行政院國家科學委員會專題研究計畫　成果報告

Osteopontin 基因在急性呼吸窘迫症候群，結核性肋膜炎及肺外結核病人之表現

計畫類別：個別型計畫
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計畫主持人：李麗娜

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行政院國家科學委員會專題研究計畫成果報告

Osteopontin基因在結核性肋膜炎及粟粒性結核病人之表現

Expression of osteopontin gene in patients with tuberculous pleurisy and miliary tuberculosis

† 八十六年度及以前的一般國科會專題計畫(不含產學合作研究計畫)亦可選擇適用，惟較特殊的計畫如國科會規劃案等，請先洽得國科會各學術處同意。

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一、中文摘要

osteopontin 是由巨噬細胞與 T 淋巴球產生的介質（matrix）蛋白. 過去已有報告顯示, 在結核病造成的肉芽腫 (granuloma), osteopontin 之表現很強. 亦有報告顯示, 肺結核病人血液中的 osteopontin 濃度比正常人血液中的值高出很多. 本計畫旨在研究結核性肋膜炎及粟粒性結核病人血液中 osteopontin 的濃度, 並與正常人血液中的值比較,osteopontin 的濃度測定是使用 ELISA 法. 研究結果顯示, 結核性肋膜炎及粟粒性結核病人血漿中的 osteopontin 比正常人血漿中的值, 有顯著之上升.

關鍵詞：osteopontin, 結核菌, 肋膜炎, 粟粒性, 肉芽腫

Abstract

Osteopontin, a noncollagenous matrix protein produced by macrophages and T lymphocytes, is expressed in granulomatous lesions caused by Mycobacterium tuberculosis infection. Circulating osteopontin levels have been found to be elevated in patients with pulmonary tuberculosis, and correlate with severity of pulmonary tuberculosis. In the present study we investigated plasma osteopontin levels in patients with tuberculous pleurisy, those with miliary tuberculosis, as there was no reports of such study, and compared them with those of normal control subjects. We found that plasma osteopontin levels, as measured by an ELISA assay, were significantly higher in patients with tuberculous pleurisy, those with miliary tuberculosis, than in healthy control subjects.

Keywords: osteopontin, *Mycobacterium tuberculosis*, pleurisy, miliary, granuloma

INTRODUCTION

Osteopontin is a phophorylated acidic glycoprotein, which contains an arginine-glycine-aspartate-binding motif to the integrin family of adhesion molecules (1-3). It is abundantly produced by T cells and macrophages during the formation of granulomatous lesions in tuberculosis (4). Ashkar and coworkers (5) found a new role for OPN, acting as a proinflammatory cytokine for inducing type 1 T helper (Th1) cell-mediated immune responses. Using osteopontin-knockout mice, they showed that osteopontin was essential for the

development of Th1 responses and granuloma formation through induction of interleukin (IL)-12 production by macrophages.

Host defense against M. tuberculosis consists largely of Th1 cell-mediated mechanism (6). In the present study we investigated the pathogenic role of osteopontin in tuberculosis by measuring plasma osteopontin levels in patients with tuberculous pleurisy and those with miliary tuberculosis.

MATERIALS AND METHODS

We studied 7 male and 3 female patients with tuberculous pleurisy, and 3 male patients with miliary tuberculosis. Their ages ranged from 40 to 72 years (61.0 ± 12.4). M. tuberculosis was isolated from sputum of all patients. Plasma samples were collected before anti-tuberculous chemotherapy was started, and were frozen at -80oC until use.

We also studied 15(10 males) healthy volunteers who had no history of pulmonary tuberculosis or other infection, and their chest radiographs showed no evidence of respiratory diseases. Their ages ranged from 31 to 65 years (54.2± 10.3).

Measurement of osteopontin

The concentration of osteopontin was measured by an antigen-capture ELISA (7).

Results

Plasma osteopontin concentrations in patients with tuberculous pleurisy and those with miliary tuberculosis

To investigate the pathogenic role of osteopontin in tuberculosis, we compared the plasma concentrations of this cytokine in patients with tuberculous pleural effusion, and those with miliary tuberculosis with healthy control subjects. As shown in Table 1, plasma osteopontin concentrations in patients with tuberculous pleurisy (n=10) were significantly higher than in control subjects (n=15). Plasma osteopontin concentrations in patients with miliary tuberculosis (n=3) was also significantly higher than in control subjects.

Table 1 Plasma osteopontin levels in patients with tuberculous pleurisy, patients with miliary tuberculosis, and healthy controls.

Diagnosis	Plasma osteopontin (M±SD)(ng/ml)	*p* value
TB pleurisy (n=10)	739.7±321.7	< 0.001
Miliary TB (n=3)	867.3±442.9	< 0.001
Healthy (n=15)	227.5±93.4	

DISCUSSION

In the present study, we measured the levels of circulating osteopontin in patients with tuberculous pleurisy, miliary tuberculosis and healthy control subjects. We found that plasma osteopontin levels were significantly higher in patients with tuberculous pleurisy, those with miliary tuberculosis, than in healthy controls.

Previous studies (8,9) showed that

infection of human alveolar macrophages and a macrophage cell line with *M. tuberculosis* caused a substantial increase in osteopontin gene expression. The expression of osteopontin was elevated in pathologic sections from patients with various granulomatous diseases including pulmonary tuberculosis (4,8). The observations suggested the possible involvement of osteopontin in the pathogenesis, especially the generation of granulomas, of tuberculosis.

Kiguchi and colleagues (10) also reported that plasma osteopontin levels were significantly higher in patients with tuberculosis than in control subjects. They also observed that osteopontin levels were higher in smear-positive than in smear-negative patients. The severity of tuberculosis, as evaluated by the size of pulmonary lesions on chest radiographs, correlated with plasma osteopontin concentrations. Osteopontin levels decreased in parallel with clinical improvement after treatment with antituberculous agents. These observations strongly support the role of osteopontin in the pathogenesis associated with pulmonary tuberculosis.

There was, however, no reports of osteopontin concentrations in patients with tuberculous pleurisy or miliary tuberculosis in the past. The results of our preliminary study were compatible with those involving patients with pulmonary tuberculosis. We need to measure circulating osteopontin levels in patients with other pulmonary diseases, such as malignant pleural effusion, parapneumonic effusions, autoimmune disease associated pleural effusions, uremic effusions, effusions associated pulmonary infaction, and miliary carcinomatosis, before deciding if circulating osteopontin levels can be of help in diagnosing tuberculous pleurisy or miliary tuberculosis.

CONCLUSION

Plasma osteopontin levels were significantly higher in patients with tuberculous pleurisy, those with miliary tuberculosis, than in healthy control subjects.

REFERENCES

1. Uede T, Katagiri Y, Iizuka J, Murakami M. Osteopontin, a coordinator of host defense system: a cytokine or an extracellular adhesive protein? Microbiol Immunol 1997;41:641-8.
2. Rittling SR, Denhardt DT. Osteopontin function in pathology: lessons from osteopontin-deficient mice. Exp Nephrol 1999;7:103-13.
3. O'Regan AW, Nau GJ, Chupp GL, Berman JS. Osteopontin (Eta-1) in cell-mediated immunity: teaching an old dog new tricks. Immunol Today 2000;10:475-8.
4. Carlson I, Tognazzi K, Manseau EJ, Dvorak HF, Brown LF. Osteopontin is strongly

expressed by histiocytes in granulomas of diverse etiology. Lab Invest 1997; 77: 103-8.

5. Ashkar S, Weber GF, Panoutsakopoulou V, Sanchirico ME, Jansson M, Zawaideh S, Rittling SR, Denhardt DT, Glimcher MJ, Cantor H. Eta-1 (osteopontin): an early component of type-1 cell mediated immunity. Science 2000;287:860-4.
6. Flynn JL, Chan J. Immunology of tuberculosis. Annu Rev Immunol 2001:19: 93-129.
7. Kon S, Maeda M, Segawa T, Hagiwara Y, Horidoshi Y, Chikuma S, Tanaka K, Rashid MM, Inobe M, Chambers AF et al. Antibodies to different peptides in osteopontin reveal complexities in the various secreted forms. J Cell Biochem 2000;77: 487-98.
8. Nau GJ, Chupp GL, Emile JF, Jouanguy E, Berman JS, Kim SJ, Kornfield H, Young RA. A chemoattractant cytokine associated with granulomas in tuberculosis and silicosis. Proc Natl Acad Sci USA 1997; 94:6414-9.
9. Ragno S, Romano M, Howell S, Pappin DJ, Jenner PJ, Colston MJ. Changes in gene expression in macrophages infected with M. tuberculosis: a combined transcriptomic and proteomic approach. Immunology 2001; 104:99-108.
10. Koguchi Y, Kawakami K, Uezu K, Fukushima K, Kon S, Maeda M, Nakamoto A, Owan I, et al. High plasma osteopontin level and its relationship with interleukin-12-mediated type 1 T helper cell response in tuberculosis. Am J Respir Crit Care Med 2003;167:1355-9.