# Tumor Necrosis Factor Blockade Differentially Affects Innate Inflammatory and Th17 Cytokines in Rheumatoid Arthritis

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***ABSTRACT.*** ***Objective.*** To evaluate the effect of a tumor necrosis factor-α (TNF-α) inhibitor (etanercept) on innate inflammatory and Th17 cytokines in patients with rheumatoid arthritis (RA).

***Methods.*** Serum samples were collected from 40 patients with active RA refractory to conventional disease-modifying antirheumatic drugs who initiated therapy with etanercept plus methotrexate (MTX). Treatment response was assessed at Week 24 according to the European League Against Rheumatism response criteria. Serum levels of interleukin 6 (IL-6), TNF-α, IL-32, IL-23, IL-17A, IL-21, and IL-22 were measured in patients with RA and 25 healthy controls.

***Results.*** Patients with RA had increased levels of IL-6 ($p < 0.001$), IL-32 ($p < 0.001$), IL-23 ($p < 0.001$), and a trend toward increased IL-21 in the sera compared to controls. At 24 weeks' posttreatment, followup serum samples of etanercept responders had decreased levels of IL-6 ($p < 0.001$) and increased IL-21 ($p < 0.05$) and IL-32 ($p < 0.001$), while there were no differences in cytokine levels in nonresponders. Serum IL-6 levels were positively correlated with levels of erythrocyte sedimentation rate ($r = 0.458$, $p < 0.01$), C-reactive protein ($r = 0.593$, $p < 0.01$), and 28-joint Disease Activity Score ($r = 0.432$, $p < 0.01$) at baseline. Serum IL-21 levels were positively correlated with levels of rheumatoid factor ($r = 0.513$, $r = 0.633$, both $p < 0.01$) and antimutated citrullinated vimentin antibodies ($r = 0.515$, $p < 0.01$; $r = 0.428$, $p < 0.05$) at baseline and after 24 weeks of treatment with etanercept.

***Conclusion.*** Multiple inflammatory pathways contribute to persistent chronic inflammation in RA. In contrast to nonresponders, etanercept therapy modulated serum cytokine levels and caused a marked decrease of IL-6 levels in responders. IL-21 might be involved in the regulation of autoantibody production in RA. (J Rheumatol First Release Dec 1 2011; doi:10.3899/jrheum.110697)

*Key Indexing Terms:*
RHEUMATOID ARTHRITIS
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Th17 CYTOKINES

The inflamed synovial tissue in rheumatoid arthritis (RA) is characterized by a complex interplay between multiple immune cells and mediators[1]. The important roles of innate inflammatory cytokines such as tumor necrosis factor-α (TNF-α), interleukin 6 (IL-6), and IL-1 are well documented[2], and an autoinflammatory loop between TNF-α and IL-32 can contribute to chronic joint inflammation in RA[3]. IL-17 and Th17-associated cytokines could be crucial in the pathogenesis of RA, as IL-17 synergizes with TNF-α in the induction of proinflammatory cytokines and destruction of cartilage and bone[4]. Th17 cells predominantly produce IL-17, IL-21, and IL-22, while IL-23 is necessary for their survival and maintenance[5]. Therapy with etanercept, a TNF-α receptor antagonist, has led to successful control of RA[6], but the reasons for inadequate response in a proportion of patients are not clear. Circulating cytokines may reflect disease activity and response to treatment with TNF-α inhibitors in RA[7].

Our aim was to evaluate the pattern of innate inflammatory and Th17-associated cytokines in serum of patients with established active RA and to study the influence of anti-TNF therapy on levels of these cytokines in relation to clinical response.

## MATERIALS AND METHODS

*Patients.* Forty patients with RA fulfilling the 1987 American College of Rheumatology (American Rheumatism Association) 1987 revised criteria for

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the classification of RA[8] (37 women, 3 men, mean age 48 yrs, range 22–70 yrs), with a mean duration of disease of 7 years (range 2–20 yrs), with an unsatisfactory response to conventional disease-modifying antirheumatic drugs including methotrexate (MTX) and presenting with active RA were involved in our study. Patients received subcutaneous etanercept 50 mg/week with MTX 10–25 mg/week and were permitted to continue taking low-dose oral corticosteroids (prednisone < 10 mg/day) and/or nonsteroidal antiinflammatory drugs at a stable dose. The control group consisted of 25 sex- and age-matched healthy individuals. The criterion of clinical response was a decrease of the 28-joint Disease Activity Score (DAS28) index by > 1.2 points with reference to baseline according to the European League Against Rheumatism (EULAR) response criteria[9].

Our study was approved by the ethics committee at the Institute of Rheumatology, School of Medicine, Belgrade, and was conducted according to The Helsinki Declaration. All patients gave written informed consent to participate.

*Clinical and laboratory assessments*. Patients underwent clinical and laboratory assessments just before and after 24 weeks of treatment with etanercept, including DAS28, Health Assessment Questionnaire-Damage Index (HAQ-DI), patient's global assessment of disease (by visual analog scale; VAS), serum C-reactive protein (CRP) levels, erythrocyte sedimentation rate (ESR), rheumatoid factor (RF) titers; and antimutated citrullinated vimentin (anti-MCV) antibodies (Organon, Oberschleissheim, Germany) were determined.

*Serum samples*. Serum samples were obtained at baseline and at 24 weeks posttreatment followup and stored at –80°C until assayed.

*Measurement of innate inflammatory and Th17 cytokines*. Serum levels of IL-6, IL-32, TNF-α, IL-17A, IL-21 (Biolegend Inc., San Diego, CA, USA), IL-23, IL-22 (Bender MedSystems, Vienna, Austria) were measured using commercial ELISA kits.

*Statistical analysis*. Statistical analysis was performed using the SSPS 12.0 program. Data were compared with the Mann-Whitney U test or Wilcoxon matched-pairs signed-rank test. Correlations were assessed by Spearman's method. A p value < 0.05 was considered significant and p < 0.01 as highly significant.

## RESULTS

*Circulating cytokine profiles in patients with RA*. Patients with RA had increased levels of IL-6, IL-32, IL-23, and a trend toward increased IL-21, and decreased levels of IL-17A and TNF-α in sera samples compared with healthy controls (Figure 1).

*Serum cytokine levels and disease activity before and after etanercept treatment*. There was a significant improvement of laboratory and clinical measures in patients with RA from baseline to 24 weeks after treatment with etanercept plus MTX, as follows: ESR (mm/h; p < 0.001), CRP (mg/l; p < 0.001), tender joint count (p < 0.001), DAS28 (p < 0.001), HAQ-DI (p < 0.001), patient's global assessment (VAS; p < 0.05), IgM RF (IU/ml; p < 0.01), and anti-MCV antibodies (U/ml; p < 0.01). A good/moderate EULAR response was acquired in 32 patients and 8 patients were nonresponders. In responders, serum IL-6 levels decreased significantly, while IL-32 and IL-21 levels increased at the 24-week posttreatment followup (Figure 2). No significant change in serum cytokine levels was found in nonresponders (Figure 2).

*Correlation of serum cytokine levels and RA disease activity markers*. Baseline serum IL-6 levels correlated positively with DAS28 (r = 0.432, p < 0.01) and levels of ESR (r = 0.458, p < 0.01) and CRP (r = 0.593, p < 0.01). Moreover, serum IL-21 levels correlated positively with levels of RF and anti-MCV antibodies both at baseline and at the 24-week posttreatment followup (Table 1).

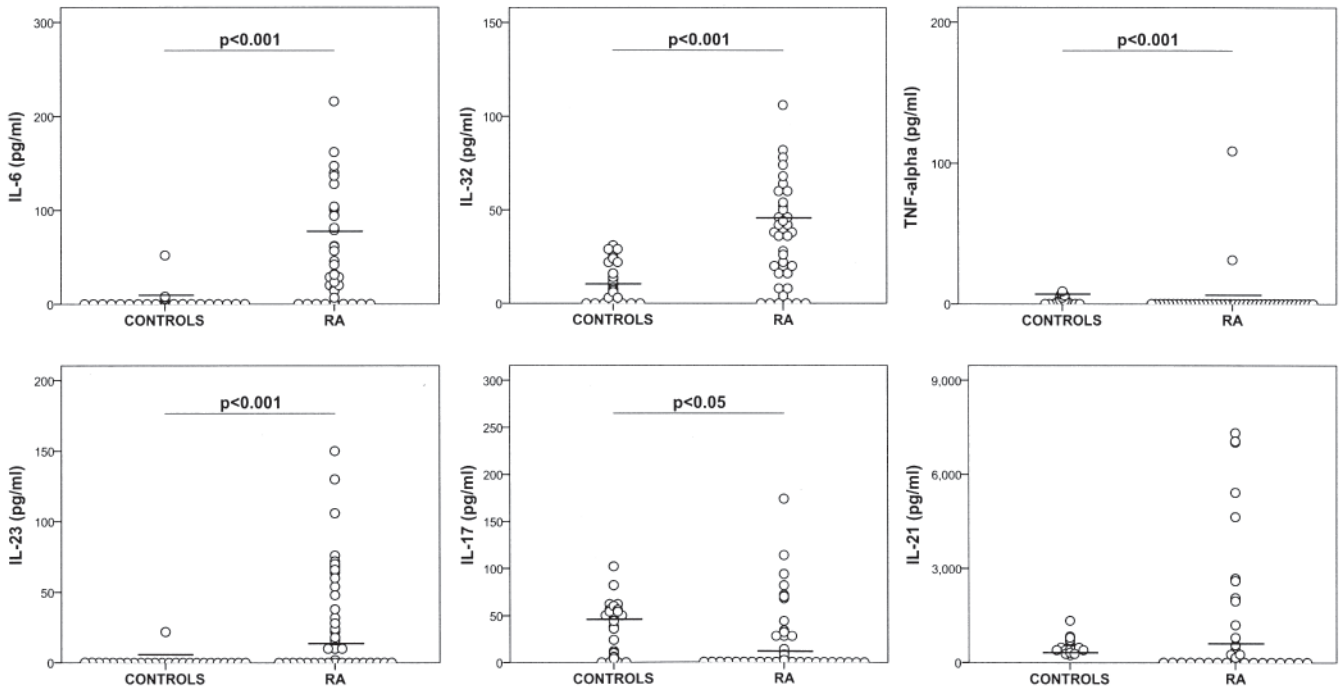


*Figure 1*. Baseline serum levels of innate inflammatory cytokines interleukin (IL)-6, IL-32, and tumor necrosis factor (TNF)-α, and Th17-associated cytokines IL-23, IL-17A, and IL-21 in healthy controls (n = 25) and patients with rheumatoid arthritis (RA; n = 40). Horizontal bars indicate the median.

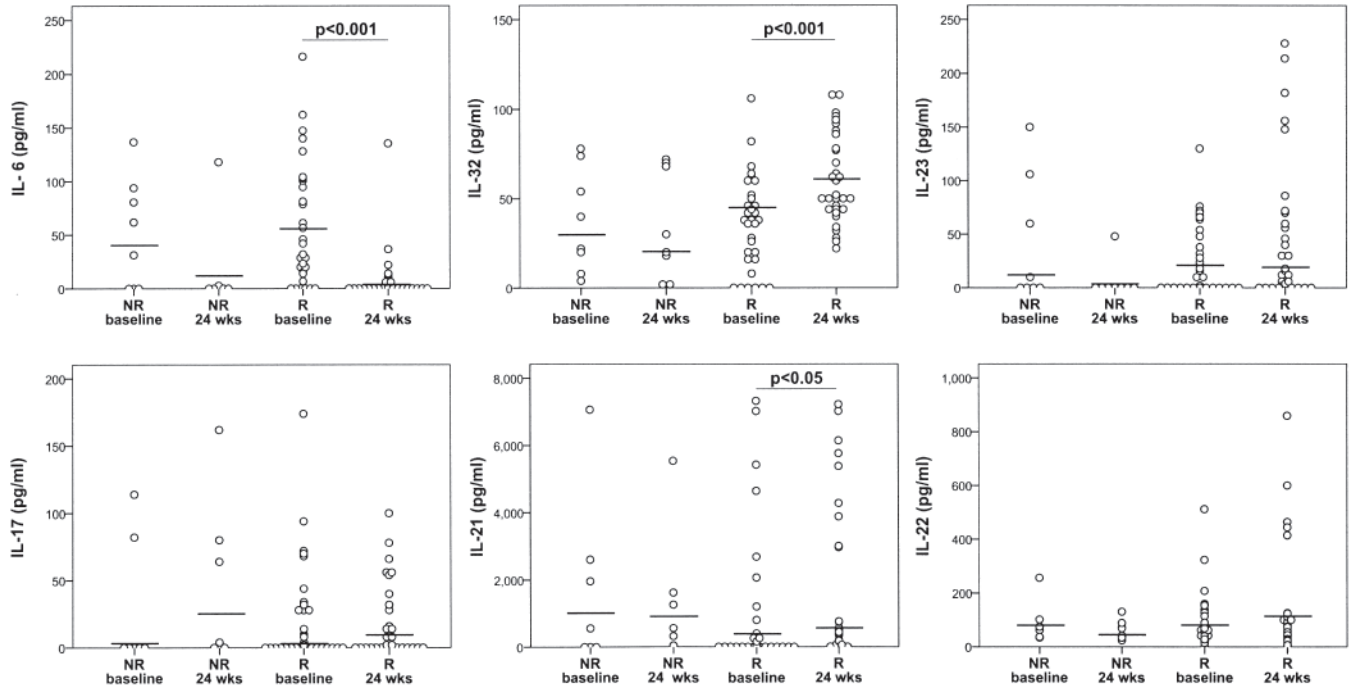


*Figure 2*. Serum levels of interleukin (IL)-6, IL-32, IL-23, IL-17A, IL-21, and IL-22 in patients with rheumatoid arthritis (n = 40) according to clinical response after 24 weeks of etanercept treatment. Horizontal bars indicate the median. NR: nonresponders; R: responders.

*Table 1*. Correlations ($r_s$) between serum IL-21 cytokine levels versus rheumatoid factor (RF) and antimutated citrullinated vimentin (anti-MCV) antibody levels in patients with rheumatoid arthritis, at baseline and at 24 weeks after treatment with etanercept.

| | Baseline RF | Anti-MCV | 24 Weeks RF | Anti-MCV |
|---|---|---|---|---|
| IL-21 | 0.513** | 0.515** | 0.633** | 0.428* |

Spearman's rank correlation; * $p < 0.05$; ** $p < 0.01$. IL: interleukin.

## DISCUSSION

We demonstrated that a heterogeneous pattern of systemic multiple proinflammatory cytokines exists in patients with chronic active RA. The cytokines that regulate TNF-α production, such as IL-32, or Th17 cell function, such as IL-23 and IL-6, were elevated in patients with RA. The findings of lower serum IL-17A and TNF-α levels in patients with RA compared to healthy controls are similar to those of another recent study[10]. In addition, Th17 cells that coexpress TNF are recruited to the inflamed synovial tissue of affected joints in RA[11]. In therapy responders, etanercept caused a marked decrease of serum IL-6 levels, which is at variance with the study showing decreased serum IL-23 levels and unchanged serum IL-6 levels in patients with RA receiving etanercept therapy[12]. We recorded a notable increase in serum levels of one of the Th17-related cytokines, IL-21, which might be in accord with results of the recent study that showed increased IL-17 production by peripheral blood Th17 cells after anti-TNF treatment[13]. In relation to increased IL-32 levels, the dissociated TNF from unstable complexes between TNF and etanercept[14] could possibly affect its production.

Our findings suggest that IL-6 could serve as a good marker of disease activity in RA. This is the first report to demonstrate the significant association between serum IL-21 levels and levels of RF and anti-MCV autoantibodies in RA. IL-21 is known to promote B cell activation and differentiation, and an important role for IL-21 in the pathogenesis of RA in animal models was recently revealed[15].

We showed that multiple immune pathways are activated in chronic active RA. Serum cytokine levels were not modulated by etanercept in nonresponders, while responders showed a marked reduction of serum IL-6 levels and increased levels of IL-21 and IL-32. Our data indicate that IL-21 might be involved in the regulation of pathogenic autoantibodies in RA.

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