行政院國家科學委員會專題研究計畫成果報告

第二型介白質-1 受體調控介白質-1 刺激人體牙齦纖維母細胞分泌分解代謝性介質的作用及其作用機轉之體外研究

Regulation of catabolic mediators synthesis in IL-1β-stimulated human gingival fibroblasts by type II interleukin-1 receptor

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一、中文摘要

介白質-1β已被牙周病學界之專家學者認定為牙周病致病機轉中一個很重要，宿主側由來之致病因子。它來自宿主。正常的生理狀況下，牙齦組織內微量存在的介白質-1β具有調節纖維母細胞修補維復細胞外基質的作用。在發炎的狀況下，被活化的巨噬細胞分泌過剩的介白質-1β會刺激纖維母細胞分泌過剩的介白質-1β、前列腺素 E_2、collagenase (MMP-1)、stromyelysin-1 (MMP-3) 及 gelatinase B (MMP-9)等分解代謝性介質，而直接或間接地造成牙周組織之細胞外基質的破壞及造成齒槽骨之吸收。因此如何調控介白質-1β對纖維母細胞的作用遂成為牙周病學研究中一個重要的課題。

本研究的目的是探討大量表現之第二型介白質-1 受體調控介白質-1β刺激纖維母細胞分泌分解代謝性介質的蛋白質表現的作用。本計畫分為二部分來進行。首先，利用基因導入的方法將第二型介白質-1 受體大量表現於牙齦纖維母細胞(大量表現轉形纖維母細胞)上。其次， 我們用介白質-1β刺激未轉形纖維母細胞及第二型介白質-1 接受器過剩表現轉形纖維母細胞之後，比較受到刺激之纖維母細胞分泌前列腺素 E_2的蛋白質分泌量之變化。

本研究的結果發現受介白質-1β刺激之未轉形細胞分泌前列腺素 E_2的蛋白質量高於第二型介白質-1 接受器過剩表現轉形細胞之分泌量。其差異有統計學上之意義。另外，研究的結果也發現隨著細胞老化, 高繼代之未轉形纖維母細胞及第二型介白質-1 接受器過剩表現轉形纖維母細胞受介白質-1β刺激之後，分泌前列腺素 E_2的量均較低繼代之未轉形纖維母細胞及第二型介白質-1 接受器過剩表現轉形纖維母細胞分泌前列腺素 E_2的量為高。這個發現可能與 Kumar 等人之研究發現高繼代之纖維母細胞比低繼代之纖維母細胞分泌較高量之介白質-1 有關。

第二型介白質-1 接受器抑制第二型介白質-1 接受器過剩表現轉形細胞受介白質-1 刺激之後，分泌前列腺素 E_2之作用機轉仍需進一步之研究來解明。

關鍵詞：牙齦纖維母細胞、 前列腺素 E_2、介白質−1、 介白質−1 接受器、 細胞衰老

Abstract

Interleukin-1β (IL-1β), an important host factor in periodontal tissue destruction, is a multifunctional cytokine. It plays important roles in the immuno-inflammatory responses of host to microbial challenge in periodontal diseases [1-10].

In healthy gingiva, low level of IL-1β regulates gingival fibroblasts (GF) proliferation [11], and may stimulate GF to produce collagen and tissue inhibitors of matrix metalloproteinase (TIMPs)[11-13]. In inflammatory gingival tissues, on the other hand, high level of IL-1β that was released

by microbial-activated macrophages stimulates GF to release potent catabolic mediators of periodontal tissues such as prostaglandin E_2 (PGE_2) and matrix metalloproteinases (MMPs). It has been suggested that elevated levels of IL-1β in disease sites might cause an unbalance of TIMPs and MMPs released from GF, which in turn, leads to the breakdown of extracellular matrix in gingiva and bone.

Because GF are the main architects of gingival tissue, IL-1β stimulated GF play important roles in amplifying the IL-1β responses in periodontal tissue destruction. It also raises important interest in modulation of IL-1β activity in GF. IL-1β acts on target cells by binding to its cognate receptors, type I IL-1 receptor (IL-1RI) and type II IL-1 receptor (IL-1RII) [28,29]. IL-1RI is suggested to be a signaling molecule, whereas IL-1RII is suggested to be a decoy target for IL-1 [34-38]. Therefore, IL-1RII has been suggested to be a unique pathway of negative regulation of the IL-1 system. In contrast to IL-1RI, IL-1RII expression is easily upregulated by chemoattractants and anti-inflammatory mediators.

In active sites of periodontal disease, the increased level of chemoattractants in gingival crevicular fluid (GCF) (43-47), and anti-inflammatory cytokines (48-53) in gingiva tissues may result in excessively elevated levels of IL-1RII expression on monocytic lineage cells in gingiva tissues. There is possibility that these IL-1RII upregulating mediators might also have effects on the expression of IL-1RII on GF. GF are the most abundant resident cells in gingival connective tissue. Their high cell numbers, in comparison to macrophages and PMNs, suggest that their collective contribution of IL-1RII expression is likely to provide a significant buffering effects on IL-1β activity in periodontal inflammation.

In this proposed study, our hypothesis is that the increased level of IL-1RII on gingival fibroblasts may have differential regulatory roles in the expression of IL-1β-induced catabolic mediators of periodontal tissue destruction. These mediators include PGE_2, and members of the MMPs family, such as MMP-1, MMP-3, and MMP-9. In this study, our strategy to test this hypothesis is firstly to overexpress IL-1RII on GF. Then the effects of IL-1β-induced expression of PGE_2 was analyzed at protein level by enzyme-linked immunosorbent assay (ELISA).

Key word: Type I interleukin-1 receptor, Type II interleukin-1 receptor, Periodontal inflammation, prostaglandin E_2.

二、緣由與目的

Interleukin-1β and Periodontal Disease

Interleukin-1β (IL-1β), an potent host factor in periodontal tissue destruction, plays important roles in the immuno-inflammatory responses of host to microbial challenge in periodontal diseases [1-10].

In healthy condition of gingiva, low level of IL-1β has regulatory functions on gingival fibroblasts (GF) to maintain functional intergrity of ginigval tissues, to regulate fibroblasts proliferation [11], and to produce collagen and tissue inhibitors of matrix metalloproteinase (TIMPs)[11-13]. In inflammatory sites of periodontal tissues, significantly elevated levels of IL-1β were released by microbial-activated macrophages [14-16]. High levels of IL-1β stimulate fibroblasts to release potent catabolic mediators of periodontal tissues such as PGE_2 and matrix metalloproteinases (MMPs) [17-38]. Previous studies have demonstrated clearly that IL-1β stimulates GF to release collagenases (MMP-1), stromelysin-1 (MMP-3), and gelatinases B (MMP-9) in inflamed gingiva [18-20.27-37]. These proteolytic enzymes play important roles in the degradation and remodeling of connective tissue matrix. It has been suggested that elevated levels of IL-1β in

disease sites might cause an unbalance of TIMPs and MMPs released from GF that leads to the breakdown of extracellular matrix in gingiva and bone[18-20].

In inflammation, IL-1β also stimulates GF to release PGE_2 . Like IL-1β, high levels of PGE_2 were detected in tissues and gingival crevicular fluid from active sites of periodontal diseases [39-45]. It has been suggested that the main sources of PGE_2 in inflamed gingiva is from macrophages and fibroblasts [43-46]. PGE_2 is a potent mediator that could initiate connective tissue destruction and bone resroption. In particular, previous studies have reported that many of the IL-1β activities on fibroblasts were regulated by PGE_2 production in response to the cytokine (46-48). El Attar et al reported that IL-1β inhibited the DNA synthesis of GF through PGE_2 release [47]. Some reports have also pointed out that IL-1β induces the production of MMP-1 via PGE_2 -dependent pathways [49]. Because GF are the main architects of gingival tissue, IL-1β stimulating GF plays important role in amplifying the IL-1β response in periodontal tissue breakdown. It also raises important interest in modulation of IL-1β activity in fibroblasts.

Modulation of IL-1β Responses by IL-1 Receptors on Target Cells

The effects of IL-1β on target cells were mediated by its receptor family which include type I IL-1 receptor (IL-1RI), type II IL-1 receptor (IL-1RII) and a growing family of IL-1 receptor accessory proteins [52,52]. Although IL-1RI and -II are often coexpressed on different cells, IL-1RI is expressed predominantly on T cells, fibroblasts, and exclusively on endothelial cells, whereas IL-1RII is expressed prominently on B cells, polymorphonuclear leukocytes (PMN), and monocytes [53]. In contrast to IL-1RI, IL-1RII expression is easily upregulated by chemoattractants such as FMLP, C5a, and IL-8 (54,55) as well as by anti-inflammatory mediators such as glucocorticoid hormones, IL-4, IL-10, and IL-13 (56). From antibody neutralization studies and cytoplasmic domain truncation studies, IL-1RI but not IL-1RII is suggested to be the primary signaling receptor, whereas IL-1RII has been thought to be a decoy target for IL-1 [57-61]. In addition, IL-1RI binds IL-1β with low affinity, and IL-1RII binds IL-1β with high affinity. Therefore, IL-1RII has been suggested to be a unique pathway of negative regulation of the IL-1β activity.

An understanding of the regulatory roles of the IL-1RII on IL-1β effects on GF is important for the development of strategies to manipulate the IL-1β effect on the progression of periodontal disease.

Hypothesis and specific aims

In this study, our hypothesis was that the increased level of IL-1RII on gingival fibroblasts may have differential regulatory roles in the expression of IL-1β-induced catabolic mediators of soft and hard tissue destruction such as PGE_2 , MMP-1, MMP-3, and MMP-9. Our strategy to test this hypothesis is to overexpress IL-1RII on GF, and then the effects of IL-1β induced expression of PGE_2 , that was used as a read-out of catabolic mediators in GF will be analyzed at protein level by enzyme-linked immunosorbent assay (ELISA).

三、結果與討論

In the present study, all experiments were done on one strain of 3 separated clones of each vector-transfected, and RII-transfected HGFs.

We first overexpressed type II IL-1R on HGFs. HGFs were obtained from biopsies of the attached gingiva of a 19-years old volunteer with clinically and radiographically normal periodontal tissues. HGFs were

transfected, selected and cloned as previously described. Positive clones with type II IL-1R overexpression were screened by RT-PCR at mRNA level. Type II IL-1R overexpression at protein level was confirmed by immunostaining with a monoclonal anti-human type II IL-1R antibody.

After the establishment of positive clones of RII-transfected GF, the production of PGE_2 were determined by using enzyme-linked immunosorbent assay (ELISA) kits purchased from R & D system. The data showed that the levels of the production of PGE_2 decreased in IL-1β stimulated RII-transfected GF than that in IL-1β stimulated vector-transfected control GF (Fig. 1).

The results also showed that the production of PGE_2 were increased in IL-1β stimulated late-passage RII-transfected GF and late-passage vector-transfected GF than those of IL-1β stimulated young-passage RII-transfected GF and vector-transfected GF . It might be related to the higher autogenous levels of age-associated IL-1 increased in the late-passage GFs as reported by Kumar et al (62-64).

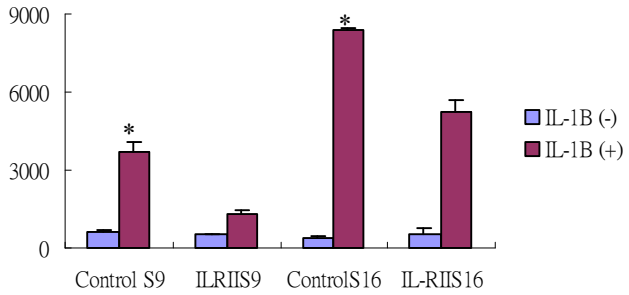
These results suggest that IL-1RII overexpressing on GF might play a role in the regulation of catabolic medators synthesis in IL-1β stimulated GF, but the underlying mechanisms of this regulation need to be further investigated.

四、計畫成果自評

於本研究計畫中，我們已完成下列之預期工作項目:

1. 利用基因導入的方法建立第二型介白質-1 接受器過剩表現之轉形牙周纖維母細胞株.
2. 藉著第二型介白質-1 接受器過剩表現之轉形牙周纖維母細胞株，來探討第二型介白質-1 接受器調控牙周纖維母細胞受介白質-1 β刺激之後未轉形纖維母細胞及第二型介白質-1 接受器過剩表現轉形纖維母細胞分泌前列腺素 E_2 的蛋白質分泌量之變化。
3. 比較高繼代與低繼代之未轉形纖維母細胞及第二型介白質-1 接受器過剩表現轉形纖維母細胞受介白質-1 β刺激之後，分泌前列腺素 E_2 的量之變化。
4. 本計畫之研究成果可以幫助瞭解第二型介白質-1 受體調控介白質-1 β刺激纖維母細胞分泌分解代謝性介質的蛋白質表現的作用，並間接幫助了解介白質-1 對老化細胞之發炎反應及對細胞外間質之破壞之影響。

Fig.1



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