รายงานวิจัยฉบับสมบูรณ์

โครงการวิจัยเรื่อง : การแสดงออกของอินโดเลมีน 2,3 ไดออกซีจีเนสโดยเซลล์ไฟโบรบลาสต์จากเหงือก

โดย ทพญ.ดร.กนกวรรณ นิสภกุลธร

มิถุนายน 2551

สัญญาเลขที่ MRG4980177

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สนับสนุนโดยสำนักงานกองทุนสนับสนุนการวิจัย

กิตติกรรมประกาศ

ผู้วิจัยขอขอบพระคุณสำนักงานกองทุนสนับสนุนการวิจัยที่ให้ทุนในการดำเนินการวิจัยนี้ ขอขอบพระคุณรองศาสตราจารย์ ทันตแพทย์หญิง รังสินี มหานนท์ ที่ให้คำปรึกษาแนะนำรวมถึงอนุเคราะห์เครื่องมือเครื่องใช้ในการวิจัย ขอขอบพระคุณศาสตราจารย์โอซามุ ทากิกาวาที่กรุณาให้ไอดีโอแอนติบอดี รวมทั้งให้คำแนะนำในการเขียนบทความ ขอขอบคุณคุณนพดล สะอาดเอี่ยม และคุณพิมพ์ประภา ฤกษ์เย็น ที่ช่วยในการทำวิจัยและช่วยงานด้านเอกสาร ทำให้การวิจัยนี้ดำเนินไปได้ด้วยดี

รหัสโครงการ: MRG4980177

ชื่อโครงการ: การแสดงออกของอินโดเลมีน 2,3 ไดออกซีจีเนสโดยเซลล์ไฟโบรบลาสต์จากเหงือก

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บทคัดย่อ

บทนำ: อินโดเลมีน 2,3 ไดออกซีจีเนส (ไอดีโอ) เป็นเอ็นไซม์ที่มีหน้าที่สลายกรดอะมิโนทริปโตเฟน และมีคุณสมบัติในการกดภูมิคุ้มกัน ปัจจุบันยังไม่มีการศึกษาถึงการแสดงออกของไอดีโอในเนื้อเยื่อเหงือก การศึกษานี้จึงมีจุดประสงค์เพื่อศึกษาการแสดงออกของไอดีโอในเนื้อเยื่อเหงือกปกติ และเนื้อเยื่อเหงือกที่เป็นโรคปริทันต์อักเสบ รวมทั้งศึกษาผลของสารสื่ออักเสบและสารจากแบคทีเรียต่อการแสดงออกของไอดีโอโดยเซลล์เส้นใยเหงือก

วิธีวิจัย: ตัวอย่างเนื้อเยื่อเหงือกได้จากผู้ป่วยที่มารับการรักษาโรคปริทันต์ ทำการตรวจการแสดงออกของไอดีโอโปรตีนในเนื่อเยื่อเหงือกโดยวิธีอิมมูโนฮิสโตเคมิคอล ทำการเพาะเลี้ยงเซลล์เส้นใยเหงือกและกระตุ้นด้วยอินเตอร์เฟอรอนแกมมา อินเตอร์ลิวคินหนึ่งเบต้า ทูเมอร์เนโครซิสแฟคเตอร์อัลฟา และไลโปโพลีแซคคาไรด์จากแบคทีเรียพอไฟโรโมแนสจินจิวาลิส จากนั้นจึงวัดระดับการแสดงออกของอาร์เอ็นเอนำรหัส โดยอาร์ทีพีซีอาร์ และระดับการทำงานของเอ็นไซม์ไอดีโอ โดยวิธีคัลเลอริเมตริก

ผลการทดลอง: พบว่าเซลล์เยื่อบุผิวเหงือก เซลล์เส้นใยเหงือก เซลล์เยื่อบุหลอดเลือด และเซลล์อักเสบมีการแสดงออกของไอดีโอ เนื้อเยื่อเหงือกที่เป็นโรคปริทันต์อักเสบมีการแสดงออกของไอดีโอสูงกว่าเนื้อเยื่อเหงือกปกติ เซลล์เส้นใยเหงือกในภาวะปกติไม่สร้างไอดีโอ อินเตอร์เฟอรอนแกมมากระตุ้นเซลล์เส้นใยเหงือกให้สร้างไอดีโอได้ดี และปริมาณไอดีโอที่ผลิตสัมพันธ์ในเชิงบวกกับปริมาณอินเตอร์เฟอรอนแกมมาที่ใช้กระตุ้น อินเตอร์ลิวคินหนึ่งเบต้า ทูเมอร์เนโครซิสแฟคเตอร์อัลฟา และไลโปโพลีแซคคาไรด์จากแบคทีเรียพอไฟโรโมแนสจินจิวาลิสก็สามารถกระตุ้นให้เซลล์เส้นใยเหงือกสร้างไอดีโอได้ การกระตุ้นด้วยอินเตอร์เฟอรอนแกมมาร่วมกับอินเตอร์ลิวคินหนึ่งเบต้า ทูเมอร์เนโครซิสแฟคเตอร์อัลฟา หรือไลโปโพลีแซคคาไรด์ ทำให้เซลล์เส้นใยเหงือกสร้างไอดีโอได้มากขึ้น

สรุป: การศึกษานี้พบการแสดงออกของไอดีโอในเนื้อเยื่อเหงือก เนื้อเยื่อเหงือกที่เป็นโรคปริทันต์อักเสบมีการแสดงออกของไอดีโอสูงกว่าเนื้อเยื่อเหงือกปกติ การที่มีไอดีโอเพิ่มขึ้นในโรคปริทันต์อักเสบส่วนหนึ่งเกิดจากการที่เซลล์เส้นใยเหงือกถูกสารสื่ออักเสบและสารจากแบคทีเรียกระตุ้นให้สร้างไอดีโอเพิ่มขึ้น

คำหลัก:

Periodontitis; Indolamine-2,3-dioxygenase; Fibroblasts; Inflammation

Project Code: MRG4980177

Project Title: Expression of indoleamine 2,3 -dioxygenase by human gingival fibroblasts.

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Project Period: 1 July 2006 - 30 June 2008

Abstract:

Background: Indoleamine 2,3-dioxygenase (IDO) is an intracellular tryptophan-oxidizing enzyme with immunosuppressive characteristics. Its expression and regulation in periodontal tissues is unknown. The aim of this study was to determine IDO expression in healthy gingiva and chronic periodontitis lesions. In addition, the effect of inflammatory cytokines and bacterial products on IDO expression and activity of human gingival fibroblasts (HGF) was assessed.

Methods: Human gingival tissue samples were obtained from patients underwent periodontal surgery. IDO expression in healthy gingiva and periodontitis lesions was determined by immunohistochemical method. HGF cells were treated with interferon-γ, interleukin-1β, tumor necrosis factor-α, and lipopolysaccharides from *P. gingivalis* (PgLPS). IDO mRNA expression was determined by RT-PCR. The IDO enzymatic activity was determined by measuring the kyneurenine level using colorimetric method.

Results: In gingival tissues, IDO expression was detected in epithelial cells, fibroblasts, endothelial cells, and inflammatory mononuclear cells. IDO expression was higher in periodontitis lesions than that of healthy gingiva. HGF did not constitutively express IDO. IFN-γ strongly induced IDO expression and activity in HGF, in a dose-dependent manner. IL-1β, TNF-α, as well as PgLPS were also able to induce IDO expression in HGF cells. IFN-γ in combination with IL-1β, TNF-α, or PgLPS showed enhanced IDO expression.

Conclusions: IDO was expressed in human gingiva and the expression was upregulated in chronic periodontitis. The increased IDO expression in periodontitis lesions was partly due to the activation of HGF by inflammatory cytokines and bacterial products.

Keywords:

Periodontitis; Indolamine-2,3-dioxygenase; Fibroblasts; Inflammation

INTRODUCTION

Indoleamine 2,3–dioxygenase is an enzyme that metabolized the amino acid tryptophan, the least abundant essential amino acid for mammals. The majority of tryptophan is metabolized along the kynurenine pathway, leading to the synthesis of nicotinamide adenine dinucleotide or the complete oxidation of the amino acid.[1] IDO was expressed in many cell types including monocytes, macrophages, dendritic cells, fibroblasts, epithelial cells, astrocytes, and several cancer cell lines.[1] Early studies showed that IDO was involved in the interferon-γ (IFN-γ)-mediated host defense to many intracellular pathogens, including Toxoplasma,[2] Chlamydia,[3] Mycobacterium,[4] Staphylococcus aureus,[5] cytomegalovirus,[6] and herpes simplex virus.[7] This antimicrobial effect was mainly mediated through IFN-γ induced IDO expression and activity.

It was shown that IDO also plays a role in immunoregulation and tolerance induction. Macrophages and dendritic cells expressing IDO can suppress T-cell responses and promote tolerance.[8] IDO–dependent T cell suppression appears to be mediated by depletion of tryptophan in the microenvironment. Excess tryptophan was able to reverse the inhibition of T cells.[9, 10] Toxic metabolites of tryptophan such as quinolinic acid and 3-hydroxy-anthranilic acid may also mediate the immunosuppressive effects of IDO.[11-13]

IDO expression is induced by several inflammatory cytokines and immunomodulating agents. IFN-γ is a potent inducer of IDO expression. IFN-α and IFN-β are also able to induce IDO expression, but at a lesser extent.[14] TNF-α, IL- 1, and lipopolysaccharide (LPS) also induce IDO expression either alone or in combination with IFN-γ.[15, 16] Since IDO expression may lead to suppression of T cell proliferation and function, induction of IDO expression by inflammatory cytokines and immunomodulating agents may limit excessive T-cell activation at local sites of inflammation, thus, serve an anti-inflammatory role.

Various cytokines that regulate IDO expression and activity were detected in periodontal tissues. High levels of IFN-γ, a strong IDO inducer, were detectable in inflammed gingival tissues.[17, 18] IL-1 and TNF-α levels have been shown to be elevated in the gingiva of chronic periodontitis and from active periodontitis sites.[19] The expression and function of IDO in periodontal tissues has not been explored. The aim of this study was to determine IDO expression in healthy and periodontitis gingiva. In addition, we studied IDO expression

and regulation of human gingival fibroblasts, one of the most abundant cell types within gingival tissues.

MATERIALS AND METHODS

Gingival tissue sample collection

The study protocol was approved by the ethics committee of Faculty of Medicine, Chulalongkorn University. Tissue samples were obtained from marginal gingiva excised during tooth extraction or periodontal surgery. Informed consent was obtained prior to tissue collection. Healthy gingival samples were collected from sites with clinically healthy gingiva, no radiographic bone loss, and probing depth < 4 mm. Periodontitis tissue samples were collected from sites with gingival inflammation, radiographic bone loss, and probing depth over 5 mm. For immunohistochemistry, tissue samples were placed in OCT compound, snap frozen in liquid nitrogen, and stored at -80°C. For cell culture, tissue samples were washed briefly in normal saline solution and placed immediately into tissue culture media. Dulbecco's modified eagle medium (DMEM) supplemented with 10% fetal calf serum (FCS), gentamycin (50 μg/ml), penicillin G (50U/ml), streptomycin (50 μg/ml),and fungizone (2.5 μg/ml)* were used for tissue collection and cell culture. The samples were kept on ice and processed within a few hours.

Immunohistochemistry

We obtained six samples from healthy gingiva and six samples from periodontitis tissues. Samples were cut at 5 μm in thickness. Cryosection were fixed with ice-cold acetone for 10 min, air-dried, and washed with phosphate buffered saline (PBS, pH 7.4). Endogenous peroxidase activity was blocked with 3% hydrogen peroxide for 10 min. Immunoperoxidase staining was performed using the VECTASTAIN ABC kit†. Non-specific binding was reduced by applying blocking serum for 20 min. Sections were then incubated overnight at 4°C with either purified IDO-specific mouse monoclonal antibody (IgG_1) (1mg/ml diluted at 1:100 in blocking serum, kind gift from Dr. Osamu Takikawa, Japan) or mouse universal negative control antibody‡. Antibody binding was detected using avidin-biotin complex detection technique, following the manufacturer's protocol. Immunostaining was visualized using 3,3-diaminobenzidine (DAB) chromogen. The slides were counterstained with hematoxylin.

Primary culture of human gingival fibroblasts

The method to obtain human gingival fibroblast (HGF) cells from the gingival tissue was that described by Murakami.[20] Briefly, the biopsy was washed twice with DMEM to remove blood clots and adherent erythrocytes. Then, it was cut into fragments of 1-3 mm^3 with a sterile scapel. These tissues were transferred to a 35-mm tissue culture dish containing 2 ml of culture media and incubated at 37 $^{\circ}C$ in a humidified 5% CO_2-air atmosphere. Culture medium was changed twice weekly. When the fibroblast cells surrounding the tissue explants were confluent, they were subcultured into a larger tissue culture dish. HGF at the passage 3 to 6 were used in this study.

Detection of IDO mRNA expression by RT-PCR

HGF ($2.5x10^5$ cells/ well) were seeded into a 6-well tissue culture plate overnight. The cells were stimulated with the following agents: IFN-γ[§](10-1000 U/ml), TNF-α[§] (0.5-50 ng/ml), IL-1β[§] (0.5-50 ng/ml), LPS from P. gingivalis strain 381 (0.1-10 µg/ ml) (PgLPS, kind gift from Dr. Robert E. Schifferle[21]). In addition, the combination of IFN-γ and each reagent (TNF-α, IL-1β, or PgLPS) were used. Un-stimulated HGF were served as a control. After 24 h, HGF were harvested to determine IDO expression. Total RNA was extracted with TRIzol Reagent[‖], according to the standard protocol. One µg of total RNA was used for reverse transcription with random hexamer and Improm-II reverse transcriptase[¶], following the manufacturer's instruction. The cDNA was used for detection of IDO mRNA by polymerase chain reaction (PCR). PCR was carried out with PCR mixture containing 15mM of $MgCl_2$, 20µM of IDO primer, 10 mM of dNTP, 5 unit/µl of Taq polymerase and the DNA template. The PCR condition consists of a first heating step (95°C for 5 min), 30 amplification cycles (95°C for 15 s, 60°C for 30s, 70 °C for 30 s) and one final extension step (72°C for 7 min). Specific primer sequences for the gene were as follow: human GAPDH: forward 5'-TCATCTCTGCCCCCTCTGCTG-3' and reverse 5'-GCCTGCTTCACCACCTTCTTG-3' (approximate size of 400 bp); human IDO: forward 5'-CTTCCTGGTCTCTCTATTGG-3' and reverse 5'-GAAGTTCCTGTGAGCTGGT-3'(approximate size of 430 bp).[22] PCR products were separated by electrophoresis in a 1.2% agarose gel containing ethidium bromide. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as an internal control. The target bands were visualized with an UV illuminator and analyzed with image analysis software.[#]

Detection of IDO enzymatic activity

HGF (3×10^4 cells/ well) were seeded into a 96-well microtiter plate overnight. The cells were stimulated with the following agents: IFN-γ, TNF-α, IL-1β, PgLPS or their combinations. Un-stimulated HGF were served as a control. After 24 and 48 h, culture supernatants were harvested and assayed for the presence of kynurenine, the first stable catabolite of tryptophan in the kynurenine pathway. Kynurenine was detected by a modified spectophotometric assay.[23] Briefly, 50 µL of 30% trichloroacetic acid was added to 100 µL culture supernatant, vortexed, and centrifuged at 8000 g for 5 minutes. Volume (75 µL) of the supernatant was then added to an equal volume of Ehrlich reagent (100 mg *p*-dimethylbenzaldehyde, 5 mL glacial acetic acid) in a 96 well microtiter plate. Optical density was measured at 492 nm. A standard curve of defined kynurenine concentration (0-100 µM) permitted analysis of unknown.

Statistical Analysis

The one sample t-test was used to compare the level of IDO activity between samples. Results were expressed as mean $\pm$ standard error of the mean (SE). Data were analyzed using SPSS version 12.0 software. Statistical differences with a *p*-value < 0.05 were considered significant.

[*] Gibco, Grand Island, NY.

[†] Vector Laboratories, Burlingame, CA.

[‡] DakoCytomation, Carpinteria, CA.

[§] R&D systems, Minneapolis, MN.

[‖] Invitrogen, Carlsbad, CA.

[¶] Improm-II ™, Promega, Madison, WI.

[#] Gene Genius Bio Imaging system, Cambridge, UK.

RESULTS

IDO expression in gingival tissues

We observed IDO expression in both healthy gingiva and periodontitis gingiva. Figure 1 showed representative findings. Within epithelium, the number of epithelial cells expressing IDO was fewer in healthy tissues (Figure 1A) than in periodontitis tissues (Figure 1B) and the level of expression appeared to be less intense. Gingival epithelial cells showed distinct nuclear staining of IDO (Figure 1C). Within connective tissues, IDO expression was observed in fibroblast cells, endothelial cells, and inflammatory mononuclear cells (Figure 1D). The number of cells staining positive for IDO was also fewer in healthy tissues (Figure 1E) than in periodontitis tissues (Figure 1F). The control sections showed no immunoreactivity (Figures 1G and 1H).

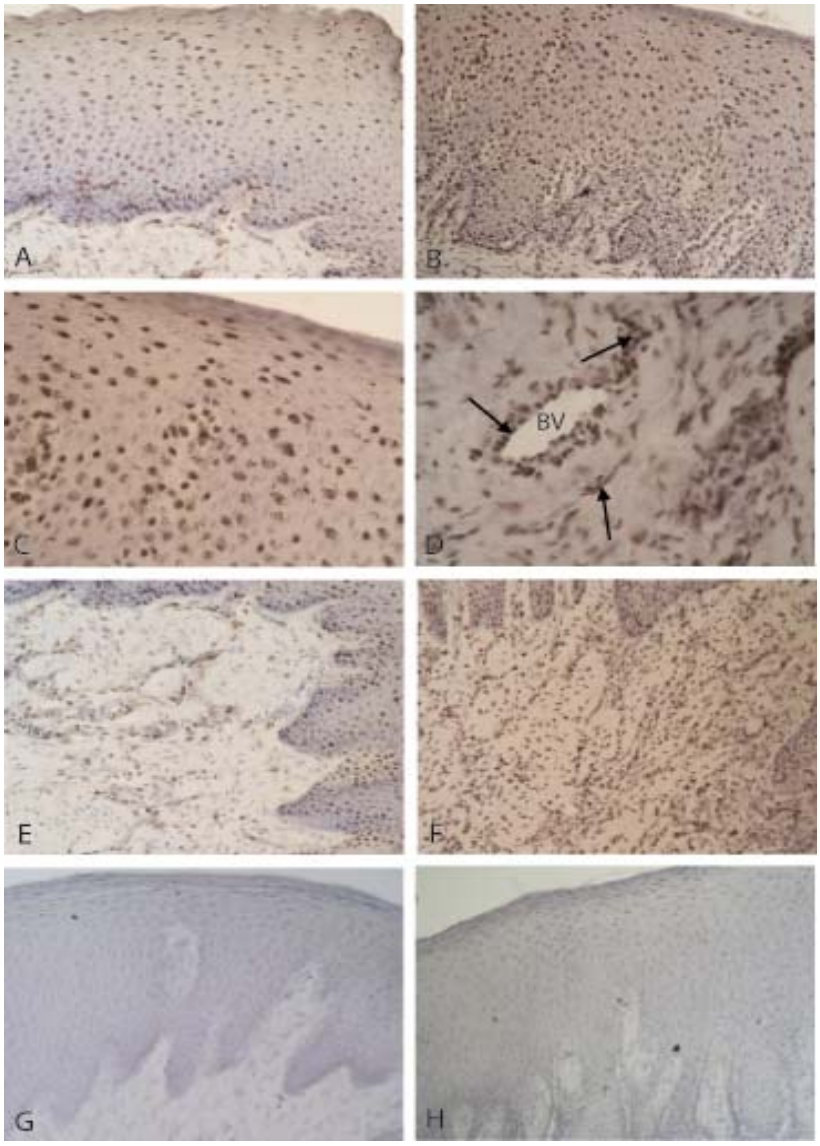

Figure 1.

Immunolocalization of IDO expression in human gingiva (A) The epithelium of healthy gingiva; magnification x200 (B) The epithelium of periodontitis gingiva; magnification x200 (C) The nuclear localization of IDO in gingival epithelial cells; magnification x400 (D) IDO expression by gingival fibroblasts, endothelial cells, and inflammatory mononuclear cells (arrows) (BV=blood vessel); magnification x400 (E) The connective tissue of healthy gingiva; magnification x200 (F) The connective tissue of periodontitis gingiva; magnification x200 (G) Negative control section of healthy gingiva stained with non-specific IgG; magnification x200 (H) Negative control section of periodontitis gingiva stained with non-specific IgG; magnification x200.

IDO mRNA expression by human gingival fibroblasts

To investigate whether inflammatory cytokines and bacterial products induce IDO mRNA expression in HGF cells, RT-PCR analysis was performed. Figure 2 depicted a representative RT-PCR result. HGF cells did not normally express detectable levels of IDO mRNA. However, IDO expression was induced upon treatment with IFN-γ, IL-1β, TNF-α, and PgLPS. IFN-γ was the strongest inducer for IDO expression. Relative IDO mRNA expression, as determined by the ratios of IDO mRNA and GAPDH mRNA, was shown in Figure 3. IFN-γ, TNF-α, and PgLPS appeared to induce IDO mRNA expression in a dose-dependent manner. Stimulation with IL-1β, however, did not show the dose-dependent effect. We also evaluated whether combinations of these cytokines showed additive effect on IDO induction. HGF cells treated with combinations of IFN-γ and IL-1β, TNF-α, or PgLPS showed increased IDO expression compared to that of IFN-γ alone (Figure 4).

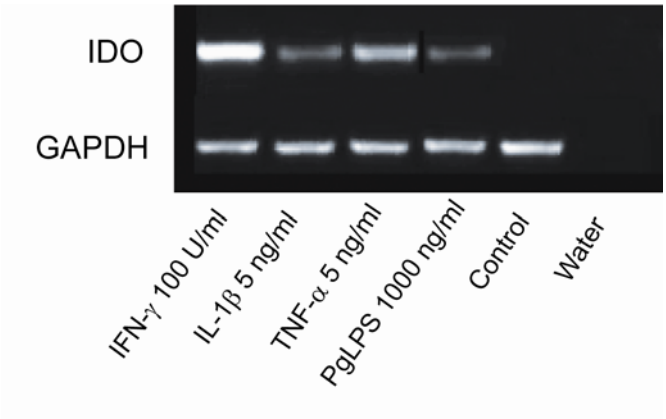

Figure 2.

IDO mRNA expression of HGF cells upon stimualtion with IFN-γ, IL-1β, TNF-α, and PgLPS. Unstimulated HGF was served as a control.

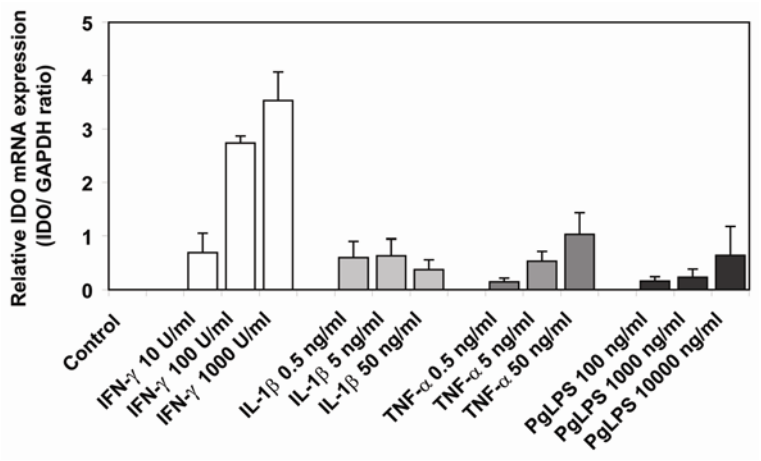

Figure 3.

Relative IDO mRNA expression in HGF cells treated with different concentrations of IFN-γ, IL-1β, TNF-α, or PgLPS. Data were shown as mean ± SE from 4 separate experiments.

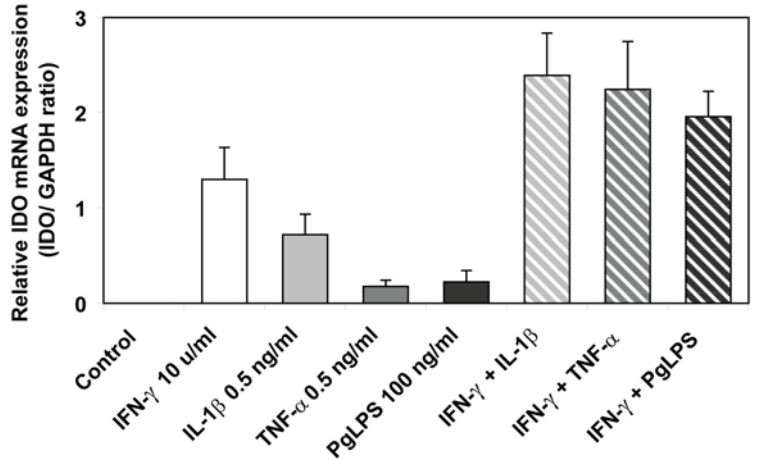

Figure 4.

Relative IDO mRNA expression in HGF cells treated with combinations of IFN-γ and IL-1β, TNF-α, or PgLPS. Data were shown as mean ± SE from 4 separate experiments.

Expression of IDO activity by human gingival fibroblasts

To evaluate whether HGF cells produced functional IDO, we detected IDO enzymatic activity by measuring kynurenine level which is the first stable catabolite in the metabolic pathway of tryptophan. IDO activity appeared to be increased over time. Significant increased IDO activity was detected in HGF cells treated with IFN-γ. Increased IDO activity was also observed in HGF cells treated with IL-1β, TNF-α, although they did not reach

statistical significance (Figure 5). The combination between IFN-γ and IL-1β as well as IFN-γ and TNF-α showed significantly higher IDO activity than that of IFN-γ alone (Figure 6).

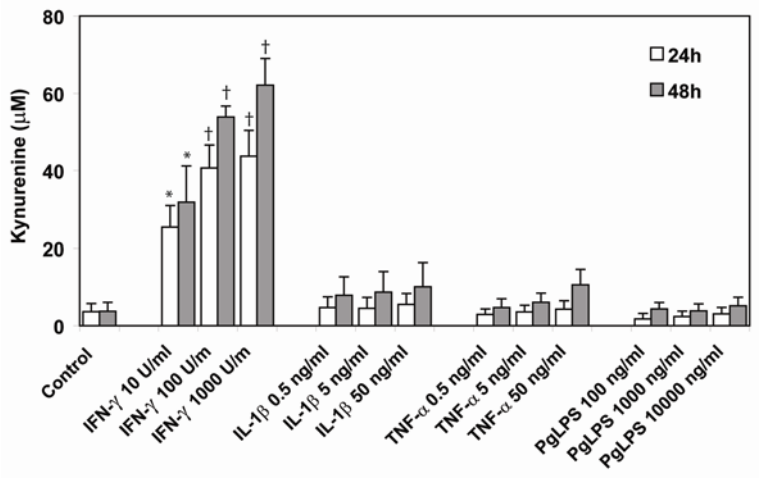

Figure 5.

IDO activity in HGF cells treated with different concentration of IFN-γ, IL-1β, TNF-α, or PgLPS. Data were shown as mean ± SE from 4 separate experiments. $^{*}p<0.05$ and $^{\dagger}p<0.01$ significant different from the untreated control.

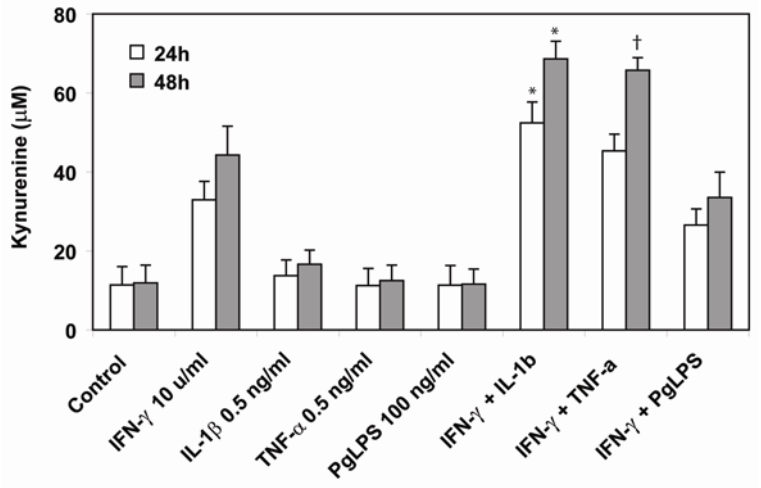

Figure 6.

IDO activity in HGF cells treated with combinations of IFN-γ and IL-1β, TNF-α, or PgLPS. Data were shown as mean ± SE from 4 separate experiments. $^{*}p<0.05$ and $^{\dagger}p<0.01$ significant different from the IFN-γ alone.

DISCUSSION

IDO is an enzyme that metabolized the amino acid tryptophan. This enzyme has a complex role in immunoregulation in infection, pregnancy, autoimmunity, transplantation, and cancer.[24] In this study we showed that IDO was expressed in human gingiva and may play a role in the pathogenesis of periodontal disease. IDO expression was detected in many cell types within gingival tissues including epithelial cells, fibroblasts, endothelial cells, and inflammatory mononuclear cells. Within epithelium, the number of epithelial cells expressing IDO was fewer in healthy tissues than in periodontitis tissues and the level of expression appeared to be less intense. Cultured foreskin keratinocytes did not express IDO mRNA unless they are induced by IFN-γ.[25] Tissue samples from inflammatory skin diseases including psoriasis and atopic dermatitis showed increased IDO mRNA expression as compared to the uninvolved skin.[26] Therefore, the IDO expression in epithelial cells appeared to be upregulated in the presence of inflammation. This was consistent with our findings that IDO expression in epithelium of periodontitis tissues was higher in than that of healthy gingiva. Within epithelial cells, we observed a strong nuclear staining pattern. IDO was reported to be an intracellular enzyme. Cytoplasmic staining of IDO was observed in peripheral blood mononuclear cells,[27] stromal cells, luminal and glandular epithelial cells of human placenta,[28] and ovarian cancer cells.[29] However, there was a report of IDO immunostaining localized primarily to the nuclei of placental endothelial cells.[30] How IDO translocate to the nucleus and whether IDO also has a nuclear function was unknown.

In gingival connective tissue, the level and extent of IDO expression was also higher in periodontitis tissues than that of healthy tissues. Periodontitis lesions had high levels of several inflammatory cytokines and bacterial products that were inducers of IDO.[17-19] Therefore, upregulation of IDO expression in periodontitis lesions may be due to the presence of these agents. In this study, we assessed IDO expression of cultured human gingival fibroblasts (HGF) upon stimulation with IFN-γ, IL-1β, TNF-α, and PgLPS. Gingival fibroblasts are the major cell type within periodontal tissues and may participate directly with several inflammatory mediators in periodontitis. We found that HGF did not constitutively express IDO. However, IDO expression was inducible in these cells. IFN-γ is a potent inducer for IDO expression in HGF. Lower levels of IDO expression was detected upon stimulation with IL-1β, TNF-α, and PgLPS. IFN-γ has been shown to be a strong inducer of IDO expression in many cell types, including dendritic cells, macrophages, epithelial cells,

skin fibroblasts, and many cancer cell lines.[14, 31, 32] It was shown that the IDO promoter contains the interferon-stimulated response element (ISRE) and gamma activated sequence (GAS). These sequences were the binding site for the transcription factor IFN-regulatory factor-1 (IRF-1) and signal transducer and activator of transcription1 (STAT1), which allows activation of IDO gene in response to IFN-γ.[33]

We showed that IL-1β, TNF-α, and PgLPS, when using as single agent, induced low levels of IDO expression in HGF. TNF-α alone was ineffective in IDO induction of peripheral blood mononuclear cells, macrophages, epithelial cell line, and an astrocytoma cell line[5, 15, 34] but showed weak IDO induction in a fibroblast cell line.[35] IL-1 alone was also unable to induce IDO expression in macrophages[36] and epithelial cells.[15] Bacterial lipopolysaccharides induced IDO expression in dendritic cells[37] and monocyte-derived macrophages,[16] but not in epithelial cells from cervix[15] and lung.[34] Therefore, IDO expression in response to inflammatory cytokines and mediators appeared to be cell type-specific.

IFN-γ in combination with IL-1β, TNF-α, or PgLPS augmented the level of IDO expression in HGF as compared to that of IFN-γ alone. Combinations of IFN-γ and IL-1β as well as IFN-γ and TNF-α also increased IDO expression in human monocyte derived macrophages and human cervical epithelial cells.[15, 36] The synergistic effect of TNF-α and IFN-γ on IDO induction has been shown to be mediated at the level of transcription through an increase in IFN-γ receptor expression which enhanced the binding of STAT1 to GAS and IRF-1 to ISRE sites.[33] The combination effect of inflammatory cytokines and bacterial products on IDO expression may be important for regulating IDO function in vivo.

The control of IDO activity appears to be complex and cell-type specific. Presence of the IDO mRNA and protein may not be correlated with its functional activity. Human dendritic cells constitutively express IDO protein, but it does not have functional enzymatic activity until these cells are activated by IFN-γ and CD80/CD86 ligation.[38] In this study, we showed that increased IDO mRNA expression in human gingival fibroblast upon treatment with IFN-γ was positively correlated with increased IDO activity. In addition, the IDO activity was increased over time. However, IDO activity in human gingival fibroblasts treated with IL-1β, TNF-α, or PgLPS was not significantly different from that of untreated controls. This may be due to the low level of IDO mRNA expression in these cells and the wide variation in the

level of response between primary cell lines. We also showed that IL-1β and TNF-α augmented the IDO activity in IFN-γ treated-HGF cells. IL-1β, TNF-α, and LPS has been shown to enhance the IDO activity induced by IFN-γ in human monocyte-derived macrophages.[16, 36] In contrast, the presence of IL-1β and TNF-α decreased the IDO activity induced by IFN-γ in uroepithelial cell line and showed no effect on the IDO activity induced by IFN-γ in astrocytoma cell line.[5] It appeared that HGF were able to produce functional IDO in response to several inflammatory cytokines and immunomodulating agents. These agents may work together or regulate each other to control IDO expression and activity in periodontal tissues.

At present, the role of IDO in periodontal disease pathogenesis is not known. A previous study from our laboratory showed that coculturing of peripheral blood mononuclear cells with HGF cells treated with IFN-γ and PgLPS resulted in suppression of T cell proliferation.[39] This effect could be reversed by the addition of 1-metyl-tryptophan, an inhibitor of IDO. Therefore, IDO expression may be one of several mechanisms to down-regulate the inflammatory process in periodontitis. This effect may be beneficial to host and prevent excessive inflammation and destruction of periodontal tissues. Further studies are needed to explore this hypothesis. In conclusion, we showed that IDO was expressed in human gingiva and the expression was upregulated in chronic periodontitis. The increased IDO expression in periodontitis lesions was partly due to the activation of HGF by inflammatory cytokines and bacterial products.

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Output

1. ผลงานเพื่อตีพิมพ์

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