RRPC89030357 (5.P)

行政院國家科學委員會專題研究計畫成果報告

第一型及第二型介白質-1 受體在人體牙齦組織之體內及體外研究：組織定位及分子調控

In vivo and *in vitro* studies of type I and -II Interleukin-1 receptor in the human gingiva : localization and molecular regulation

計畫編號：NSC 89-2314-B-038-027
執行期限：88 年 8 月 01 日至 89 年 7 月 31 日

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一、中文摘要

介白質-1β為牙周病致病機轉中一個很重要的宿主側由來之致病因子。牙周炎患者之活性病灶部位的牙齦組織內及牙齦溝液內均可檢出高濃度之介白質-1β。介白質-1β對細胞的生物活性是經由細胞膜上的介白質-1 受體來傳遞訊息。介白質-1 受體有兩型，名為第一型及第二型介白質-1 受體。兩型受體可同時表現於同一種細胞上，但第一型介白質-1 受體主要表現於 T 淋巴細胞、纖維母細胞及內皮細胞上。第二型介白質-1 受體主要表現於 B 淋巴細胞、多形性白血球及巨噬細胞上。目前許多研究均指出第一型介白質-1 受體具有訊息傳遞的功能而第二型介白質-1 受體雖能與介白質-1β結合但不具訊息傳遞的功能，因此第二型介白質-1 受體被認為可能具有中和介白質-1β生物活性的功能，因而被認為具有潛力可供發展為抗介白質-1 活性的藥劑。

相對於第一型介白質-1 受體，第二型介白質-1 受體非常容易受到趨化性介質及抗炎性介質之作用而大量表現於細胞上。在發炎的牙齦組織中，有高濃度之趨化性介質及抗炎性介質之存在。這些介質除了可能造成牙齦組織中的 B 淋巴細胞、多形性白血球及巨噬細胞大量表現第二型受體之外，也可能有調控纖維母細胞表現第二型介白質-1 受體之作用。

關於第一型及第二型介白質-1 受體於牙齦組織中之表現、功能及其表現之調控機轉等之相關研究到目前為止仍非常少。因此，本研究計畫的目的是利用免疫染色法探討在正常及牙周炎的牙齦組織中，第一型及第二型介白質-1 受體的表現分布情形。另外並利用 ELISA 法探討正常的及牙周炎的牙齦組織中，第二型介白質-1 受體蛋白質的表現量之變化。

本研究的結果發現在正常的及牙周炎的牙齦中第一型及第二型介白質-1 受體均大量表現於表皮層中。在結締組織中，第一型及第二型介白質-1 受體主要表現於淋巴細胞、纖維母細胞及內皮細胞上。但在正常的及牙周炎的牙齦中第一型介白質-1 受體之表現分布情形及表現量並無差異。第二型介白質-1 受體之表現在牙周炎的牙齦之內皮細胞、纖維母細胞及巨噬細胞上均較正常的牙齦之表現強。但是，利用 ELISA 法分析第二型介白質-1 受體之表現，則發現在牙周炎及正常的牙

齦中，無統計學上有意義之差異($p<0.05$)。儘管如此，值得注意的是 histogram 中可看出牙周炎的樣本中有較多第二型介白質-1 受體濃度高於 1000 μg/ml 之樣本。因此本研究結果之原因可能是樣本數不夠多(牙周炎 n=22, 正常的牙齦 n=18)而 standard error 大，因而無法檢測出 mean difference。因此，將於未來繼續收集樣本進行分析。

關鍵詞：牙齦纖維母細胞,介白質-1, 介白質-1 接受器, 牙齦組織, 調控機轉

Abstract

Keywords：Type I interleukin-1 receptor, Type II interleukin-1 receptor, periodontal inflammation

Interleukin-1β, a potent proinflammatory and inflammatory mediator, plays important roles in the pathogenesis of periodontal disease. 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). IL-1 activity is mediated by IL-1RI, whereas IL-1RII is suggested to be a decoy target for IL-1. 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 the present study, the results of the immunocytochemical staining of IL-1RI and IL-1RII showed that both receptors were strongly expressed in the epithelial layers of inflamed and healthy gingiva with the most intensive staining in the basal layer. As the epithelial cells differentiated, the staining became less strong. In the connective tissue layers, IL-1RI was stained on lymphocytes, endothelial cells, and fibroblasts. However, there is no difference in the expression pattern and level of IL-1RI in healthy and inflamed gingiva. The expression of IL-1RII on endothelial, fibroblasts and macrophages were more strong in inflamed gingiva than those cells in healthy gingiva. Therefore, the levels of IL-1RII in inflamed and healthy gingival were further analyzed by ELISA. The results of ELISA revealed no significant difference of IL-1RII expression in healthy and inflamed gingiva with $p<0.05$ (Wilcoxon rank sum test). However, it is noteworthy that the histogram analysis showed an increased samples from the inflamed gingival population than those from healthy population were in the concentration above 1000 to

2500 μg/ml. Based on this observation, it is possible that the sample size were not big enough to detect the mean difference due to large standard error resulted from small sample size. To further clarify the physiologic and pathologic role of IL-1RII in gingiva, we will continue to collect inflamed and healthy gingiva to analyzed the expression level of IL-1RII by ELISA and RNase protection assay.

二、緣由與目的

Interleukin-1β (IL-1β), a potent multifunctional cytokine plays important roles in the pathogenesis of periodontal disease and is generally accepted to serve as a marker of periodontal disease severity and activity (1-7). The detection of significantly elevated levels in periodontal diseased tissues (1-6) and the potent bone-resorbing effects made IL-1β an important host factor in periodontal tissue destruction (7-9).

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) [10, 11]. Both receptors are possibly co-expressed on IL-1 responding cells, but their relative amount is dependent on cell types. IL-1RI was predominantly expressed on T cells, fibroblasts, and endothelial cells, whereas IL-1RII was prominent on B cells, polymorphonuclear leukocytes, and monocytes [12].

An understanding of the physiologic and pathologic roles of the IL-1RI and -II in periodontal tissue is important for the development of strategies to manipulate the IL-1β effect on the progression of periodontal disease. In a recent study, an increase of IL-1R (the author did not point out IL-1RI or –II) expression was found on fibroblasts derived from inflamed gingiva (13). However, there is little information available about the physiologic and pathologic localization of IL-1R-1 and -II in periodontal tissues *in vivo*.

The purpose of this study is to characterize and to identify the IL-1RI and –II bearing cells in normal and inflamed gingiva tissues. Then the correlation of IL-1RI and -II expression with periodontal inflammation will be analyzed at protein level in tissue extracts of normal and inflamed gingiva tissues.

The proposed research will help us to get insight into the physiologic and pathologic roles of IL-1RI and –II in both normal and inflamed periodontal tissues.

三、結果與討論

The study population includes 40 patients, 22 patients served as sources of inflamed gingival tissues, were diagnosed as moderate or severe periodontitis. These patients have pocket depths greater than 5 mm, bleeding on probing, and radiographic evidence of 30% to 50% bone loss at several sites. The 18 healthy adult volunteers, served as sources of normal gingival tissues, require tooth extraction for reasons other than periodontal involvement, have probing pocket depth $\leq$ 3 mm at all sites and no bleeding on probing. Informed consents were obtained from all participants.

The results of the immunocytochemical staining of IL-1RI and IL-1RII showed that both receptors were strongly expressed in the epithelial layers of inflamed and healthy gingival with the most intensive staining in the basal layer. As the epithelial cells differentiated, the staining became less strong. In the connective tissue layers, IL-1RI was stained on lymphocytes, endothelial cells, and fibroblasts. However, there is no difference in the expression pattern and level of IL-1RI in healthy and inflamed gingiva. The expression of IL-1RII on endothelial, fibroblasts and macrophages were more strong in inflamed gingiva than those cells in healthy gingival. Therefore, the level of IL-1RII in inflamed and healthy gingival was further analyzed by ELISA. The results of ELISA revealed no significant difference of IL-1RII expression with $p<0.05$ (Wilcoxon rank sum test). However, the histogram analysis showed an increased samples from the inflamed gingival population than those from healthy population were in the concentration above 1000 to 1500 g/ml. Based on this observation, it is possible that the sample size were not big enough to detect the mean difference due to large standard error resulted from small sample size. To further clarify the physiologic and pathologic role of IL-1RII in gingiva, we will continue to collect inflamed and healthy gingival to analyzed the

expression level of IL-1RII by ELISA and RNase protection assay.

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本研究之結果預定於 79th General Session & Exhibition of the International Association for Dental Research (2001, June, Chiba, Japan) 發表.

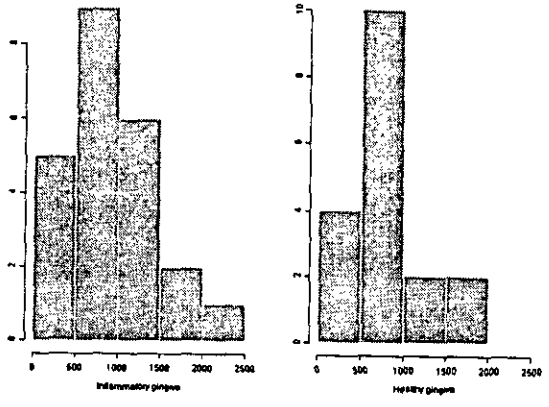

Fig. 1 IL-1RII concentration in inflammatory and healthy gingiva. The horizontal axis was the concentration of IL-1RII in gingiva. The vertical axis was the number of patients distributed in the indicated ranges of IL-1RII concentration.