamine 2,3-dioxygenase (*IDO*) mRNA in human gingival fibroblasts (HGFs) and periodontal ligament (PDL) cells. Cells were treated with 0.1 μg/mL of LPS for the indicated times. Expression of *IDO* (A) and interleukin-1beta (*IL1B*) (B) expression were analyzed using real-time RT-PCR and were visualized on a 1.2% agarose gel. The GFs were prepared from the gingiva of the same donors from whom PDL cells were obtained. The data from a representative donor among four donors are shown and are expressed as mean ± standard error for experiments performed in triplicate. $*p < 0.01$, compared with the control group.

or at any study time-point in PDL cells. This demonstrated that LPS induced IDO expression through an IFN-γ-independent manner in human PDL. Therefore, IDO expression in response to bacterial products and inflammatory mediators seems to be cell-type specific.

It has been demonstrated that T cells play an important role in the pathogenesis of periodontitis (29,51,52). Fibroblasts could prospectively offer an immunosuppressive function by increasing the expression of CD274, a down-regulator of T-cell activation and proliferation, after treatment of inflammatory cytokines (29). In addition, we here supported the immunosuppressive function of PDL cells that expressed IDO by LPS. Therefore, LPS-induced IDO expression in PDL may be one of several mechanisms involved in the down-regulation of the inflammatory process, which may be beneficial in preventing excessive inflammation and the destruction of periodontal tissue.

Conclusion

This study showed that human PDL cells expressed IDO and respond to LPS through an IFN-γ-independent process. Moreover, strong IDO expression was observed in the PDL of the maxilla after systemic administration of LPS, but not in the gingiva. These data suggest that PDL cells play a role in oral inflammatory diseases.

Acknowledgements

This research was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea Government (MEST) (2012-0009421).

References

1. Cochran DL. Inflammation and bone loss in periodontal disease. *J Periodontol* 2008;**79**:1569–1576.
2. Lopez NJ. Occurrence of *Actinobacillus actinomycetemcomitans*, *Porphyromonas gingivalis*, and *Prevotella intermedia* in progressive adult periodontitis. *J Periodontol* 2000;**71**:948–954.
3. Holzhausen M, Spolidorio LC, Vergnolle N. Role of protease-activated receptor-2 in inflammation, and its possible implications as a putative mediator of periodontitis. *Mem Inst Oswaldo Cruz* 2005;**100** (suppl 1):177–180.
4. Uehara A, Muramoto K, Takada H, Sugawara S. Neutrophil serine proteinases activate human nonepithelial cells to produce inflammatory cytokines through protease-activated receptor 2. *J Immunol* 2003;**170**:5690–5696.
5. Okugawa T, Kaneko T, Yoshimura A, Silverman N, Hara Y. NOD1 and NOD2 mediate sensing of periodontal pathogens. *J Dent Res* 2010;**89**:186–191.
6. Kusumoto Y, Hirano H, Saitoh K *et al*. Human gingival epithelial cells produce chemotactic factors interleukin-8 and monocyte chemoattractant protein-1 after stimulation with Porphyromonas gingivalis via toll-like receptor 2. *J Periodontol* 2004;**75**:370–379.
7. Hatakeyama J, Tamai R, Sugiyama A, Akashi S, Sugawara S, Takada H. Contrasting responses of human gingival and periodontal ligament fibroblasts to bacterial cell-surface components through the CD14/Toll-like receptor system. *Oral Microbiol Immunol* 2003;**18**:14–23.
8. Hans M, Hans VM. Toll-like receptors and their dual role in periodontitis: a review. *J Oral Sci* 2011;**53**:263–271.
9. Munn DH, Shafizadeh E, Attwood JT, Bondarev I, Pashine A, Mellor AL. Inhibition of T cell proliferation by macrophage tryptophan catabolism. *J Exp Med* 1999;**189**:1363–1372.
10. Frumento G, Rotondo R, Tonetti M, Damonte G, Benatti U, Ferrara GB. Tryptophan-derived catabolites are responsible for inhibition of T and natural killer cell proliferation induced by indoleamine 2,3-dioxygenase. *J Exp Med* 2002;**196**:459–468.
11. Terness P, Bauer TM, Rose L *et al*. Inhibition of allogeneic T cell proliferation by indoleamine 2,3-dioxygenase-expressing dendritic cells: mediation of suppression by tryptophan metabolites. *J Exp Med* 2002;**196**:447–457.
12. Neumeister A. Tryptophan depletion, serotonin, and depression: where do we stand? *Psychopharmacol Bull* 2003;**37**:99–115.
13. Paster BJ, Boches SK, Galvin JL *et al*. Bacterial diversity in human subgingival plaque. *J Bacteriol* 2001;**183**:3770–3783.
14. Ghahary A, Li Y, Tredget EE *et al*. Expression of indoleamine 2,3-dioxygenase in dermal fibroblasts functions as a local immunosuppressive factor. *J Invest Dermatol* 2004;**122**:953–964.
15. Fallarino F, Grohmann U, You S *et al*. The combined effects of tryptophan starvation and tryptophan catabolites down-regulate T cell receptor zeta-chain and induce a regulatory phenotype in naive T cells. *J Immunol* 2006;**176**:6752–6761.
16. Miwa N, Hayakawa S, Miyazaki S *et al*. IDO expression on decidual and peripheral blood dendritic cells and monocytes/macrophages after treatment with CTLA-4 or interferon-gamma increase in normal pregnancy but decrease in spontaneous abortion. *Mol Hum Reprod* 2005;**11**:865–870.

17. Nisapakultorn K, Makrudthong J, Sa-Ard-Iam N, Rerkyen P, Mahanonda R, Takikawa O. Indoleamine 2,3-dioxygenase expression and regulation in chronic periodontitis. *J Periodontol* 2009;**80**:114–121.
18. Sedlmayr P, Semlitsch M, Gebru G *et al*. Expression of indoleamine 2,3-dioxygenase in carcinoma of human endometrium and uterine cervix. *Adv Exp Med Biol* 2003;**527**:91–95.
19. Okamoto A, Nikaido T, Ochiai K *et al*. Indoleamine 2,3-dioxygenase serves as a marker of poor prognosis in gene expression profiles of serous ovarian cancer cells. *Clin Cancer Res* 2005;**11**:6030–6039.
20. Pfefferkorn ER, Eckel M, Rebhun S. Interferon-gamma suppresses the growth of *Toxoplasma gondii* in human fibroblasts through starvation for tryptophan. *Mol Biochem Parasitol* 1986;**20**:215–224.
21. Hayashi T, Rao SP, Takabayashi K *et al*. Enhancement of innate immunity against *Mycobacterium avium* infection by immunostimulatory DNA is mediated by indoleamine 2,3-dioxygenase. *Infect Immun* 2001;**69**:6156–6164.
22. Adams O, Besken K, Oberdorfer C, MacKenzie CR, Russing D, Daubener W. Inhibition of human herpes simplex virus type 2 by interferon gamma and tumor necrosis factor alpha is mediated by indoleamine 2,3-dioxygenase. *Microbes Infect* 2004;**6**:806–812.
23. Babcock TA, Carlin JM. Transcriptional activation of indoleamine dioxygenase by interleukin 1 and tumor necrosis factor alpha in interferon-treated epithelial cells. *Cytokine* 2000;**12**:588–594.
24. Hissong BD, Byrne GI, Padilla ML, Carlin JM. Upregulation of interferon-induced indoleamine 2,3-dioxygenase in human macrophage cultures by lipopolysaccharide, muramyl tripeptide, and interleukin-1. *Cell Immunol* 1995;**160**:264–269.
25. Kaneda T, Miyauchi M, Takekoshi T *et al*. Characteristics of periodontal ligament subpopulations obtained by sequential enzymatic digestion of rat molar periodontal ligament. *Bone* 2006;**38**:420–426.
26. Lekic P, McCulloch CA. Periodontal ligament cell population: the central role of fibroblasts in creating a unique tissue. *Anat Rec* 1996;**245**:327–341.
27. Melcher AH. On the repair potential of periodontal tissues. *J Periodontol* 1976;**47**:256–260.
28. Sun Y, Shu R, Li CL, Zhang MZ. Gram-negative periodontal bacteria induce the activation of Toll-like receptors 2 and 4, and cytokine production in human periodontal ligament cells. *J Periodontol* 2010;**81**:1488–1496.
29. Konermann A, Beyer M, Deschner J *et al*. Human periodontal ligament cells facilitate leukocyte recruitment and are influenced in their immunomodulatory function by Th17 cytokine release. *Cell Immunol* 2012;**272**:137–143.
30. Gorska R, Gregorek H, Kowalski J, Laskus-Perendyk A, Syczewska M, Madalinski K. Relationship between clinical parameters and cytokine profiles in inflamed gingival tissue and serum samples from patients with chronic periodontitis. *J Clin Periodontol* 2003;**30**:1046–1052.
31. Graves DT, Cochran D. The contribution of interleukin-1 and tumor necrosis factor to periodontal tissue destruction. *J Periodontol* 2003;**74**:391–401.
32. Ukai T, Mori Y, Onoyama M, Hara Y. Immunohistological study of interferon-gamma- and interleukin-4-bearing cells in human periodontitis gingiva. *Arch Oral Biol* 2001;**46**:901–908.
33. Mahanonda R, Sa-Ard-Iam N, Montreekachon P *et al*. IL-8 and IDO expression by human gingival fibroblasts via TLRs. *J Immunol* 2007;**178**:1151–1157.
34. Yamaji Y, Kubota T, Sasaguri K *et al*. Inflammatory cytokine gene expression in human periodontal ligament fibroblasts stimulated with bacterial lipopolysaccharides. *Infect Immun* 1995;**63**:3576–3581.
35. Braun D, Longman RS, Albert ML. A two-step induction of indoleamine 2,3 dioxygenase (IDO) activity during dendritic-cell maturation. *Blood* 2005;**106**:2375–2381.
36. Lestage J, Verrier D, Palin K, Dantzer R. The enzyme indoleamine 2,3-dioxygenase is induced in the mouse brain in response to peripheral administration of lipopolysaccharide and superantigen. *Brain Behav Immun* 2002;**16**:596–601.
37. MacKenzie CR, Heseler K, Muller A, Daubener W. Role of indoleamine 2,3-dioxygenase in antimicrobial defence and immuno-regulation: tryptophan depletion versus production of toxic kynurenines. *Curr Drug Metab* 2007;**8**:237–244.
38. Holmes EW. Expression and regulation of interferon-gamma-induced tryptophan catabolism in cultured skin fibroblasts. *J Interferon Cytokine Res* 1998;**18**:509–520.
39. Sarkhosh K, Tredget EE, Uludag H *et al*. Temperature-sensitive polymer-conjugated IFN-gamma induces the expression of IDO mRNA and activity by fibroblasts populated in collagen gel (FPCG). *J Cell Physiol* 2004;**201**:146–154.
40. Konermann A, Deschner J, Allam JP *et al*. Antigen-presenting cell marker expression and phagocytotic activity in periodontal ligament cells. *J Oral Pathol Med* 2012;**41**:340–347.
41. Konermann A, Stabenow D, Knolle PA, Held SA, Deschner J, Jager A. Regulatory role of periodontal ligament fibroblasts for innate immune cell function and differentiation. *Innate Immun* 2012;**18**:745–752.
42. Morandini AC, Sipert CR, Gasparoto TH *et al*. Differential production of macrophage inflammatory protein-1alpha, stromal-derived factor-1, and IL-6 by human cultured periodontal ligament and gingival fibroblasts challenged with lipopolysaccharide from *P. gingivalis*. *J Periodontol* 2010;**81**:310–317.
43. de Vries TJ, Schoenmaker T, Wattanaroonwong N *et al*. Gingival fibroblasts are better at inhibiting osteoclast formation than periodontal ligament fibroblasts. *J Cell Biochem* 2006;**98**:370–382.
44. Hormdee D, Nagasawa T, Kiji M *et al*. Protein kinase-A-dependent osteoprotegerin production on interleukin-1 stimulation in human gingival fibroblasts is distinct from periodontal ligament fibroblasts. *Clin Exp Immunol* 2005;**142**:490–497.
45. Daubener W, MacKenzie CR. IFN-gamma activated indoleamine 2,3-dioxygenase activity in human cells is an antiparasitic and an antibacterial effector mechanism. *Adv Exp Med Biol* 1999;**467**:517–524.
46. Bodaghi B, Goureau O, Zipeto D, Laurent L, Virelizier JL, Michelson S. Role of IFN-gamma-induced indoleamine 2,3 dioxygenase and inducible nitric oxide synthase in the replication of human cytomegalovirus in retinal pigment epithelial cells. *J Immunol* 1999;**162**:957–964.
47. Pantoja LG, Miller RD, Ramirez JA, Molestina RE, Summersgill JT. Inhibition of Chlamydia pneumoniae replication in human aortic smooth muscle cells by gamma interferon-induced indoleamine 2, 3-dioxygenase activity. *Infect Immun* 2000;**68**:6478–6481.
48. Connor TJ, Starr N, O'Sullivan JB, Harkin A. Induction of indolamine 2,3-dioxygenase and kynurenine 3-monooxygenase in rat brain following a systemic inflammatory challenge: a role for IFN-gamma? *Neurosci Lett* 2008;**441**:29–34.
49. Fujigaki S, Saito K, Sekikawa K *et al*. Lipopolysaccharide induction of indoleamine 2,3-dioxygenase is mediated dominantly by an IFN-gamma-independent mechanism. *Eur J Immunol* 2001;**31**:2313–2318.
50. Wang Y, Lawson MA, Dantzer R, Kelley KW. LPS-induced indoleamine 2,3-dioxygenase is regulated in an interferon-gamma-independent manner by a JNK signaling pathway in primary murine microglia. *Brain Behav Immun* 2010;**24**:201–209.
51. Honda T, Aoki Y, Takahashi N *et al*. Elevated expression of IL-17 and IL-12 genes in chronic inflammatory periodontal disease. *Clin Chim Acta* 2008;**395**:137–141.
52. Lester SR, Bain JL, Johnson RB, Serio FG. Gingival concentrations of interleukin-23 and -17 at healthy sites and at sites of clinical attachment loss. *J Periodontol* 2007;**78**:1545–1550.