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行政院國家科學委員會專題研究計畫成果報告

計畫編號：NSC 93－2314－B－038－039
執行期限：93 年 08 月 01 日 至 94 年 07 月 31 日
主持人：周幸華　台北醫學大學牙醫學系
共同主持人：賴銘堂　何元順

一、中文摘要

牙周病是經由 *Porphyromonas gingivalis* 感染所引發的慢性發炎反應，近年來許多文獻報導指出，牙周病會增加患者罹患心血管疾病的機率，發炎反應的三個指標：C-reactive protein (CRP)、fibrinogen(Fb)、interleukin-6 皆對於增加心衰竭與中風之危險有相關聯性；其中 interleukin-6 又爲冠狀動脈疾病的獨立性危險因子。本研究藉由探討人類臍靜脈內皮細胞在受到牙周病主要致病菌 *Porphyromonas gingivalis* (以下簡稱 381) 感染，誘發 interleukin-6 及其接受器表現之訊息傳遞來討論牙周病增加心血管疾病危險因子之機制。另外，利用另一突變菌株insertionally inactivated *fimA* (*fimA* mutants，以下簡稱 DPG3)，比較細胞受到侵入型與非侵入型細菌感染後訊息傳遞機制之差異。結果顯示， 細胞分別受到兩菌株感染後皆可誘發interleukin-6 產生及調控 IL-6 receptor complex (IL-6α、gp130)的表現，但是兩者有程度上的差異。在我們的結果顯示JAKs/STAT3 路徑只參與在由 381 所調控的 receptor 表現。在我們的實驗模式中，經由牙周病致病菌 *P.gingivalis* 感染人類臍靜脈內皮細胞後誘導 IL-6 的產生並且調控IL-6 receptor 的表現造成訊息傳遞的迴路，使得細胞本身自我調控產生 IL-6 的大量分泌；然而，過度的刺激會讓免疫反應所造成的損害性大過於修復性。因此，藉由探討牙周病菌對於臍靜脈細胞的感染發炎機制，可進一步的了解牙周病與心血管疾病間的相關聯性，並期望在將來可由致病機轉中找出減低牙周病患者罹患心血管疾病的危險

Keywords: *Porphyromonas gingivalis*, IL-6, IL-6 receptor, gp130

二、英文摘要

Porphyromonas gingivalis is an oral pathogen that causes a chronic local inflammatory disease, periodontal disease, which results in the destruction of the periodontal ligament and alveolar bone. Recent studies have focused on the association of *P. gingivalis*-mediated periodontal infection and systemic diseases. Several reports support a definite relationship between periodontal infections and certain systemic conditions including atherosclerosis and cardiovascular disease. Therefore, markers of systemic inflammation, such as C-reactive protein (CRP), fibrinogen, different cytokines; especially interleukin-6 (IL-6) have been studied as potential new risk factors. It has established that the periodontal pathogen *Porphyromonas gingivalis* is capable of invading aortic, heart, and human umbilical vein endothelial cells (HUVEC). Interactions of *P. gingivalis* with endothelial cells and the subsequent host cell response to infection may be important in the

pathogenesis of atherosclerosis. In this study, we coculture HUVEC with live *P. gingivalis* strain, 381, and insertionally inactivated *fimA* mutant, DPG3 respectively to compare the results. We demonstrated that *P. gingivalis* upregulated IL-6 and IL-6 receptor in HUVEC. The results showed that both *P. gingivalis* strains can modulate IL-6 expression in endothelial cell, but there is difference in the expression level. Our data showed that STAT3 activation was only involved in 381-regulated IL-6 receptor expression. Our results revealed the signaling pathway involved in regulation of IL-6 receptor complex expression in HUVEC by invasive and non-invasive *P. g*. Our data showed an experimental link between *P. gingivalis* infection and vein endothelial cells. It would activate IL-6 signaling transduction and result in excess IL-6 production in HUVEC infected by *P. g*. The results suggested that *P. gingivalis* infection would induce inflammatory response in endothelial cells; therefore accelerates atherosclerotic changes.

Keywords: *Porphyromonas gingivalis*, IL-6, IL-6 receptor, gp130

三、計畫緣由與目的

Porphyromonas gingivalis (*P. gingivalis*) has been implicated as a major etiological agent in the development of adult periodontitis, which is a bacterially induced chronic inflammatory disease that leads to the inflammation of the gingiva, loss of alverolar bone with eventual loss of teeth (16). Recently, several epidemiological and pathological studies have demonstrated a role for *Porphyromonas gingivalis* infection in atherosclerosis and human coronary heart disease (2, 4, 6, 8, 9, 12). *In vitro* experiments of cell culture had shown that bacterial components of *P. gingivalis* such as fimbriae, LPS and gingipains can induce IL-6 production in gingival fibroblasts, epithelial cells and KB cells, respectively (5, 7, 11, 13, 17). IL-6 is an important mediator of the acute phase response and can increase plasma levels of C-reactive protein (CRP) that are correlated with prognosis in patients with unstable angina (3, 10), and with the risk of myocardial infarction in healthy subjects (15). The results of *in vitro* studies also suggested the potential direct involvement of IL-6 in atherogenesis (18). Ogawa et al reported that *P. gingivalis* fimbriae induced IL-6 production in human peripheral monocytes (13). Lourbakos et al reported that purified Arginine-specific protease of *P. gingivalis* induces IL-6 secretion in human oral epithelial cells (11), while Protempa's group reported that purified *P. gingivalis* gingipain degrade IL-6 and IL-6R in a cell-free model (1, 14). These results indicated that purified molecule of *P. gingivalis* gingipain can degrade IL-6 in a cell-free model, but the results from cell culture experiment is contrary and suggested that *P. gingivalis* gingipain stimulate IL-6 expression. In the past few years, studies had focused only on the effect of *P. gingivalis* bacterial components on IL-6, but whether this extends to the modulation of IL-6 and its receptor (IL-6R) and signaling subunit (gp130) expression in human endothelial cells following active invasion of live *P. gingivalis* remained to be investigated.

In the present study, we investigated how active invasion of *P.*

gingivalis modulates IL-6R and gp130 expression and gp130 signaling in endothelial cells.

四、結果與討論

In this study, we demonstrated that *P. gingivalis* upregulated IL-6 and IL-6 receptor in HUVEC. The results showed that both *P. gingivalis* strains can modulate IL-6 expression in endothelial cell, but there is difference in the expression level (Fig.1). *P. gingivalis*381 upregulated IL-6 receptor (IL-6Rα, gp130) expression in HUVEC cells (Fig.2). The JAK1/STAT3 activation were involved in *P. gingivalis* induced IL-6 receptor (IL-6Rα, gp130) expression (Fig.3). Our data showed that STAT3 activation was only involved in 381-regulated IL-6 receptor expression. Our results revealed the signaling pathway involved in regulation of IL-6 receptor complex expression in HUVEC by invasive and non-invasive *P. g*. Our data showed an experimental link between *P. gingivalis* infection and vein endothelial cells. It would activate IL-6 signaling transduction and result in excess IL-6 production in HUVEC infected by *P. g*. The results suggested that *P. gingivalis* infection would induce inflammatory response in endothelial cells; therefore accelerates atherosclerotic changes.

Figures and Legends.

Figure 1

P. gingivalis upregulated IL-6 expression in HUVEC cells. ELISA analysis of the IL-6 secretion in HUVEC infected with *P. gingivalis* 381 and DPG3. The HUVEC cells were treated with MOI = 100 of 381, DPG3 for 24 hour; then the culture medium supernatant was measured by ELISA. The relative level was calculated as ratio of control level. Results were presented as mean ± S.E. *, $p < 0.05$ as compared with control.

Figure 2

*P. gingivalis*381 upregulated IL-6 receptor (IL-6Rα, gp130) expression in HUVEC cells. The HUVEC cells were treated with MOI = 100 of 381, DPG3 for the indicated times (0.5, 4, 6, 16, 24 hour). The relative level was calculated as ratio of control level. Results of IL-6R and gp130 were presented as mean ± S.E. *, $p < 0.05$ as compared with control (A, B). One experiment representative of 3~6 different experiments is shown.

Figure 3

JAK1 inhibitor inhibited*P. gingivalis*381 induced IL-6 receptor (IL-6Rα, gp130) expression in HUVEC cells. HUVEC cells were pretreated with 1μM JAK inbibitor 1 for 1 hour before incubated with MOI = 100 of 381, DPG3 for 6 hour. It was been observed that both 381, DPG3 induce shedding of IL-6Rα and gp130 from HUVEC cells at 6 hour, but only 381-induced IL-6 receptor expression could be reversed by JAK inhibitor 1 (A, B). The relative level was calculated as ratio of control level. Results were presented as mean ± S.E. *, $p < 0.05$ as compared with control; # < 0.05 as compared with only P. g treated group. One experiment representative of 3~6 differentexperiments is shown.

Fig.1

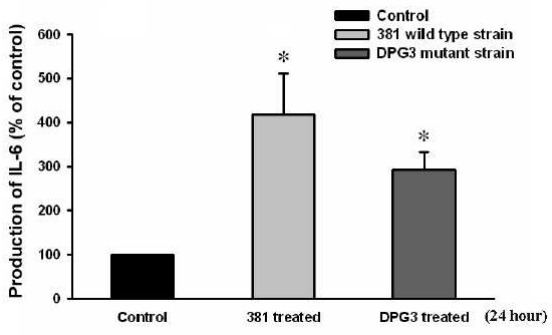


Fig. 2A

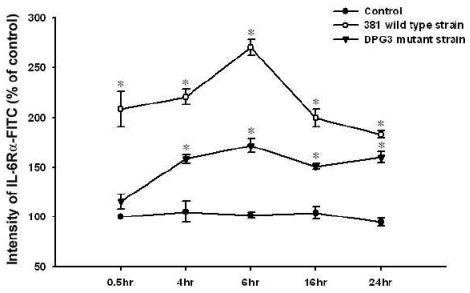


Fig. 2B

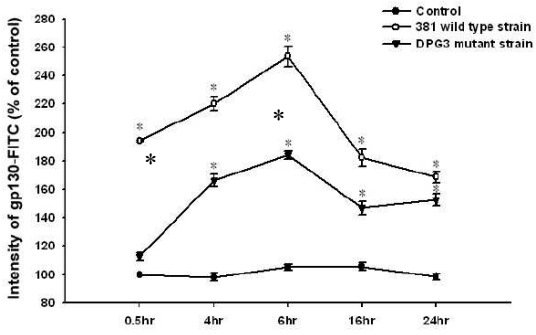


Fig.3A

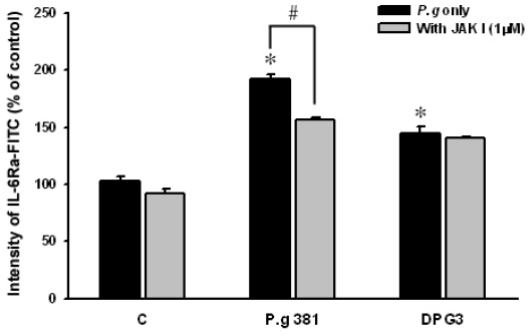
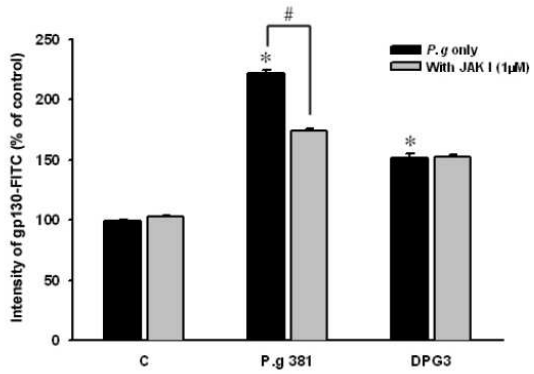


Fig.3B



五、計畫成果自評

在我們的實驗模式中，經由牙周病致病菌 *P.gingivalis* 感染人類臍靜脈內皮細胞後誘導 IL-6 的產生並且調控 IL-6 receptor 的表現造成訊息傳遞的迴路，使得細胞本身自我調控產生 IL-6 的大量分泌；然而，過度的刺激會讓免疫反應所造成的損害性大過於修復性。因此，藉由探討牙周病菌對於臍靜脈細胞的感染發炎機制，可進一步的了解牙周病與心血管疾病間的相關聯性，並期望在將來可由致病機轉中找出減低牙周病患者罹患心血管疾病的危險。

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