Disease Activity Improvement in Rheumatoid Arthritis Treated with Tumor Necrosis Factor-α Inhibitors Correlates with Increased Soluble Fas Levels

Eloisa Romano, Riccardo Terenzi, Mirko Manetti, Francesca Peruzzi, Ginevra Fiori, Francesca Nacci, Silvia Bellando-Randone, Marco Matucci-Cerinic, and Serena Guiducci

ABSTRACT. Objective. Rheumatoid arthritis (RA) is characterized by chronic synovial inflammation and hyperplasia. Tumor necrosis factor-α (TNF-α) plays a pivotal role in RA by interfering with the Fas–Fas ligand (FasL) proapoptotic pathway. We investigated the circulating levels of soluble Fas (sFas) and soluble FasL (sFasL), and their possible correlation with disease activity and improvement after anti-TNF-α treatment in RA.

Methods. Serum levels of sFas and sFasL were measured by quantitative ELISA in 52 patients with RA before and after 3 months of anti-TNF-α treatment (adalimumab, n = 32; infliximab, n = 20). Disease activity measures [Disease Activity Score at 28 joints-erythrocyte sedimentation rate (DAS28-ESR), C-reactive protein (CRP)] were recorded before and after treatment. Forty age-matched and sex-matched healthy subjects served as controls.

Results. No significant differences in serum sFas levels were detected between anti-TNF-α-naive patients with RA and controls. After anti-TNF-α treatment, serum sFas levels significantly increased in patients with RA compared to both anti-TNF-α-naive patients and controls. Increased sFas levels inversely correlated with disease activity variables (DAS28-ESR: r = –0.739, CRP: r = –0.636, both p < 0.001). No significant differences in sFasL levels were detected in patients with RA before and after anti-TNF-α treatment.

Conclusion. In RA, an increase in sFas levels closely correlates with improvement in disease activity induced by TNF-α inhibitors, suggesting their ability to modulate Fas-mediated synoviocyte apoptosis. (First Release Sept 1 2014; J Rheumatol 2014;41:1961–5; doi:10.3899/jrheum.131544)

Key Indexing Terms:
RHEUMATOID ARTHRITIS   SOLUBLE FAS   APOPTOSIS
TNF-α INHIBITORS   DISEASE ACTIVITY

Rheumatoid arthritis (RA) is characterized by chronic joint inflammation and synovial hyperplasia caused by the influx of activated inflammatory cells, abnormal activation of fibroblast-like synoviocytes, and induction of angiogenesis with reduced rates of programmed cell death[1].

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Tumor necrosis factor-α (TNF-α) plays a pivotal role in RA pathogenesis by interfering with various apoptotic pathways[2]. Among these, the Fas (CD95/TNFRSF6)-Fas ligand (FasL, CD178/TNFSF6) death receptor pathway is inhibited by TNF-α in a dose-dependent manner[3]. Indeed, TNF-α significantly increases the levels of Fas pathway inhibitors, such as soluble Fas (sFas) and decoy receptor 3[4,5]. Both Fas and FasL are found as membrane (mFas and mFasL, respectively) and soluble (sFas and sFasL, respectively) forms, and the engagement of mFas by FasL leads to the activation of caspase-8 and caspase-3, eventually resulting into apoptosis. In RA, several cytokines and endogenous inhibitors may protect fibroblast-like synoviocytes from mFas-induced apoptosis, and increased intrasynovial sFas appears to compete with mFas, preventing synoviocyte apoptosis[6]. However, it has also been shown that recombinant sFas can undergo oligomerization and stimulate apoptotic cell death, while the sFas monomeric form is not cytotoxic[7]. Moreover, the ability of sFas receptor to oligomerize *in vitro* suggests the existence of native sFas oligomeric forms[7].

In RA, sFas is found at high concentrations in proapoptotic oligomeric form and displays cytotoxic effects on primary cultures of lymphocytes and transformed cell lines[7,8]. It has been shown that the proapoptotic effect of sFas oligomers can be fulfilled by the so-called FasL-mediated "reverse signaling" pathway, in which sFas acts as an inducer of cytotoxic signals (ligand) and transmembrane FasL as an acceptor (receptor)[9]. In RA, conflicting data have been reported concerning the serum levels of sFas[10,11]. TNF-α inhibitors can induce synovial cell apoptosis, and Fas–FasL death receptor pathway may be modulated by anti-TNF-α therapy[8,12].

On this basis, the aim of our present study was to investigate whether in RA circulating sFas and sFasL levels may correlate with improvement in disease activity upon anti-TNF-α therapy.

MATERIALS AND METHODS

The charts of 52 patients with RA diagnosed according to the 2010 American College of Rheumatology revised criteria (37 women, 15 men; mean ± SD age: 52.1 ± 16.6 yrs) were reviewed. All subjects gave written informed consent and our study was approved by the institutional review board. Before starting the anti-TNF-α treatment, all patients with RA were receiving conventional disease-modifying antirheumatic drug treatment and were naive to TNF-α inhibitors. Clinical characteristics at baseline are reported in Table 1. Patients were treated with adalimumab (ADA; n = 32) or infliximab (IFX; n = 20) for 3 months. Disease activity was evaluated by Disease Activity Score-28 joints (DAS28)-erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP). Forty age-matched and sex-matched healthy subjects (28 women, 12 men) were used as controls. Circulating levels of sFas and sFasL were measured by commercial quantitative colorimetric sandwich ELISA (Quantikine ELISA kits, catalog numbers DFS00 and DFL00, respectively; R&D Systems). The assay detection range was 31.2–2000 pg/ml for sFas and 15.6–1000 pg/ml for sFasL. For the sFas assay, serum samples were diluted 1:6. Serum levels of sFas and sFasL were determined in the same patients before and after 3 months of anti-TNF-α treatment. Each sample was measured in duplicate. For both sFas and sFasL assays, the interassay and intraassay variances were < 10%. Because rheumatoid factor (RF) may interfere with ELISA, we added known quantities of recombinant sFas or sFasL to some RF-positive serum

Table 1. Clinical characteristics of the 52 patients with RA at baseline. Except where indicated otherwise, values are the number (%) of subjects.

Characteristic	Patients with RA
Age, yrs, mean ± SD	52.1 ± 16.6
Sex	
Male	15 (28.9)
Female	37 (71.1)
Disease duration (mos), mean ± SD	78.2 ± 51.4
DAS28-ESR, mean ± SD	5.3 ± 1.5
CRP, mg/l, mean ± SD	10.8 ± 4.2
RF positivity	40 (76.9)
Immunosuppressants	
Glucocorticoids	35 (67.3)
Methotrexate	43 (82.7)

RA: rheumatoid arthritis; DAS28-ESR: Disease Activity Score at 28 joints-erythrocyte sedimentation rate; CRP: C-reactive protein; RF: rheumatoid factor.

samples to determine whether this altered the assay ability to measure the relevant molecule. The presence of RF had no significant effect on the measurement. Statistical analysis was performed using the SPSS software for Windows Version 20.0 (SPSS). Descriptive statistics were expressed as median and interquartile range (IQR). According to not-normally distributed data, circulating levels of sFas and sFasL were compared by the nonparametric Mann-Whitney U test for independent samples and by Wilcoxon test for related samples. The Spearman rank correlation coefficient (r) was used to examine the relationship between sFas and sFasL serum levels and disease activity variables (DAS28-ESR and CRP). A multivariate linear regression analysis including DAS28-ESR as dependent variable and sFas and CRP as independent variables was performed. All p values are 2-tailed, and p values < 0.05 were considered statistically significant.

RESULTS

Before starting anti-TNF-α therapy, in 37/52 (71%) patients with RA, disease activity was severe (DAS28-ESR: median 6.0, IQR 5.7–6.8; CRP: median 11 mg/l, IQR 8–15 mg/l), while in 15/52 (29%) patients, disease activity was moderate (DAS28-ESR: median 3.6, IQR 2.6–4.1; CRP: median 9 mg/l, IQR 5–11 mg/l). In the whole group of patients with RA, a significant reduction in DAS28-ESR (median 2.6, IQR 1.99–3.05) and CRP (median 0.8 mg/l, IQR 0.3–1.1 mg/l) was observed after 3 months of anti-TNF-α therapy. In particular, after treatment, 26 out of 52 patients had DAS28-ESR < 2.6, which is indicative of clinical remission as described by Fransen and van Riel[13].

No significant differences in serum sFas levels were detected between anti-TNF-α-naive patients with RA (median 5284.7 pg/ml, IQR 5079.4–5602.5 pg/ml) and controls (median 5566.0 pg/ml, IQR 4755.2–5973.5 pg/ml; Figure 1A). However, we observed a significant inverse correlation between sFas levels and both DAS28-ESR (r = –0.64, p < 0.001) and CRP (r = –0.35, p = 0.01) in anti-TNF-α-naive patients with RA. After 3 months of anti-TNF-α therapy, serum sFas levels were significantly increased in patients with RA (median 9580.1 pg/ml, IQR 7597.7–10,759.1 pg/ml) compared to both anti-TNF-α-naive patients and healthy subjects (p < 0.001 for both; Figure 1A). In particular, serum sFas levels in patients with RA treated with IFX (median 9541.9 pg/ml, IQR 7000.7–10,925.5 pg/ml) or ADA (median 9580.1 pg/ml, IQR 7597.7–10,652.0 pg/ml) were both significantly higher than in the corresponding anti-TNF-α-naive patients and healthy individuals (p < 0.001 for all comparisons; Figure 1B). After anti-TNF-α treatment, increased sFas levels correlated inversely with disease activity variables (DAS28-ESR: r = –0.739 and CRP: r = –0.636, both p < 0.001). These significant correlations were also confirmed when the 2 different TNF-α inhibitors were analyzed separately (DAS28-ESR: r = –0.752 and CRP: r = –0.743 for IFX; DAS28-ESR: r = –0.684 and CRP: r = –0.601 for ADA; all p < 0.001). Moreover, when patients were stratified according to DAS remission cutpoints[13], we found that patients who achieved clinical remission had significantly higher sFas levels (median 10,677.5 pg/ml, IQR
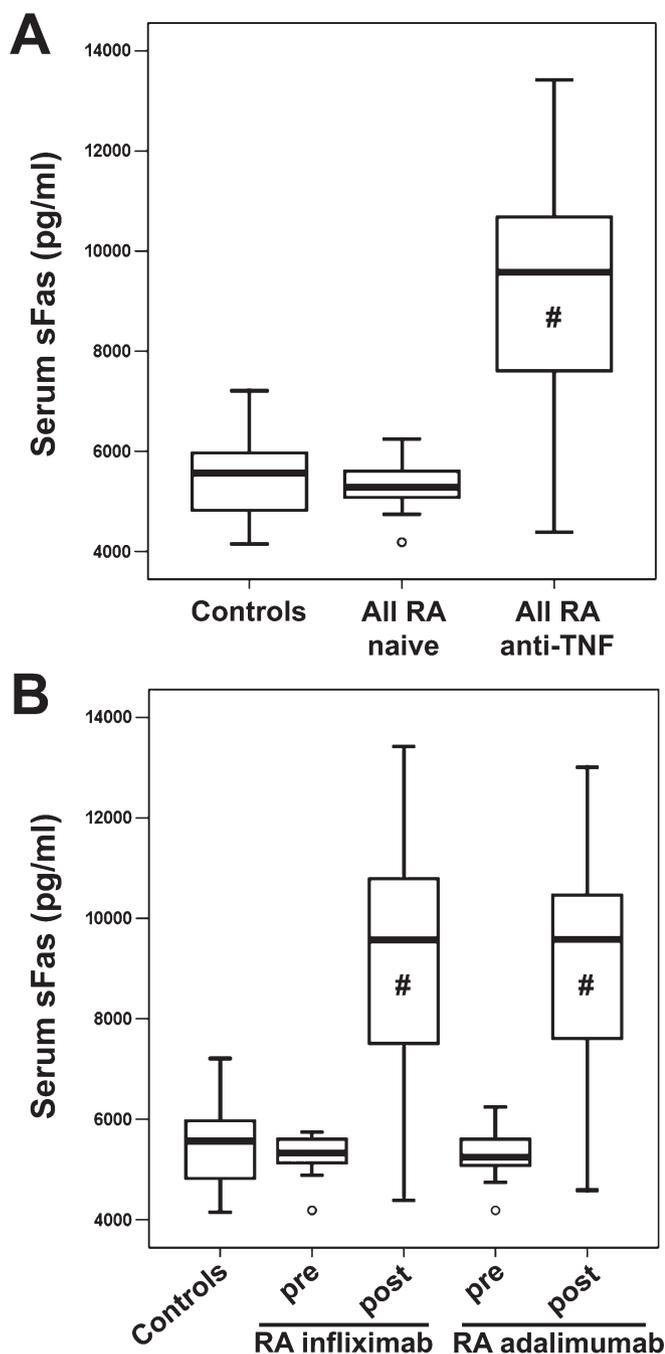
Figure 1. A. Serum levels of soluble Fas (sFas) are significantly increased in patients with RA after 3 months of anti-TNF-α treatment compared to both naive patients and healthy controls (p < 0.001 for both). B. Serum sFas levels in patients with RA treated with infliximab or adalimumab are both significantly higher than in controls and the corresponding naive patients (p < 0.001 for all comparisons). Serum levels of sFas were measured by quantitative colorimetric sandwich ELISA. Data are shown as box plots. Each box represents the 25th to 75th percentiles. Lines outside the boxes represent the 10th and 90th percentiles. Lines inside the boxes represent the median, and circles the outliers. sFas: soluble Fas; RA: rheumatoid arthritis; TNF-α: tumor necrosis factor-α.

9584.9–11,313.6 pg/ml) compared with patients who had disease activity improvement without reaching remission (median 8080.9 pg/ml, IQR 6339.3–9160.6 pg/ml, p < 0.001). We also investigated the correlation between the pretreatment to posttreatment change in sFas levels and the changes in either DAS28-ESR or CRP, both in the whole patient cohort and in the subgroups of patients who achieved or did not achieve clinical remission. A significant inverse correlation between the change in sFas levels and the change in DAS28-ESR was found in the whole cohort (r = –0.473, p < 0.001), as well as in the subgroups of patients stratified according to clinical remission (r = –0.460, p = 0.01 for remission subgroup; r = –0.410, p = 0.03 for non-remission subgroup). A trend toward an inverse correlation between the change in sFas levels and the change in CRP was observed, but this analysis did not reach statistical significance. Finally, multivariate linear regression analysis revealed that sFas was an independent determinant of DAS28-ESR (β = –0.674, p < 0.001) in a model including CRP.

Circulating sFasL levels were similar in anti-TNF-α-naive patients with RA (median 48.4 pg/ml, IQR 43.9–57.8 pg/ml) and controls (median 50.4 pg/ml, IQR 38.0–59.0 pg/ml; Figure 2A). Similar sFasL levels were also found in patients with RA before and after anti-TNF-α therapy (median 51.6 pg/ml, IQR 37.5–64.8 pg/ml; Figure 2A), as well as when the IFX and ADA groups were analyzed separately (Figure 2B). No significant correlation between sFasL levels and disease activity variables was found in patients with RA either before or after anti-TNF-α therapy.

DISCUSSION

To the best of our knowledge, our data show for the first time that circulating sFas levels significantly increase in patients with RA who achieved an improvement in disease activity following treatment with the TNF-α inhibitors IFX and ADA. Moreover, serum sFas levels inversely correlated with disease activity outcome measures DAS28-ESR and CRP either before or after anti-TNF-α therapy. Interestingly, stratification of patients according to DAS remission cutpoints[13] revealed that sFas levels were significantly higher in patients who achieved clinical remission than in those who did not reach remission, despite an improvement in disease activity.

These data may suggest that in RA, the increase in sFas levels after anti-TNF-α treatment may contribute to foster cell apoptosis through the so-called FasL-mediated "reverse signaling" pathway[9]. In agreement with our findings, Dubikov and Kalinichenko have shown that patients with RA with high disease activity have low rate of synovial apoptotic cell death, which correlates with low levels of serum sFas[14]. Moreover, serum sFas levels were reported to be significantly correlated with a reduction in RA disease activity and an increase in synovial apoptotic cell index[14].
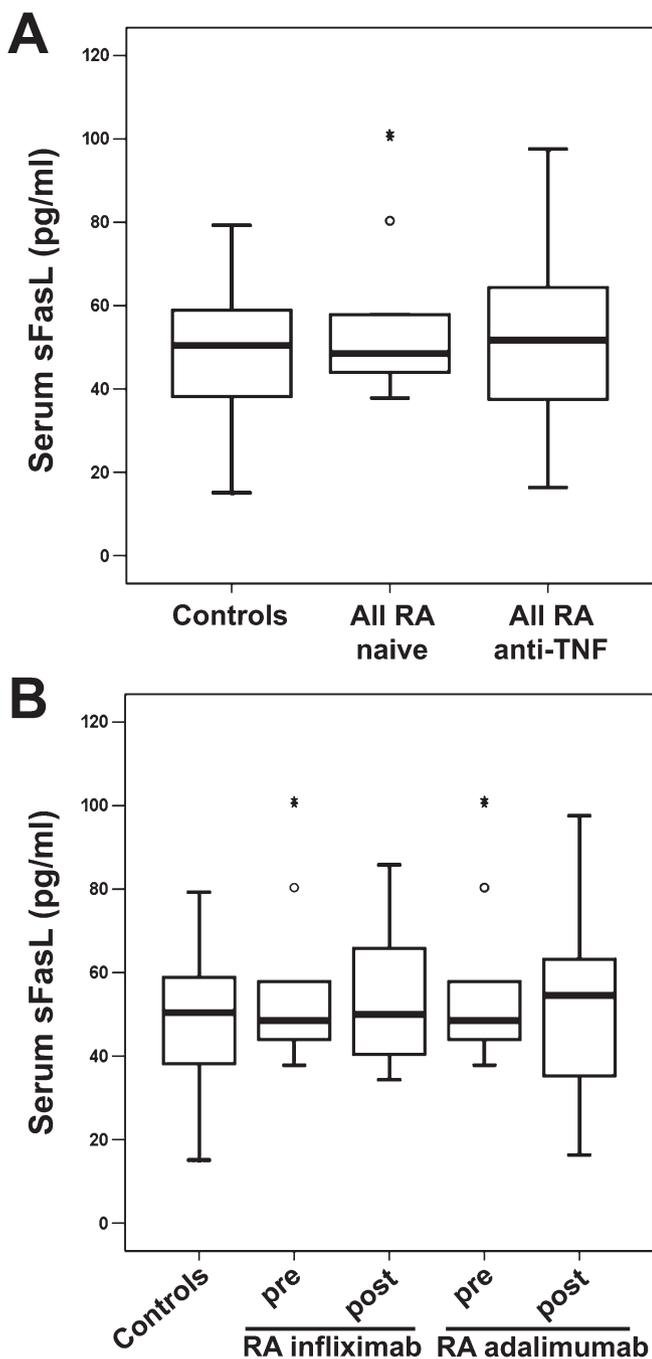
Figure 2. A. Circulating soluble FasL (sFasL) levels were similar in anti-TNF-α–naive patients with RA and healthy controls. No significant differences were detected in patients with RA before and after anti-TNF-α treatment. B. No difference was detected between sFasL levels in patients with RA treated with IFX or ADA and both controls and the corresponding naive patients. Serum levels of sFasL were measured by quantitative colorimetric sandwich ELISA. Data are shown as box plots. Each box represents the 25th to 75th percentiles. Lines outside the boxes represent the 10th and 90th percentiles. Lines inside the boxes represent the median, circles the outliers, and asterisks the extreme values. sFasL: soluble Fas ligand; RA: rheumatoid arthritis; TNF-α: tumor necrosis factor-α; IFX: infliximab; ADA: adalimumab.

On the contrary, serum sFasL levels were similar in patients with RA before and after anti-TNF-α therapy, further suggesting that TNF-α inhibitors might act through the "reverse signaling" mechanism, which is specifically mediated by the binding of sFas to the transmembrane, but not soluble form of FasL[9].

RA is characterized by high levels of TNF-α, one of the most important cytokines leading to synovial inflammation and hyperplasia, and protecting the proliferating synovial tissue from apoptosis[1,12]. The treatment of RA with TNF-α inhibitors results in a decrease of cellularity at sites of inflammation. This effect may be attributable to the induction of apoptosis, a decrease in the influx or an increase in efflux of inflammatory cells, or by direct cyto-toxicity[15]. *In vitro* studies on human-activated peripheral blood lymphocytes and monocytes showed that both IFX and ADA may increase the proportion of cells undergoing apoptosis[16]. In a recent report, both IFX and ADA had the ability to induce apoptosis of RA fibroblast-like synovio-cytes even in the presence of autologous peripheral blood mononuclear cells[17]. In this context, our results suggest that in RA, TNF-α inhibitors might exert a proapoptotic action through an increase in sFas levels. Indeed, in our work, we clearly observed a significant correlation between the reduction in disease activity (DAS28-ESR and CRP) and the increase in circulating sFas levels after treatment both with IFX and ADA, without obvious differences between the 2 drugs.

Treatment of RA with TNF-α–blocking agents provided a significant improvement in symptoms and signs of disease activity in parallel with a marked increase in circulating levels of sFas. These findings might be related to the ability of TNF-α inhibitors in inducing synoviocyte apoptosis through the modulation of the Fas/FasL death receptor pathway, ultimately contributing to an improvement in disease activity or even clinical remission. Therefore, sFas might even be used as a potential biomarker to provide additional information regarding disease activity along with the traditional indices such as DAS28-ESR and CRP. The investigation of a larger sample size is warranted to confirm the correlation between sFas levels and response to anti-TNF-α therapy in RA. It will also be of interest to study the possible correlation between sFas and disease activity variables in patients with RA treated with other TNF-α inhibitors. Moreover, further functional studies will help to understand the molecular mechanisms by which TNF-α inhibitors may modulate the Fas/FasL proapoptotic pathway.

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