CONCISE REPORT

Soluble tumour necrosis factor receptor treatment does not affect raised transforming growth factor β levels in rheumatoid arthritis

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Objective: To further elucidate the immunomodulating effects of anti-tumour necrosis factor α treatment in rheumatoid arthritis (RA) by studying changes in plasma levels of transforming growth factor β (TGFβ) in patients with RA undergoing etanercept treatment.
Methods: Plasma levels of TGFβ1 and TGFβ2 were determined in 26 patients with RA during six months of etanercept treatment and compared with disease activity and laboratory parameters, including matrix metalloproteinase-3 (MMP-3) and interleukin 6 (IL6).
Results: Before treatment all patients had raised TGFβ1, IL6, and MMP-3 levels. In the course of treatment IL6 and MMP-3 levels decreased significantly, accompanied by a drop in serological markers (C reactive protein and erythrocyte sedimentation rate) and clinical disease activity (visual analogue scale and Thompson joint score). By contrast, high levels of latent TGFβ1 were present in all specimens over the entire six months. TGFβ2 levels did not change during treatment.
Conclusions: Etanercept treatment induces subtle changes in the cytokine network. Although the proinflammatory cytokine IL6 is down regulated, the persistence of high TGFβ plasma levels indicates the existence of as yet unknown mechanisms for TGFβ overexpression in RA. This may predispose to severe infections and can cause an altered tumour defence.

Rheumatoid arthritis (RA) is a chronic autoimmune disease characterised by complex interactions between proinflammatory cytokines such as interleukin 1 (IL1), IL6, and tumour necrosis factor α (TNFα) and anti-inflammatory cytokines such as IL4, IL10, and IL13, with an imbalance in favour of the former.[1] TNFα, produced primarily by activated monocytes and macrophages, plays a central part in inducing and sustaining inflammatory processes in RA. This cytokine induces downstream events, such as the up regulation of IL1 and IL6, and the release of prostaglandin E_2 and matrix metalloproteinases (MMPs).[1 2] This led to the development of anti-TNF compounds as therapeutic agents for the treatment of RA.

While TNFα has been shown to increase the incidence and severity of experimental arthritis, an opposite effect was found for systemically administered and endogenously produced transforming growth factor β (TGFβ).[3] In RA plasma levels of TGFβ1 have been shown to be raised.[4] TGFβ is one of the cytokines which exerts both pro- and anti-inflammatory effects. Its possible beneficial effects in RA are the inhibition of cartilage degradation mediated by proinflammatory cytokines—that is, the regulation of MMP transcription, the inhibition of lymphocyte proliferation, and suppression of macrophage superoxide production.[5] On the other hand, TGFβ has chemotactic properties at the site of inflammation and stimulates cells to produce proinflammatory cytokines such as IL1, IL6, and TNFα.[6] Differential effects of TGFβ make it difficult to conclude whether the anti- or proinflammatory properties of this cytokine predominate in RA.

Earlier reports suggested that the proinflammatory cytokines TNFα, IL1, and IL6 induce TGFβ expression. The question arises whether TNF neutralising agents such as etanercept have an influence on the expression of TGFβ.

To our knowledge, no data have been published on the effects of anti-TNFα treatment on raised TGFβ levels in patients with RA. We sought therefore to further elucidate the immunomodulating effects of TNF neutralising treatment on the complex network of cytokines by determining TGFβ levels in patients with RA.

PATIENTS AND METHODS

Patients

Twenty six patients (mean age 52.3 years, six male/20 female, 15 rheumatoid factor positive, 11 rheumatoid factor negative) who met the American College of Rheumatology (ACR) criteria for RA were studied.[7] On average, three or more different disease modifying antirheumatic drugs had previously failed to control disease activity.

Each patient was given 2×25 mg etanercept (Enbrel) subcutaneously each week. Seven patients also received methotrexate (10–15 mg/wk), and one patient azathioprine (100 mg/day). Initial co-medication consisted of steroids (between 10 and 20 mg/day), which was tapered according to clinical improvement.

Blood samples were taken before treatment, and one, three, and six months after the first application of etanercept. Plasma samples were obtained within one hour after blood collection by two centrifugations (700 *g* for 10 minutes, 1300 *g* for 10 minutes) and stored at −80°C until analysis.

Clinical evaluation

The clinical course was assessed by routine laboratory parameters such as erythrocyte sedimentation rate (ESR) and C reactive protein (CRP). Disease activity was measured using the patient's assessment of pain (visual analogue scale; VAS) and the Thompson joint score.

Quantification of TGFβ

Plasma samples were analysed for TGFβ1 and 2 levels by two different TGFβ isoform-specific enzyme linked immunosorbent assays (ELISAs), as described elsewhere.[8 9] To determine concentrations of total TGFβ (that is, bioactive and

Abbreviations: CRP, C reactive protein; ESR, erythrocyte sedimentation rate; IL, interleukin; MMP, matrix metalloproteinase; RA, rheumatoid arthritis; TGFβ, transforming growth factor β; TNFα, tumour necrosis factor α; VAS, visual analogue scale

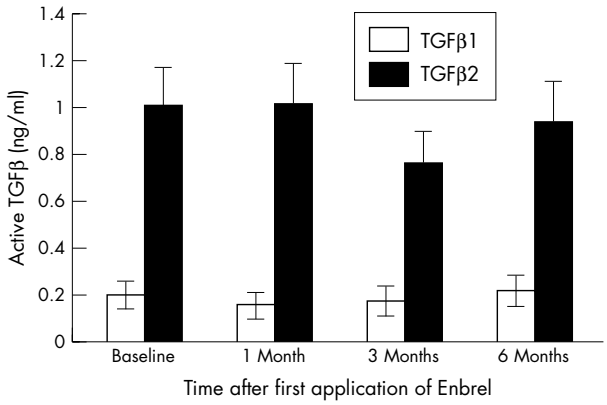

Figure 1 Course of plasma levels of spontaneously active TGFβ in the etanercept treated patients as determined by isoform-specific ELISAs. Bars represent the mean values (SEM).

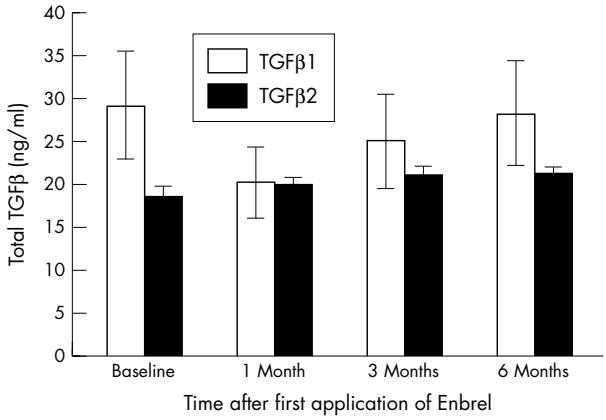

Figure 2 Course of plasma levels of total TGFβ in the etanercept treated patients as determined by isoform-specific ELISAs after acid activation of latent TGFβ. Bars represent the mean values (SEM).

latent TGFβ), samples were acidified with 5 M HCl at pH 1.5, incubated for 30 minutes at 37°C, and then neutralised with 1.4 M NaOH in 0.7 M Hepes.

Determination of MMP-3 and IL6

MMP-3 was measured in patient plasma by a sandwich ELISA (Amersham Pharmacia Biotech, UK) according to the manufacturers instructions. IL6 was determined in plasma samples applying the Quantikine ELISA of R&D Systems.

Statistical analysis

Mann-Whitney's U test was used for unpaired comparisons between patients and healthy controls. To compare variables in the course of treatment, the Wilcoxon matched pairs test was applied.

RESULTS

Before etanercept treatment total TGFβ1 levels (mean (SEM)) were significantly raised in the patients with RA compared with healthy controls (29.9 (6.2) *v* 5.3 (0.6) ng/ml, p<0.01). Total TGFβ2 levels were similar in both groups (18.0 (1.0) *v* 21.2 (1.0) ng/ml). Levels of active TGFβ1 and 2 were significantly raised in RA and remained unchanged during etanercept treatment (fig 1). Total TGFβ1 and TGFβ2 concentrations remained constant in all specimens throughout the six month study period (fig 2).

Before treatment all 26 patients had raised IL6 levels of 63.4 (24.0) pg/ml, whereas levels in healthy controls (n=19) were below 3.1 pg/ml (p<0.01). IL6 levels in the patient group decreased during the first three months of etanercept treatment (table 1). After three months IL6 levels rose again, but did not attain the high concentrations present before etanercept treatment. Like the IL6 concentrations, plasma MMP-3 levels declined significantly (109.2 (18.5) ng/ml before etanercept, 66.1 (10.2) ng/ml after six months, p<0.01). The drop in IL6 and MMP-3 plasma levels was accompanied by a significant reduction in ESR and CRP values and in clinical disease activity (table 1).

A comparison of the individual variables over the course of treatment showed significant correlations (Pearson correlation, p<0.01) between IL6 and MMP-3 concentrations and CRP and VAS. MMP-3 levels correlated strongly with IL6 and CRP (p<0.01), less strongly with the Thompson score and VAS (p<0.05).

DISCUSSION

The present findings show that etanercept has differential effects on the cytokine network in patients with RA. Neutralisation of TNFα resulted in the suppression of various proinflammatory cytokines, as shown here for IL6, accompanied by a reduction in the acute phase protein CRP and disease activity, while etanercept treatment had no effect on the very potent anti-inflammatory cytokine TGFβ, which is overexpressed in RA.[4] Results of TGFβ protein determination have been confirmed for TGFβ1 at the mRNA level in reverse transcriptase-polymerase chain reaction according to Szymkowiak and coworkers.[10] No change in the TGFβ1-specific polymerase chain reaction product was found in polymorphonuclear cells, peripheral blood mononuclear cells, and synovial fluid cells either before or after etanercept treatment (data not shown). This supports the thesis that etanercept acts selectively on the immune system.

TGFβ is of considerable interest in RA owing to its immunomodulatory properties and its unidirectional effect on proinflammatory cytokines such as TNFα, IL6, and IL1.[6] The failure of anti-TNFα treatment to influence TGFβ levels was somewhat unexpected as IL1 and IL6 have been described as inducers of TGFβ. Both cytokines are down regulated by anti-TNFα treatment. As a secondary effect TGFβ levels were expected to decrease. The persisting high TGFβ levels indicate the existence of as yet unknown mechanisms for TGFβ overexpression in RA that act independently of IL1 and IL6.

Table 1 Improvement of laboratory parameters (CRP, ESR, IL6, and MMP-3) and clinical activity (VAS and Thompson score) with etanercept treatment (mean (SEM)). The Wilcoxon matched pairs test was used to compare changes over the course of treatment

Time point	Before treatment	After 1 month	After 3 months	After 6 months
Patients (n)	26	24	22	19
ESR (mm/1st h)	66.5 (7.3)	40.8 (5.8)**	35.7 (5.3)**	39.6 (9.0)
CRP (mg/l)	52.4 (6.3)	26.6 (5.7)**	27.3 (6.7)**	20.0 (5.5)**
IL6 (pg/ml)	63.4 (24.0)	19.0 (4.5)**	33.5 (8.1)	25.6 (5.7)
MMP-3 (ng/ml)	109.2 (18.5)	80.5 (11.3)	71.3 (9.9)**	66.1 (10.2)**
VAS (mm)	60.9 (4.8)	31.6 (4.7)**	26.6 (6.3)*	17.7 (4.7)*
Thompson score	228.9 (28.2)	123.5 (22.4)	93.2 (24.0)	70.5 (20.9)

*p<0.05 *v* baseline; **p<0.01 *v* baseline.

This study confirms previous reports that MMP-3 levels are raised and correlate with measures of inflammation in RA.[11 12] It also shows that serum MMP-3 levels are down regulated after etanercept treatment. The strong association of MMP-3 concentrations with parameters of disease activity suggests that plasma MMP-3 is a useful indicator of the effect of anti-TNFα treatment in RA.

In patients with RA, high TGFβ levels may be beneficial for the autoimmune process, as shown in experimental autoimmune disease in animals.[13] TGFβ exerts strong immunosuppressive effects that limit the inflammatory response. However, high TGFβ1 levels may have the adverse effects of predisposing to severe infections and altering tumour defence.

It remains speculative whether persisting high TGFβ levels in patients with RA undergoing anti-TNFα treatment account for a further reduction in the severity of the disease.[4] Future long term studies will have to take these observations into account.

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