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

計畫編號：NSC 89-2314-B-038-064
執行期限：89 年 8 月 01 日至 91 年 2 月 28 日
主持人：周幸華
執行機構及單位名稱:台北醫學院牙醫學系

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-1 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-1 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).

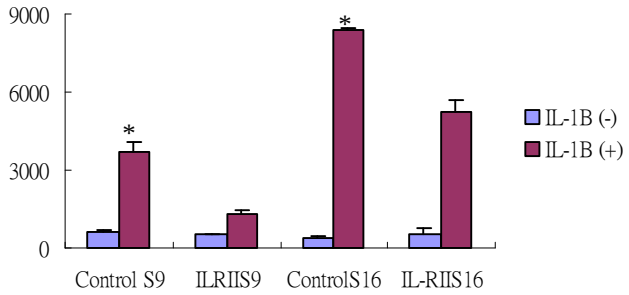
These results suggest that IL-1RII overexpressing on GF might play a role in the regulation of catabolic medators synthesis in IL-1β stimulated GF, but the underlying mechanisms of this regulation need to be further investigated.

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-1β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-1β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-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，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



五、參考文獻

1. Stashenko P, Jandinski JJ, Fujiyoshi P, Rynar J, and Socransky SS. Tissue levels of bone resorptive cytokine in periodontal disease. J Periodontol 1991; 62:504-509.
2. Jandinski JJ, Stashenko P, Feder LS, Leung CC, Peros WJ, Rynar JE, and Deasy MJ. Localization of Interleukin-1 in human periodontal tissue. J Periodontol 1991; 62:504-509.
3. Stashenko P, Fujiyoshi P, Obernesser MS, Prostak L, Haffajee AD, and Socrasky SS. Levels of Interleukin-1 in tissue from sites of active periodontal disease. J Clin Periodontol 1991; 18:548-554.
4. Masada MP, Persson R, Kenney JS, Lee SW, Page RC, and Allison AC. Measurement of Interleukin-1 and -1 in gingival crevicular fluid: Implications for the pathogenesis of periodontal disease. J Periodont Res 1990; 25: 156-163.
5. Kinane DF, Winstanley FP, Adonogianaki E, and Moughal NA. Bioassay of Interleukin-1 in human gingival crevicular fluid during experimental gingivitis. Archs Oral Biol. 1992; 37: 153-156.
6. Hillmann G., Hillmann B., and Geurtsen W. Immunohistological determinationof Interleukin-1 in inflamed human gingival epithelium. Archs Oral Biol. 1995; 40 (4): 353-359.
7. Grossi SG, Genco RJ, Machtei EE, HO Aw, koch G, Dunford R, Zambon JJ. Assessment of risk indicators for alveolar bone loss. J. Periodontol. 1995; 66:23-29.
8. Tatakis DN. Interleukin-1 and bone metabolism: a review. J Periodontol 1993; 64:416-431
9. Birkedal-Hansen H. Roles of cytokines and inflammatory mediators in tissue destruction. J Periodontol Res 1993; 28: 500-510.
10. Okamatsu Y, Kobayashi M, Nishihara T., Hasegawa K. Interleukin-1 produced in human gingival fibroblasts induces several activities related to the progression of periodontitis by direct contact. J Periodontol Res 1996; 31: 355-364.
11. Havemose-Poulsen, A. and Holmstrup, P. Factors affecting IL-1-mediated collagen metabolism by fibroblasts and the pathogenesis of periodontal disease: a review of the literature. Crit Rev Oral Biol Med 1997; 8, 217-36.
12. Page, R.C. The role of inflammatory mediators in the pathogenesis of

periodontal disease. J Periodontal Res 1991; 26, 230-42.

13. Postlethwaite, A.E., Raghow, R., Stricklin, G.P., Poppleton, H., Seyer, J.M. and Kang, A.H. Modulation of fibroblast functions by interleukin 1: increased steady-state accumulation of type I procollagen messenger RNAs and stimulation of other functions but not chemotaxis by human recombinant interleukin 1 alpha and beta. J Cell Biol 1988, 106, 311-8.
14. Jandinski JJ, Stashenko P, Feder LS, Leung CC, Peros WJ, Rynar JE, Deasy MJ Localization of interleukin-1 in human periodontal tissue. J. Periodontol. 1991; 62:36-43.
15. Matuski Y, Yamamoto T, Hara K. Interleukin-1 mRNA-expressing macrophages in human chronically inflamed gingival tissues. Am J Pathol. 1991; 138:1299-1305.
16. Meikle MC, McAlpine CG, Heath Jk, Newman HN, Reynolds JJ. Interleukin-production by peripheral blood mononuclear cells from patients with severe periodontitis. In: Lehner T., CimasoniGed. The borderland between caries and periodontal disease. Geneva: Editions Medecine et Hygiene. 1986: 283-290.
17. Birkedal-Hansen H., Moore WGI, Bodden MK, Windsor LJ, Birkedal-Hansen B, Decarlo A., Engler JA. Matrix Metalloproteinases: A Review. Crit. Rev. Oral. Biol. Med. 1996: 7(4):300-318.
18. Meikle MC, Heath Jk, Reynolds JJ. Advances in understanding cell interactions in tissue resorption. Relevance to the pathogenesis of periodontal diseases and a new hypothesis. J. Oral Pathol. 1986: 15:239-250.
19. Page R. C., Offenbacher S., Schroeder HE, Seymour GJ, Kornman KS. Advances in the pathogenesis of periodontitis: summary of developments, clinical implications and future directions. Periodontology 2000 (14):216-248.
20. Reynolds JJ, Meikle MC. Mechanisms of connective tissue matrix destruction in periodontitis. Periodontol 2000 1997: 14:144-157.
21. Guerne, P.A., Carson, D.A. and Lotz, M. IL-6 production by human articular chondrocytes. Modulation of its synthesis by cytokines, growth factors, and hormones in vitro. J Immunol 1990; 144, 499-505.
22. Harigai, M. et al. Interleukin 1 and tumor necrosis factor-alpha synergistically increase the production of interleukin 6 in human synovial fibroblast. 1991 J Clin Lab Immunol 34, 107-13.
23. Kent, L.W., Rahemtulla, F., Hockett, R., Jr., Gilleland, R.C. and Michalek, S.M. Effect of lipopolysaccharide and inflammatory cytokines on interleukin-6 production by normal human gingival fibroblasts. Infect Immun 1998; 66, 608-14.
24. Larsen, C.G., Anderson, A.O., Oppenheim, J.J. and Matsushima, K. Production of interleukin-8 by human dermal fibroblasts and keratinocytes in response to interleukin-1 or tumour necrosis factor. Immunology 1989; 68, 31-6.
25. Odake, H., Koizumi, F., Hatakeyama, S., Furuta, I. and Nakagawa, H. Production of cytokines belonging to the interleukin-8 family by human gingival fibroblasts stimulated with interleukin-1 beta in culture. Exp Mol Pathol 1993; 58, 14-24.
26. Takigawa, M., Takashiba, S., Takahashi, K., Arai, H., Kurihara, H. and Murayama, Y. Prostaglandin E2 inhibits interleukin-6 release but not its transcription in human gingival fibroblasts stimulated with interleukin-1 beta or tumor necrosis factor-alpha. J Periodontol 1994; 65, 1122-7.
27. Takigawa, M., Takashiba, S., Myokai, F., Takahashi, K., Arai, H., Kurihara, H. and Murayama, Y. Cytokine-dependent synergistic regulation of interleukin-8 production from human gingival fibroblasts. J Periodontol 1994; 65, 1002-7.
28. Zitnik, R.J., Kotloff, R.M., Latifpour, J., Zheng, T., Whiting, N.L., Schwalb, J. and Elias, J.A. Retinoic acid inhibition of IL-1-induced IL-6 production by human lung fibroblasts. J Immunol 1994; 152, 1419-27.
29. DiBattista, J.A., Pelletier, J.P., Zafarullah, M., Iwata, K. and Martel-Pelletier, J. Interleukin-1 beta induction of tissue inhibitor of metalloproteinase (TIMP-1) is functionally antagonized by prostaglandin E2 in human synovial fibroblasts. J Cell Biochem 1995; 57, 619-29.
30. Lark, M.W., Walakovits, L.A., Shah, T.K., Vanmiddlesworth, J., Cameron, P.M. and Lin, T.Y. Production and purification of prostromelysin and procollagenase from IL-1 beta-stimulatedhuman gingival fibroblasts. Connect Tissue Res 1990; 25, 49-65.
31. Meikle, M.C., Atkinson, S.J., Ward, R.V., Murphy, G. and Reynolds, J.J. Gingival fibroblasts degrade type I collagen films when stimulated with tumor necrosis factor and interleukin 1: evidence that breakdown is mediated by metalloproteinases. J Periodontal Res 1989; 24, 207-13.
32. Elias, J.A., Gustilo, K., Baeder, W. and Freundlich, B. Synergistic stimulation of fibroblast prostaglandin production by recombinant interleukin 1 and tumor necrosis factor. J Immunol 1987; 138, 3812-6.
33. Gitter, B.D., Labus, J.M., Lees, S.L. and Scheetz, M.E. Characteristics of human synovial fibroblast activation by IL-1 beta and TNF alpha. [Journal Article] Immunology. Immunology 1989; 66, 196-200.
34. Saito, S., Ngan, P., Rosol, T., Saito, M., Shimizu, H., Shinjo, N., Shanfeld, J. and Davidovitch, Z. Involvement of PGE synthesis in the effect of intermittent pressure and interleukin-1 beta on bone resorption. J Dent Res 1991; 70, 27-33.
35. Meikle MC., Hembry RM., Holley J., Horton C., McFarlane CG., Reynolds JJ. Immunolocalization of matrix metalloproteinases and TIMP-1(tissue inhibitor of metalloproteinases) in human gingival tissues from periodontitis patients. J. Periodont Res 1994; 29:118-126
36. Makela M, Salo T., Uitto VJ, Larjava H. Matrix metalloproteinases (MMP-2 and MMP-9) of the oral cavity: cellular origin and relationship to periodontal status. J. Dent. Res. 1994;73:1397-1406.
37. Kubota T., Nomura T., Takahashi T., Hara K. Expression of mRNA for matrix metalloproteinase and tissue inhibitors of metalloproteinases in periodontitis-affected human gingival tissue. Arch Oral Biol 1996:41:253-262.
38. Pinchback JS., Gibbins JR., Hunter N. Vascular co-localization of proteolytic enzymes and proteinase inhibitors in advanced periodontitis. J. Pathol. 1996; 179:326-332.
39. El Attar TMA, Lin HS. Prostaglandins in gingiva of patients with periodontal disease. J Periodontol. 1981; 52:16-19.
40. Offenbacher S., Farr DH., Goodson JM. Measurement of prostaglandin E in crevicular fluid. J Clin. Perodontol. 1981; 8:319-327.
41. Dewhirst FE., Moss DE., Offenbacher S., Goodson JM. Levels of PGE_2 , thromboxane and prostacyclin in periodontal tissue. J. Periodont. Res. 1983; 18:156-163.
42. Offenbacher S, Odle BM., Van dyke TE. The use of crevicular fluid PGE_2 levels as a predictor of periodontal attachment loss. J. Periodont. Res 1986: 21:101-112.
43. Attar El. TMA. PGE_2 in human gingiva in health and disease and its stimulation by female sex steroids. Prostaglandins 1976; 11:331-342.
44. Garrison SW., Holt SC., Nichols FC. Lipopolysaccharide-stimulated PGE_2 release from human monocytes. J. Periodontol 1988; 59:684-687.
45. Offenbacher S., Heasman PA., Collins JG. Modulation of host PGE_2 secretion as a determinant of periodontal disease expression. J. Periodontol. 1993:64:432-444.
46. Gemmell E., Marshall RI., Seymour GJ. Cytokine and prostaglandins in immune homeostasis and tissue destruction in periodontal disease. Periodontology 2000 (14):112-144.
47. El Attar T., Lin H. Prostaglandin E2 antagonizes gingival fibroblasts proliferation stimulated by interleukin-1b. Prostaglandins Leukotr Essen Fatty Acids 1993: 49:847-850.
48. Takigawa M., Takashiba S., Takahashi K, Arai H., Kurihara H., Murayama Y. Prostaglandin E2 inhibits interleukin-6 release but not transcription in human gingival fibroblasts stimulated with interleukin-1 or tumor necrosis factor- . J. Periodontol 1994: 65: 1122-1127.
49. Zhang Y. Mcluskey K, Fujii K, Wahl LM. Differential regulation of monocyte matrix metalloproteinase and TIMP-1 production by TNF- , granulocyte-macrophage CSF, and IL-1 through prostaglandin-dependent and –independent mechanism.J. Immunol. 1998;161(6):3071-6.
50. Smith WL. Prostanoid biosynthesis and mechanisms of action. Am. J. Physiol. 1992; 263: F181-F191.
51. Sims, J.E. et al. cDNA expression cloning of the IL-1 receptor, a member of the immunoglobulin superfamily. Science 1988; 241, 585-9.
52. Sims, J.E., Acres, R.B., Grubin, C.E., McMahan, C.J., Wignall, J.M., March, C.J. and Dower, S.K. Cloning the interleukin 1 receptor from human T cells. Proc Natl Acad Sci U S A 1989; 86, 8946-50.
53. Dinarello, C.A. Biologic basis for interleukin-1 in disease. Blood 1996; 87, 2095-147.
54. Colotta, F., Simone O, Fadlon EJ, Sozzani S, Matteucci C, and Mantovani, A. Chemoattractants induce rapid release of the Interleukin-1 type II decoy

receptor in human polymorphonuclear cells. J Exp Med 1995;181: 2181-2188.

55. Colotta, F. et al. Interleukin-1 type II receptor: a decoy for IL-1 that is regulated by IL-4. Science 1993; 261, 472-5.

56. Colotta, F., Dower, S.K., Sims, J.E. and Mantovani, A. The type II 'decoy' receptor: a novel regulatory pathway for interleukin 1. Immunol Today 1994;15, 562-6.

57. Sims, J.E., Gayle MA, Slack J, Alderson MR, Bird TA, Giri JG, Colotta F, Re F, Mantovani A, Shanebeck K, Kenneth HG and Dower, S.K. Interleukin-1 signaling occurs exclusively via type I receptor. Proc Natl Acad Sci U S A 1993; 90, 6155-59.

58. Curtis BM, Gallis B, Overell RW, McMahan CJ, Deroos P, Ireland R, Eisenman J, Dower SK, and Sims JE. T-cell interleukin-1 receptor cDNA expressed in Chinese hamster ovary cells regulates functional responses to Interleukin-1. Proc Natl Acad Sci U S A 1989; 86, 3045-49.

59. Heguy A, Baldari CT, Censini S, Ghiara P and Telford JL. Chimeric type II/typeI Interleukin-1 receptor can mediate interleukin-1 induction of gene expression in T cells. J. Biol. Chem. 1993; 268:10490-94.

60. Bossu, P. et al. Transfected type II Interleukin-1 receptor impairs responsiveness of human keratinocytes to Interleukin-1. Am J Pathol 1995; 147, 1852-61.

61. Re, F. et al. Inhibition of Interleukin-1 responsiveness by type II receptor gene transfer: a surface “receptor” with anti-interleukin-1 function. J Exp Med 1996; 183, 1841-50.

62. Kumar S, Millis AJT, and Baglioni C. Expression of interleukin 1-inducible genes and production of interleukin 1 by aging human fibroblasts. Proc. Natl. Acad. Sci. USA. 1992; 89:4683-4687.

63. Kumar S, Vinci JM, Millis AJT, Baglioni C. Expression of interleukin-1□ and □ in early passage fibroblasts from aging individuals. Exp. Geronto. 1993; 28: 505-513.

64. Zeng G, Millis AJT. Differential regulation of collagenase and stromelysin mRNA in late passage cultures of human fibroblasts. Exp. Cell Res. 1996; 222:150-156.