Detection of Tumor Necrosis Factor-α and Receptors in the Serum and Synovial Fluid of Patients With Rheumatoid Arthritis and Osteoarthritis

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Tumor necrosis factor (TNF) induces the production of two forms of soluble receptors (p55 and p75) that are present in human serum at concentrations that increase in inflammatory rheumatic disease. The purpose of this study was to evaluate the usefulness of TNF-α and soluble TNF receptor (sTNFR) for distinguishing between rheumatoid arthritis (RA), osteoarthritis (OA) and normal individuals. Serum and synovial fluid (SF) from patients with RA and OA, and serum from normal control subjects were analyzed for p55, p75 and TNF-α by enzyme-linked immunosorbent assays (ELISA). TNF-α was detectable in 20% of RA sera. However, it could not be measured in OA or control sera. Levels of TNF-α were significantly higher in SF of RA group when compared with the OA group. Although p55 sTNFR concentrations in serum showed a significant increase in RA than in the controls, there was no difference between patients with OA and the controls. Measurement of p55 sTNFR in SF showed a significant difference between the RA and OA groups. Furthermore, p75 sTNFR in serum was markedly higher in RA and OA groups than in the controls. p75 in SF was significantly different between RA and OA patients. There was no significant correlation between p55, p75, TNF-α and disease activity, including clinical and laboratory parameters (erythrocyte sedimentation rate and C-reactive protein levels). In conclusion, our study demonstrates that TNF-α and sTNFR are up-regulated in patients with RA and produced locally in the joints. In addition, serum and SF levels of sTNFR and TNF-α do not seem to be related to disease activity. **(Mid Taiwan J Med 1999;4:109-15)**

Key words

disease activity, rheumatoid arthritis, soluble tumor necrosis factor receptor, tumor necrosis factor- α

INTRODUCTION

Tumor necrosis factor (TNF)-α is an inducible cytokine produced primarily by monocytes and macrophages. Many biological activities of TNF-α appear to be involved in the pathogenesis of chronic inflammation and arthritis. TNF-α can induce resorption of cartilage and bone [1], endothelial adherence and activation of granulocytes [2], and class I

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major histocompatibility complex expression, as well as stimulation of fibroblast growth via platelet-derived growth factor [3], synovial cells prostaglandin E2 and collagenase production [4]. The beneficial effect of an anti-TNF-α antibody in an animal model of type II collagen-induced arthritis supports the hypothesis that TNF-α is pivotal in the pathogenesis of rheumatoid arthritis (RA) [5,6].

One mechanism involved in the regulation of TNF function is the inducible proteolytic cleavage of cell soluble TNF receptors (sTNFR), which results in down-regulation of the membrane receptors and formation of soluble forms of the receptors. These soluble forms of the receptors can block TNF function, by competing for TNF [7-10]. Studies of serum levels of sTNFR have indicated that cleavage of the TNF receptors takes place constantly *in vivo* even in normal individuals, and is enhanced in some disease states. Recently, soluble binding proteins identical to the extracellular portions of the two sTNFR were characterized [11-13]. These receptors are commonly known as the 75 kDa or type A and 55 kDa or type B sTNFR. sTNFR are differentially expressed on macrophages, granulocytes, activated T and B lymphocytes, and various other cell lines [14]. They have been shown to increase after administration of TNF [15]. Production of TNF in disease condition is therefore likely to result in increased serum concentrations of sTNFR.

Both p55 and p75 sTNFR levels were significantly higher in RA patients as compared with osteoarthritis (OA) patients from previous reports [16-18]. Differences are apparent in the serum, but are most markedly in the synovial fluid (SF) samples. Furthermore, several studies have reported a correlation between an elevation in sTNFR in serum or SF and disease activity in some patients [16,17]. However, other reports disprove against such an association [18]. In Taiwan, Lee et al. have demonstrated the concentration of TNF-α and sTNFR in serum of patients with RA, OA and gout [19]. However, SF levels were not tested in their study. In our study, we were interested in the production of sTNFR in the synovial joints at sites of enhanced TNF-α and sTNFR production in RA. We determined the concentrations of TNF-α and sTNFR in serum and SF of patients with RA and OA to investigate whether these could be used clinically to distinguish patients from different forms of arthritis and to research for the relationship between TNF-α, sTNFR and clinical disease activity of RA.

MATERIALS AND METHODS

Patients

Thirty patients with rheumatic disease, who visited the Division of Immunology and Rheumatology at China Medical College Hospital, were studied. Among those patients, 15 patients (median age 61 years, range 41-73) had a definite RA according to the 1987 Revised American College of Rheumatology Criteria [20] and 15 patients (median age 59 years, range 45-70) had clinically and radiologically diagnosed OA predominantly involving the knee joints. An age-matched control group included 15 healthy volunteers with a median age of 57 years (range 39 - 70 years). The majority of patients with RA and OA were treated with nonsteroidal anti-inflammatory drugs (NSAIDs). In addition, several of the patients with RA were treated with low dose steroids (n = 12) and/or disease-modifying anti-rheumatic drug (DMARD), i.e., methotrexate (n = 5), sulfasalazine (n = 6), hydroxychloroquine (n = 8), D-penicillamine (n = 1), or azathioprine (n = 1). No patient had been injected with steroids in the same joint in the three months previous to the study.

Clinical Evaluation

To evaluate the relationship between TNF-α, sTNFR and clinical disease activity of RA, we used a summation of scores based on six features [duration of morning stiffness for more than 1 hour, warmth, joint tenderness,

Table 1. p55 sTNFR (pg/ml) in serum and synovial fluid from patients with RA, OA and control subjects

Group	No.	Serum		Synovial fluid	
		Mean ±SD	Range	Mean ±SD	Range
RA	15	2036.5 ±864.0*	939 –3280	7310.8 ±2678.9†	2910 –10000
OA	15	1303.8 ±630.2	494 –2380	5535.0 ±2731.2 †	2750 –10000
Control	15	1100.0 ±366.8*	400 –1600		

*$p < 0.05$; †$p < 0.05$. RA= rheumatoid arthritis; OA= osteoarthritis.

Table 2. p75 sTNFR (pg/ml) in serum and synovial fluid from patients with RA, OA and control subjects

Group	No.	Serum		Synovial fluid	
		Mean ±SD	Range	Mean ±SD	Range
RA	15	4041.2 ±1026*	2200 –5000	6814.0 ±2885.7‡	2320 –10000
OA	15	3149.1 ±1603.1†	1000 –5000	5382.5 ±2701.5‡	2180 –10000
Control	15	2250.0 ±988.5*†	500 –3500		

*$p < 0.005$; †$p < 0.01$; ‡$p < 0.05$. RA= rheumatoid arthritis; OA= osteoarthritis.

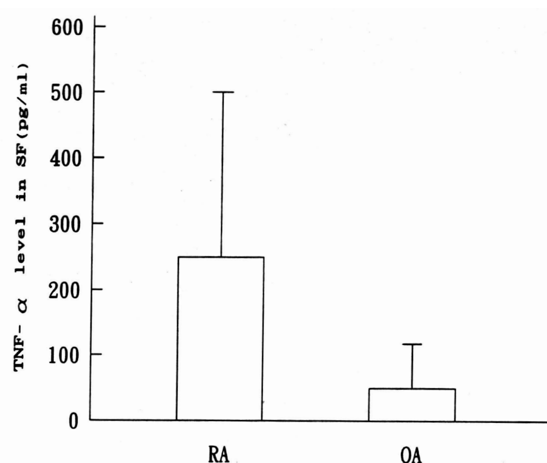

Fig.1 TNF-α level in synovial fluid (SF) from patients with rheumatoid arthritis (RA) and osteoarthritis (OA) ($p <$ 0.01).

effusion, synovial thickening and levels of erythrocyte sedimentation rate (ESR), C-reactive protein (CRP)]. The joint was globally defined as having "high inflammatory activity" if 4-6 parameters were present, and "low inflammatory activity" if < 2 were present [21].

Sample Preparation

SF and peripheral venous blood samples were collected at the same time. All SF samples were aspirated from the knee joint and treated with sheep hyaluronidase (2000 U/ml). SF and blood samples were spun at 2000 rpm for 10 minutes. Then the SF supernatant and plasma were collected and stored at -20°C until being assayed for cytokines.

Assays for TNF-α and sTNFR

sTNFR was measured by an enzyme-linked immunosorbent assay (ELISA) (R&D Systems, Minneapolis, MN, USA) [22]. TNF-α was also measured by the ELISA method, (Endogen, Woburn, MA, USA). All samples were tested in duplicate. The interassay variations were 6.1% and 4.1% for the p55 and p75 assays, respectively, and < 10% for the TNF-α assay. The lower limits of detection for p55 and p75 were 30 pg/ml and 10 pg/ml, respectively. The sensitivity of the TNF-α ELISA was < 5 pg/ml.

Statistical Analysis

For paired samples, differences were tested by Mann-Whitney U test. Spearman coefficient was used to assess a correlation between cytokine (TNF-α, p55 and p75 sTNFR) and disease activity parameters (ESR and CRP). Results were expressed as mean ± standard deviation (SD). A p value of less than 0.05 was considered significant.

RESULTS

TNF-α in Serum and SF

TNF-α was detectable in only three (20%) of 15 serum samples from the RA patients and

none of those from the OA group and the control subjects. Thirteen (86%) of the 15 SF samples from RA patients, and eight (53%) of 15 SF samples from the OA patients had measurable TNF-α. TNF-α in SF of RA patients was significantly higher when compared with SF of OA patients (270.2 ± 236.9 pg/ml *vs* 61.6 ± 57.9 pg/ml, $p < 0.01$) (Fig. 1).

p55 sTNFR in Serum and SF

As shown in Table 1, serum p55 sTNFR levels in patients with RA, OA and control subjects were 2036.5 ± 864 pg/ml, 1303.8 ± 630.2 pg/ml, and 1100 ± 366.8 pg/ml, respectively. The serum level of p55 sTNFR in RA patients was significantly higher than those in the control subjects ($p < 0.05$).

The p55 sTNFR levels in SF between the RA and OA groups were significantly different (7310.8 ± 2678.9 pg/ml *vs* 5535 ± 2731.2 pg/ml, $p < 0.05$).

p75 sTNFR in Serum and SF

No significant difference in serum p75 sTNFR was found between patients with RA (4041.2 ±1026 pg/ml) and OA (3149.1 ±1603.1 pg/ml, Table 2). On the contrary, the serum sTNFR levels in RA and OA patients were significantly higher than in the controls (2250 ± 988.5 pg/ml, $p < 0.005$ and $p < 0.01$, respectively). The p75 sTNFR level in SF was significantly higher in RA patients (6814 ± 2885.7 pg/ml) than in OA patients (5382.5 ± 2701.5 pg/ml, $p < 0.05$).

Correlation Between Cytokine and Disease Activity

The correlation between TNF-α, p55 and p75 sTNFR levels and laboratory variables as well as with clinical variables were determined. Serum and SF levels of TNF-α, p55 and p75 sTNFR were not significantly correlated with the clinical disease activity and laboratory parameters (data not shown).

DISCUSSION

It has been well established that some cytokines play key roles in inflammatory processes, and particular interest has been focused on elucidating the pathways regulated by these mediators and their corresponding antagonists and endogenous binding proteins [23,24]. Measuring cytokines and their soluble receptors in biological fluids may not only be helpful in investigating pathogenic mechanisms but may also have a potential value for evaluation of disease activity and prognosis.

TNF-α has been demonstrated to inhibit the proteoglycan synthesis in cartilage and increase bone erosion. In this study, TNF-α was found in the SF of 86% of RA patients and 53% of OA patients. In addition, levels of TNF-α were significantly higher in SF of RA group when compared with OA group. On the other hand, TNF-α was detectable the sera of 20% of the RA patients, but none of the OA patients contained TNF-α. Our results are lower than those obtained by Saxne et al. [25] and Lee et al. [19], they have demonstrated that 50% to 79% RA patients had detectable TNF-α levels in sera. The reason for the discrepancy is uncertain, possible explanations include differences in patient groups or methods of assay.

In our study, the serum concentrations of p75 sTNFR in RA and OA patients were higher than those in control subjects. The serum levels of p55 sTNFR in patients with RA were higher than those in the control groups. However, the SF concentrations of p55 and p75 sTNFR in RA patients were significantly higher than those in OA patients. These results were in agreement as reported by Steiner et al. [16] and Cope et al. [17]. This suggests that increased levels of sTNFR generally occur in inflamed joints (in RA, and even in OA patients), which may be due to increased shedding of the receptor from the cell surface in the joints. It is not clear which factors (cytokines), apart from TNF-α itself, regulate the release of TNF-α receptor [26].

As the report of Roux-Lombard et al. [18], our results did not show any correlation

between disease activity and the levels of p55 and p75 sTNFR or TNF-α in RA patients. This lack of correlation with established disease activity variables may indicate that, rather than disease activity, sTNFR may more likely reflect the disease process of RA.

In summary, we observed higher levels of TNF-α and sTNFR in SF, but not in serum, in patients with RA than OA. This may suggest that TNF-α and sTNFR are up-regulated in RA patients and produced locally in the joints. Moreover, serum and SF levels of TNF-α and sTNFR do not seem to be related to disease activity.

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類風濕性關節炎和退化性關節炎病患之血清和關節液腫瘤壞死因子及接受體之偵測

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腫瘤壞死因子可誘發兩種游離性接受體（p55和p75）之產生，其在發炎性風濕病人身上濃度會有上升情形。本研究目的在於探討類風濕性關節炎、退化性關節炎及正常人其腫瘤壞死因子及接受體之差異及其臨床應用。我們使用酵素免疫吸收法測量類風濕性關節炎及退化性關節炎病人血清及關節液和正常人的血清之腫瘤壞死因子及其接受體p55、p75的濃度。結果顯示，血清之腫瘤壞死因子在類風濕性關節炎有20%患者可測到，然而退化性關節炎和正常人卻都偵測不出來。關節液之腫瘤壞死因子在類風濕性關節炎患者明顯高於退化性關節炎患者。p55腫瘤壞死因子接受體之血清濃度在類風濕性關節炎患者高於正常人，然而在退化性關節炎患者和正常人在統計上則無明顯差異。類風濕性關節炎患者關節液中之p55卻較退化性關節炎患者來得高。而血清p75腫瘤壞死因子接受體在類風濕性關節炎和退化性關節炎病人皆比正常人高。在關節液方面，類風濕性關節炎則較退化性關節炎有明顯增高情形。另外，我們發現包括腫瘤壞死因子、p55、p75和疾病活動度，ESR及CRP數值相比皆沒有明顯之相關性存在。本研究顯示，腫瘤壞死因子和腫瘤壞死因子接受體在類風濕性關節炎患者扮演一調節角色，尤其是在關節內。另外，腫瘤壞死因子和腫瘤壞死因子接受體似乎和疾病活動度並沒有相關性存在。（中台灣醫誌　1999;4:109-15）

關鍵詞

疾病活動度，類風濕性關節炎，可溶性腫瘤壞死因子接受體，甲型腫瘤壞死因子

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