

In vivo Pro- and Anti-inflammatory Cytokines in Normal and Patients with Rheumatoid Arthritis

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Abstract

Introduction: Rheumatoid arthritis (RA) is a chronic, deforming arthritis that can lead to disabilities and poor quality of life. Cytokines are protein mediators of inflammation and are produced as a result of the activation of various cellular reactions. They are the final mediators and/or regulators of the inflammatory process. **Materials and Methods:** The sera from 64 RA patients were assayed for both Th-1 and Th-2 related cytokines and soluble TNF- α receptors (IFN- γ , TGF- β , TNF- α , IL-1 β , IL-2, IL-4, IL-6, IL-8, IL-10, IL-12, IL-18, sTNF-R1 and sTNF-R2) using ELISA. **Results:** The pro-inflammatory cytokines (IL-1, IL-6, IL-8, IL-18 and TNF- α) were significantly elevated in RA patients, while TGF- β , an immunomodulatory cytokine, was elevated in control individuals. When the RA patients were categorised as active or inactive based on DAS scores, similar cytokines profiles were observed in both RA sub-groups. However, assays of sTNF-R1 and sTNFR-2 were noted to be significantly elevated in inactive RA patients when compared to active patients. **Conclusion:** Our findings indicate that local production of cytokine inhibitors is capable of diminishing disease activity and cytokine activity.

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Key words: Cytokines, Inflammation, Rheumatoid arthritis soluble receptors

Introduction

Rheumatoid arthritis (RA) is a chronic autoimmune disease characterised by severe joint deformities due to bony erosions and tendon damage. The chronic inflammatory process is mediated through a complex cytokine network. The clinical expressions and outcome of the disease can vary among different ethnic groups, possibly depending upon the differential expressions of MHC and cytokine genes. Cytokines are protein messengers that convey information between and within cells via specific cell surface receptor molecules. The release of specific cytokines into the systemic circulation has been observed in a variety of inflammatory disease including RA. Their concentration levels usually reflect disease severity and prognosis. However, since most cytokines are expressed transiently and can be induced or inhibited by other cytokines, it has been suggested that a "cytokine network" may exist in which cytokines regulate each other.^{1,2}

Cytokines are also divided into pro-inflammatory cytokines (TNF- α , IFN- γ , IL-1, IL-2, IL-6, IL-8, IL-12 and IL-18) and anti-inflammatory cytokines (IL-4, IL-10, TGF- β). In RA, the balance between pro-inflammatory and anti-inflammatory cytokines determines the degree and extent of inflammation, and thus can lead to different clinical effects. Anti-inflammatory cytokines or cytokine antagonists counteract the effects of pro-inflammatory cytokines and therefore the relative concentration of a cytokine to its inhibitor or antagonist will determine its final effect.

Soluble TNF- α , IL-1 receptors and the IL-1 receptor antagonist specifically inhibit the functions of TNF- α and IL-1 respectively.³ The soluble p55 (TNF-R1) and p75 (TNF-R2) TNF receptors are elevated in RA synovial fluid and are spontaneously released in rheumatoid synovial membrane cultures.^{4,5} Both TNF receptors are subject to proteolytic cleavage by members of the matrix metalloprotease family and are shed from the surface of the

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cells in response to inflammatory signals such as TNF- α ligand-receptor binding. The shed extracellular domains of both receptors retain their ability to bind TNF- α and therefore act as natural inhibitors of TNF- α bioactivity.^{6,7} Pro-inflammatory cytokines like TNF- α and IL-1 β have been shown to be elevated in both synovial fluid as well as in the blood.

In recent years TGF- β has been shown to play a part in rheumatic conditions like RA and systemic lupus erythematosus (SLE). TGF- β , which was initially discovered as a growth factor, has emerged as a pivotal immunoregulatory cytokine.⁸ It is generally considered to have an inhibitory effect on the inflammatory processes. Hence, the upregulation of TGF- β can lead to a dampening of the inflammatory processes. In *in vivo* studies, fibroblast-like synoviocytes from rheumatoid synovium, when pre-incubated with TGF- β , demonstrated downregulation of IFN- γ -induced DR protein and DRB mRNA expression. TGF- β levels in the blood have also been noted to be low when compared to controls in a small group of RA patients with active disease. Hence, determination of cytokines profiles provides useful data on the roles the different cytokines play in the rheumatoid disease process. This study aims to demonstrate the cytokine profiles in RA patients with active and inactive joint disease in a cohort of Chinese RA patients.

Materials and Methods

Recruitment of Subjects and Collection of Samples

Patients of Chinese origin, satisfying the American College of Rheumatology 1987 revised criteria for classification of RA,⁹ were recruited for the study. In total, 64 RA patients were recruited. Exclusion criteria included (i) a haemoglobin level lower than 10 g/dL, (ii) pregnant, (iii) demented, (iv) refused consent for venepuncture, and (v) with concomitant autoimmune diseases, e.g. SLE, or Graves' disease. Forty healthy, unrelated individuals were recruited from among laboratory personnel, undergraduates and blood donors as controls for this study. Informed consent was obtained from both patients and controls before venepuncture. For patients, this was timed to coincide with their regular blood testing sessions. All subjects were informed that blood samples would be anonymised prior to genotyping. Clinical data of patients were obtained by chart review prior to anonymisation. RA disease activity was assessed using the DAS28,¹⁰ a validated and widely used measure of disease activity.

Determination of Cytokines and Cytokine Receptors Concentration

The serum obtained from RA patients and healthy controls were analysed for IFN- γ , TGF- β , TNF- α , IL-1 β , IL-2, IL-4, IL-6, IL-8, IL-10, IL-12, IL-18, sTNF-R1 and sTNF-R2

using commercially available ELISA kits following the manufacturers' instructions (MBL and R&D systems). All samples were assayed in duplicate and equivocal results were repeated. Cytokine concentration was calculated from a standard curve of the corresponding recombinant human cytokine.

Statistics

The results are expressed as mean $\pm$ SD. Paired and unpaired Student's *t*-tests were used to determine the significance of differences; a value of *P* < 0.05 was considered statistically significant.

Results

Sixty-four Chinese RA patients (55 females and 9 males) were recruited for the study. Their mean age was 60 years (range, 40 to 78), with a mean duration of RA exceeding 10 years. All patients were being treated with a disease-modifying anti-rheumatic drug, methotrexate, at the time of the study. At recruitment, 33 patients were assessed as having active joint disease and 31 patients were in remission according to the DAS28 scoring. The healthy controls had a mean age of 38 years (range, 20 to 52).

Cytokine Profiles

The serum cytokines profiles were analysed both in RA patients and control individuals. The serum levels of pro-inflammatory cytokines such as IL-1 β , IL-6, IL-8, IL-18 and TNF- α were significantly (*P* < 0.05) elevated (Figs. 1 and 2) in RA patients (mean [pg/mL]: IL-1 β = 1.09, IL-6 = 13.7, IL-8 = 297, IL-18 = 385 and TNF- α = 39.67) compared to controls (mean [pg/mL]: IL-1 β = 0.25, IL-6 = 2.10, IL-8 = 112, IL-18 = 124 and TNF- α = 9.18). Conversely, the mean concentration of TGF- β , an immunomodulatory cytokine, was significantly increased in control individuals. We did not observe any difference in the serum levels of anti-inflammatory cytokines such as IL-4 and IL-10 between patients and controls. Elevated serum levels of soluble forms of TNF receptors (sTNF-R1 and sTNF-R2) were also observed in patients (sTNF-R1 = 1656 pg/mL and sTNF-R2 = 5622 pg/mL) when compared to controls (sTNF-R1 = 754 pg/mL and sTNF-R2 = 1430 pg/mL) (Fig. 3). When the RA patients were categorised according to active or inactive status, similar cytokines profiles were observed in both RA sub-groups. However, assays of sTNF-R1 and sTNF-R2 were noted to be significantly elevated in inactive RA (sTNF-R1 = 1870 pg/mL and sTNF-R2 = 7003 pg/mL) patients when compared to active patients (sTNF-R1 = 1492 pg/mL and sTNF-R2 = 4044 pg/mL) (Fig. 4).

Discussion

In the present study, high serum concentrations of TNF-

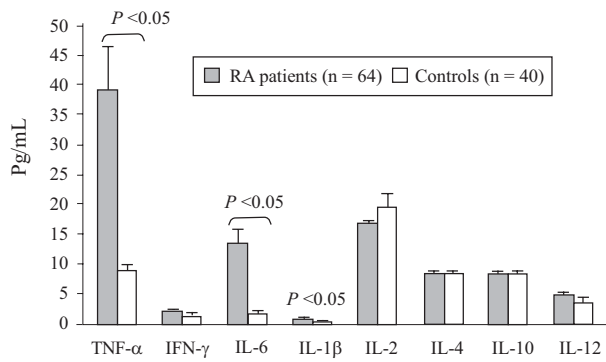


Fig 1. Serum cytokine profiles in controls and RA patients. Mean concentrations of cytokines in serum from RA patients (n = 64) and healthy controls (n = 40) were quantified by ELISA. Values are expressed as mean $\pm$ SEM and $P < 0.05$ is statistically significant.

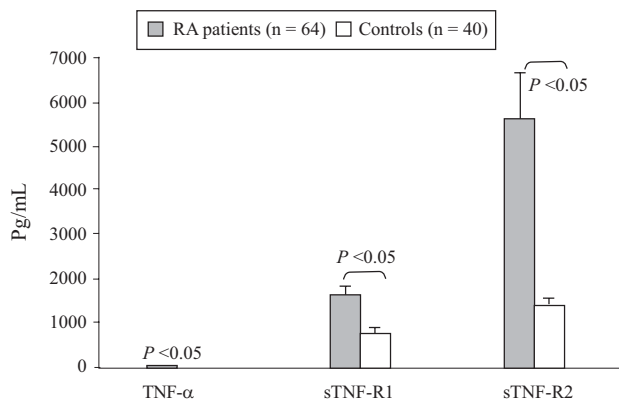


Fig 3. Serum cytokine profiles of TNF- α , sTNF-R1 and sTNF-R2. Mean concentrations of TNF- α , sTNF-R1 and sTNF-R2 from RA patients (n = 64) and healthy controls (n = 40) were quantified by ELISA. Values are expressed as mean $\pm$ SEM and $P < 0.05$ is statistically significant.

α , IL-1 β , IL-6 and IL-8 were detected in patients with RA. In contrast, low levels of IL-4 and IL-10 (anti-inflammatory cytokines) were detected. Great variation was seen in the patterns of cytokine concentration between different RA patients, although, interestingly, we observed that not all cytokines were elevated in the same samples. This variation among different cytokines in the same sample reflects the intricate cytokine network and its regulatory functions. The balance between pro-inflammatory and anti-inflammatory cytokines in RA determines the degree and extent of inflammation which can lead to major clinical effects. In line with our findings, high concentrations of TNF- α , IL-1 β , IL-6 and IL-8 have also been reported by others.¹¹⁻¹³ In addition, interleukin-18 (IL-18) is also reported to play an important role during the chronic inflammatory phase of RA.¹⁴ It is predominantly secreted by activated monocytes/macrophages¹⁵⁻¹⁷ and is a pleiotrophic cytokine which acts synergistically with IL-12 to promote Th-1 activation. We also observed levels of IL-18 to be significantly elevated in the serum of RA patients compared

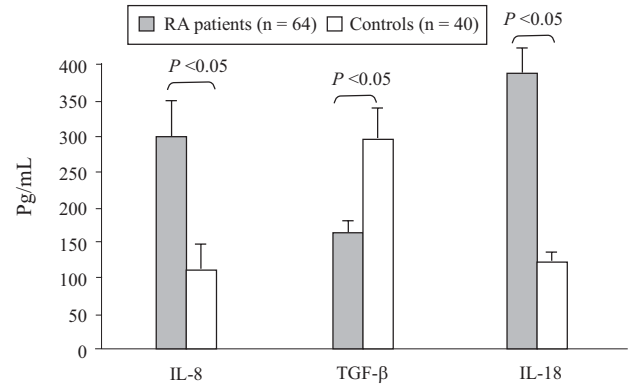


Fig 2. Serum cytokine levels of IL-8, TGF- β and IL-18 in controls and RA patients. Mean concentration of IL-8, TGF- β and IL-18 from RA patients (n = 64) and healthy control (n = 40) were quantified by ELISA. Values are expressed as mean $\pm$ SEM and $P < 0.05$ is statistically significant.

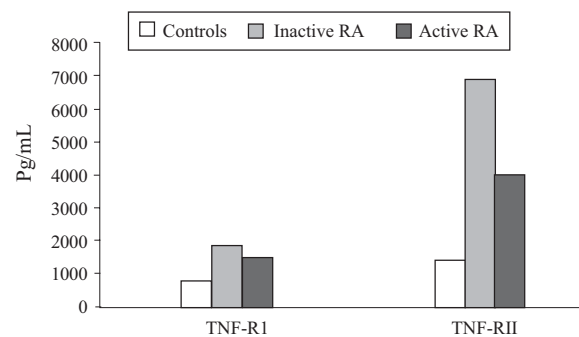


Fig 4. Soluble levels of sTNF-R1 and sTNF-R2 in controls and RA patients (active and inactive). Mean serum concentrations of sTNF-R1 and sTNF-R2 in active and inactive RA patients compared with controls. The mean concentrations of sTNF-R1 and sTNF-R2 were increased in inactive RA patients than the active. Data represent mean $\pm$ SEM. A $P < 0.05$ is statistically significant.

to controls. TNF- α is an autocrine stimulator as well as a potent paracrine inducer of other inflammatory cytokines, including IL-1, IL-6, IL-8, and granulocyte-monocyte colony-stimulating factor.¹⁸⁻²¹ Interestingly, the serum levels of TGF- β were found to be decreased in patients compared to controls. TGF- β is a potent regulator of pro-inflammatory cytokines²² and exerts its anti-inflammatory effect by inhibiting the synthesis of pro-inflammatory cytokines such as IL-1 α , IL-1 β , and TNF- α . The action of TGF- β on cells is dependent on the cell type, its state of differentiation and the total milieu of cytokines present. Early studies of the effects of TGF- β on human lymphocyte functions revealed that activated T cells can self-synthesise TGF- β upon re-stimulation. Exogenous TGF- β favours Th2 differentiation. Conditions favouring Th2 differentiation were also found to induce the subsequent production of high levels of TGF- β .

We also observed increased levels of soluble TNF receptors, both sTNF-R1 and sTNF-R2, in RA patients when compared to controls. Similar to published findings,

we found that the concentration of sTNF-R1 was lower than sTNF-R2^{2,4} in RA patients. Soluble receptors have a physiologic role in neutralising many cytokines, as exemplified by soluble TNF receptors. Soluble forms of both sTNF-R1 and sTNF-R2 are part of a feedback loop that can modulate the inflammatory action of TNF- α . The transmembrane domain of both TNF receptors is susceptible to lysis by proteases, including TNF- α -converting enzyme,⁶ leading to the release of the soluble form of the receptor. Hence, both types of receptors are present in body fluids. Soluble TNF receptors are found in high concentrations in the synovial fluids and sera of patients with RA.⁴ However, excessive TNF production can overwhelm the sTNF-R present in the blood, resulting in prolonged inflammation. The increased concentrations of TNF inhibitors in RA are of interest,²³ as they exclude the possibility that a major contribution to the pathogenesis of RA is the failure to produce inhibitory factors normally. We also found that inactive RA patients have higher levels of TNF inhibitors when compared to those with active disease activity. These results indicate that local production of cytokine inhibitors is capable of diminishing disease activity and cytokine activity. In view of the above findings, we suggest that TGF- β and TNFR could be exploited as potential candidates to inhibit cytokine activity and hence the disease process in RA.

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