

Tumour necrosis factor in serum and synovial fluid of patients with active and severe rheumatoid arthritis

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Abstract

Fifteen serum samples and 29 synovial fluids of patients with rheumatoid arthritis (RA) were examined for the presence of tumour necrosis factor (TNF). The assay for TNF was based on the cytotoxic activity of this cytokine for human melanoma cells in tissue culture. High concentrations of TNF were found in serum samples of patients with severe RA, who had increased erythrocyte sedimentation rate and serum α_2 macroglobulin, but decreased haemoglobin and serum iron concentrations. Tumour necrosis factor was also found in the synovial fluid of 16 out of 29 patients. High TNF concentrations were found in fluids with $>10^{10}$ leucocytes/l. Tumour necrosis factor was not detected in the serum of normal subjects or in synovial fluid of patients with osteoarthritis. A mediator of inflammation, such as TNF, may contribute to the severity of RA.

Rheumatoid arthritis (RA) is a chronic and systemic inflammatory disease of the joints. This inflammatory arthritis of unknown cause is characterised by a chronic synovitis, resulting in cartilage erosion, bone injury, and fibrous ankylosis of the joints, which are often associated with systemic manifestations.¹ The severity and progression of this synovitis depend on the local accumulation and activation of lymphomononuclear cells that release lymphokines and cytokines.² Such primary mediators of inflammation modulate the migration and proliferation of chondrocytes and fibroblastic cells that synthesise matrix components.² Furthermore, the local production of lymphokines and cytokines by activated lymphocytes and macrophages may regulate growth, differentiation, and activity of other cells participating in inflammatory and immunological reactions of the joints.

Wood *et al* reported the isolation of an interleukin-1-like factor from human joint effusions,³ and Miossec *et al* recently identified a chemotactic activity in RA synovial fluid with interleukin-1.⁴ While our investigation was in progress Saxne *et al* reported the detection of tumour necrosis factor α (TNF), but not lymphotoxin, in the synovial fluid of six out of 12 patients with RA and in seven of 12 serum samples of these patients.⁵ Furthermore, his patients with detectable TNF had a higher erythrocyte sedimentation rate and synovial fluid leucocyte count than those with undetectable TNF.⁵ These findings suggest that TNF is a mediator of inflammation affecting RA. In fact, TNF is produced by activated monocytes/

macrophages⁶ and can itself induce the synthesis of interleukin-1,⁷ prostaglandin E_2 ,⁸ and platelet activating factor.⁹ These secondary mediators can amplify the inflammatory reaction.

Our investigation was aimed at establishing whether TNF is present locally in the synovial fluid and systemically in the serum of patients with RA. Tumour necrosis factor was readily detected in both synovial fluid and serum of patients with RA. The severity of this disease correlated with the concentration of TNF present.

Patients and methods

PATIENTS

Thirty nine patients with RA (three men, 36 women; mean age (SD) 63 (11) years) were studied. These patients fulfilled the New York criteria for the diagnosis of RA.¹⁰ Serum TNF concentrations were determined in 15 patients with RA of different severity, as indicated by clinical and laboratory data (erythrocyte sedimentation rate, haemoglobin, serum iron, and α_2 macroglobulin concentration). Functional and radiological assessment was carried out according to Steinbroker.¹¹ Fifteen healthy subjects were taken as controls. Tumour necrosis factor concentration and leucocytes were measured in the synovial effusion of 29 patients with RA and five patients with osteoarthritis (three men, two women; mean age 58 (11) years).

TNF ASSAY

Tumour necrosis factor was measured with a sensitive biological assay, based on the cytotoxic activity of this cytokine in the presence of an inhibitor of protein synthesis.¹² Fresh serum or synovial fluid samples were added together with 0.1 mg/ml of cycloheximide to cultures of human SK-MEL-109 melanoma cells that are sensitive to the cytotoxic activity of TNF concentrations as low as 20 pg/ml.¹³ These cells were grown as monolayers in 24-well cluster plates, incubated with appropriate dilutions of serum or synovial fluid samples, and after 18 hours washed with phosphate buffered saline before staining with crystal violet, which was eluted and measured as described.¹³ A calibration curve was constructed with human recombinant TNF to convert the cytotoxic activity of biological samples into ng/ml of TNF. Furthermore, these samples were assayed on a TNF resistant cell line (designated R4) selected from SK-MEL-109 cells, as recently described for HeLa cells.¹⁴ The lack of cytotoxicity for TNF

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resistant R4 cells in such control experiments showed that the cell death observed with sensitive SK-MEL-109 cells was specifically caused by TNF. The cytotoxicity assays were carried out in triplicate and gave a standard error of <5%.

Results

The presence of TNF in serum was examined in 15 patients with RA subdivided into three groups on the basis of the severity of their disease: (1) patients with moderately active RA; (2) with active RA; and (3) with severe RA. The table shows the clinical and laboratory data for these patients. The severity of the RA correlated with an increased erythrocyte sedimentation rate and serum α_2 macroglobulin, and decreased haemoglobin and serum iron concentration. Detectable amounts of TNF were present in the serum of most of these patients (9/15), but high concentrations of TNF were only found in serum samples of patients with severe RA (group 3; fig 1A).

The statistical significance of differences in serum TNF concentration among the three groups of patients was evaluated by unpaired *t* test analysis, with $2p < 0.1$ considered significant. The TNF concentration was significantly increased in patients of group 3 compared with patients in the other groups and with healthy control subjects. No significant difference ($2p > 0.1$) was found between patients of groups 1 and 2, or between these patients and healthy subjects. These data show that the high concentrations of TNF found in the serum of patients with severe RA are significantly different from those found in other patients with RA, despite the small number of samples examined. On the

other hand, TNF concentrations found in patients of group 2 may lack statistical significance because only a few serum samples were examined. The high serum TNF concentrations correlated with an increased erythrocyte sedimentation rate (fig 2A). The statistical significance of these data was examined by correlation analysis, which gave $r = 0.55$.

The presence of TNF in the synovial fluid of patients with RA was also examined (fig 1B). Significant concentrations of TNF (> 0.3 ng/ml) were found in 16 of 29 patients. The patients were arbitrarily subdivided into two groups on the basis of the leucocyte count in the synovial fluid: $> 10^{10}$ or $< 10^{10}$ leucocytes/l. There were more patients with increased TNF in the first group (9/12) than in the second group (7/17). The mean TNF concentration was also significantly higher in the first group than the second (fig 1B). Statistical analysis of the TNF concentration *v* leucocyte count (fig 2B) showed a marginally significant correlation ($r = 0.38$). The patients with osteoarthritis had low TNF concentrations and leucocyte count (fig 2B). These data show that very high concentrations of TNF are commonly present in the synovial fluid of patients with RA with a high leucocyte count, whereas moderate to low concentrations are detected in patients with a low count.

Figure 1 Tumour necrosis factor (TNF) concentration in serum (A) and synovial fluid (B) of three groups of patients with RA and controls (C). Average TNF concentrations shown by bars. The difference between the mean TNF concentration in synovial fluid for patients with low and high leucocyte count was highly significant ($2p < 0.05$) (fig B).

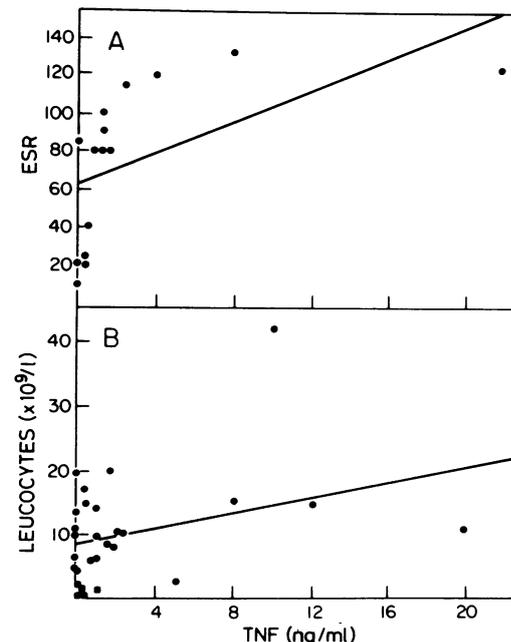
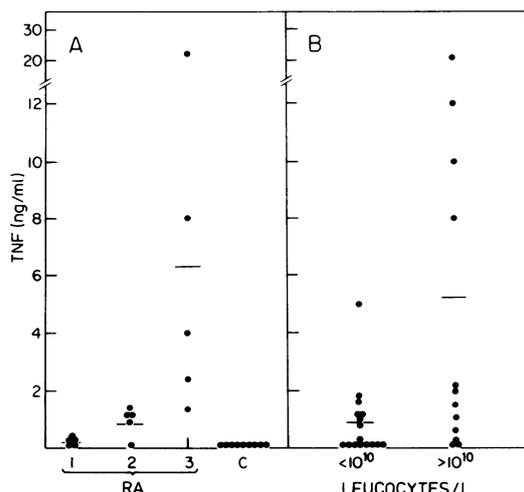


Figure 2 Correlation between serum tumour necrosis factor (TNF) concentrations and erythrocyte sedimentation rate (ESR) (A) or between TNF concentration in synovial fluid and leucocyte count (B). ● = patients with rheumatoid arthritis; ■ = patients with osteoarthritis.

Clinical and laboratory data of 15 patients with RA. Results show mean (SEM)

RA classification		Stages†		ESR‡ (mm/h)	Hb‡ (g/l)	α_2 M‡ (mg/l)	Serum iron (μmol/l)	Treatment (daily)
Group*	Activity	Functional	Radiological					
1	Moderate	2-4	2-4	23 (11)	137 (25)	137 (38)	15.6 (7.0)	NSAID‡
2	Active	2-3	1-3	67 (4)	103 (18)	131 (7)	5.7 (0.7)	NSAID+5 mg prednisone
3	Severe	3-4	3-4	118 (11)	97 (7)	162 (47)	7.6 (2.1)	NSAID+10 mg prednisone

*Five patients from each group were studied.

†Determined according to Steinbroker *et al.*¹¹

‡ESR=erythrocyte sedimentation rate; Hb=haemoglobin; α_2 M= α_2 macroglobulin; NSAID=non-steroidal anti-inflammatory drug.

Discussion

Tumour necrosis factor was initially detected in the serum of animals treated with endotoxin and was identified as a mediator of the necrosis of some transplantable tumours.¹⁴ It is cytostatic or cytotoxic for some tumour cells,¹⁵ but can stimulate fibroblast proliferation^{16,17} and shows a wide range of biological activities. It is pyrogenic,¹⁸ affects lipid metabolism,¹⁸ and modulates several functions of the vascular endothelium.^{19,20} Furthermore, recent evidence suggests that TNF is one of the primary mediators of the inflammatory reaction as it is chemotactic for monocytes and polymorphonuclear leucocytes,^{21,22} and stimulates phagocytosis²³ and superoxide anion generation by neutrophils.²⁴

We have recently shown that TNF induces synthesis and release of platelet activating factor from macrophages, polymorphonuclear neutrophils, and cultured human endothelial cells.⁹ Platelet activating factor and other autacoids may mediate the induction by TNF of inflammatory reactions. Therefore, production of TNF may be at least in part responsible for several acute and chronic inflammatory processes, including RA. The present findings that high concentrations of TNF are present in the serum and synovial fluid of patients with severe RA support the role proposed for this cytokine. These observations are in substantial agreement with those recently published by Saxne *et al.*⁵

In our investigation the highest concentrations of TNF were detected in patients with severe RA, high leucocyte count in the synovial fluid, and a disease state resulting in high ESR and α_2 macroglobulin, but low haemoglobin and serum iron. These data are indicative of an extensive and systemic disease. High concentrations of TNF were present in some of these patients despite daily administration of 10 mg of prednisone (table). Anti-inflammatory corticosteroids, such as dexamethasone, were shown to prevent the production of TNF elicited by endotoxin in murine macrophages, but did not abolish TNF secretion when added after endotoxin.²⁵ Therefore, the fairly low dose of prednisone given to our patients in this investigation may be ineffective in lowering the continuing production of TNF. Higher doses of glucocorticoids would be more effective, but the side effects of these anti-inflammatory steroids may outweigh the benefit of reducing the production of TNF and other mediators of inflammation, such as interleukin-1. The presence of high concentrations of serum TNF may indicate that the dose of glucocorticoids should be increased in an attempt to reduce the systemic effects of these cytokines.

Treatment of bone cultures with TNF stimulates osteoclast proliferation and decreases the amount of mineralised bone matrix.²⁶ Therefore, like interleukin-1,²⁷ TNF is an osteoclast activating factor and stimulates synovial cells to release prostaglandin E₂ and collagenase.⁸ These observations suggest that TNF mediates the recruitment of inflammatory cells and the vasoactive phenomena characteristic of inflammatory synovitis, but at the same time promotes the cartilage and bone erosion that eventually leads to pathological alterations of the joints characteristic of RA. Therefore, it may be quite important in the management of this disease to reduce production of TNF and interleukin-1.

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