CONCISE REPORT

Anti-tumour necrosis factor (TNF) α treatment of rheumatoid arthritis (infliximab) selectively down regulates the production of interleukin (IL) 18 but not of IL12 and IL13

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Objective: To measure interleukin (IL)18 serum concentrations in patients with rheumatoid arthritis (RA) undergoing infliximab treatment (tumour necrosis factor (TNF) α blockade) and to evaluate the concomitant modification of IL12 and IL13 serum concentrations, two cytokines belonging to the Th1 and Th2 profile respectively and biologically related to IL18.

Methods: Ten patients with RA not responding to disease modifying antirheumatic drugs (DMARDs) received intravenous infliximab at a dose of 3 mg/kg at baseline and after two and six weeks. Serum samples were collected from all patients before each infusion and assayed for IL18, IL12, and IL13 by enzyme linked immunosorbent assay (ELISA); IL18 was also measured eight weeks after the last infusion.

Results: Serum concentrations of IL18 in all patients were already markedly reduced from baseline after two weeks ($p<0.005$). Serum IL18 was also decreased in a stable manner after six ($p<0.01$) and 14 weeks ($p<0.01$) compared with baseline concentrations. No significant modifications were found in serum concentrations of IL12 and IL13 at any time point.

Conclusion: There was a rapid and persistent decrease in serum concentrations of IL18 in all the patients studied. This result provides evidence of an in vivo regulation of IL18 by TNFα and suggests that anti-TNFα therapy is likely to interrupt the synergistic effect between these two cytokines.

The high concentration of interleukin (IL) 18 in synovial fluids and serum samples of patients with rheumatoid arthritis (RA) and its wide expression in rheumatoid synovitis suggest this newly identified cytokine may be a relevant mediator of joint inflammation.[1] Moreover, it has recently been suggested that serum concentrations of IL18 are increased in patients with more severe adult onset Still's disease.[2] Although mainly characterised as a major inducer of interferon (IFN) γ in synergy with IL12 and IL15, recent in vitro experimental evidence indicates that IL18 also exerts its inflammatory action in an IFNγ independent way—that is, by direct effect on macrophages or the up regulation of other cytokines such as IL1, IL6, and especially TNFα.[3] On the basis of the structural homology, receptor family, intracellular transduction pathway, and biological effects, IL18 is considered a member of the IL1 family and, in common with this cytokine, participates in both innate and acquired immunity. Although there is no evidence that IL18 binds to IL1 receptor 1 (IL1R1), IL18 interacts with a complex receptor (IL18R) comprising a binding chain (IL18Rα), previously identified as IL1R accessory protein (IL1RAcP), and a signal chain (IL18Rβ), both members of the IL1R family[4]; IL18R activates IL1R associated kinase (IRAK) and TNF associated factor (TRAF6), which is then able to phosphorylate the nuclear factor inducing kinase κB (NFκB) with subsequent activation of this factor.[5 6] Moreover, IL18 is a powerful co-inducer, with IL2, of IL13 a mediator which mainly has anti-inflammatory functions within rheumatoid synovia. This effect of IL18 is independent of IL12 although influenced by the production of IFNγ.[7 8] On the other hand, some findings suggest that IL12 is widely involved in IFNγ dominant cytokine production by infiltrating T cells in joints with chronic RA and thereby this cytokine has a crucial role in maintaining the imbalance towards a predominant Th1 cytokine profile in the course of RA.[9]

Treatments blocking TNFα in patients with RA often result in rapid clinical improvement, decrease in serum C reactive protein (CRP) concentrations, and down regulation of inflammatory cytokines—for example, IL6—stimulated by TNFα.[10 11]

The aim of this study was to measure IL18 serum concentrations in patients with RA undergoing infliximab treatment and to evaluate the concomitant modification of the concentrations of two related cytokines, IL12 and IL13.

PATIENTS AND METHODS

We measured serum IL18, IL12, and IL13 concentrations in 10 patients (seven females and three males, mean age 50.5 (SD 16.5) years, mean disease duration 142.8 (SD 78.3) months) with active RA, classified according to American Rheumatism Association (ARA) criteria, not responding to disease modifying antirheumatic drugs (DMARDs). Patients were allowed to continue DMARDs, steroids, and non-steroidal anti-inflammatory drugs at a stable dose for at least four weeks before and during infliximab administration. Patients received intravenous infliximab at a dose of 3 mg/kg at baseline and after two and six weeks. Serum samples were collected from all patients before each infusion and stored at –20°C until tested. Interleukin-18 was also measured eight weeks after the last infusion. All samples were tested in duplicate. An enzyme

Abbreviations: BSA, bovine serum albumin; CRP, C reactive protein; DMARDs, disease modifying antirheumatic drugs; ELISA, enzyme linked immunosorbent assay; HAQ, Health Assessment Questionnaire; IFNγ, interferon γ; IL1R1, interleukin 1 receptor 1; PBS, phosphate buffered saline; TNFα, tumour necrosis factor α

Table 1 Clinical and laboratory variables (mean (SEM)) of RA patients during treatment with infliximab

	Weeks			
	0	2	6	14
Number of patients	10	10	8	8
Serum IL18 (pg/ml)	1364 (384)	739 (317) p<0.005*	353 (165) p<0.01*	196 (26.4) p<0.01*
Serum IL12 (pg/ml)	36.4 (26.4)	41.2 (32)	24.9 (19.1)	
Serum IL13 (pg/ml)	43.4 (12.2)	50.9 (10.7)	32.9 (11.8)	
ESR (mm/1st h)	46.2 (5.9)	23.4 (2.6) p<0.005*	36.2 (6.6) p<0.02†	34 (7.9)
CRP (mg/l)	26.4 (6.4)	8.7 (4.6) p<0.02*	14.2 (4.2) p<0.03†	17.6 (7.2)
Number of tender joints	31.5 (7.9)	27.7 (8.3)	19 (8) p<0.02*	27.5 (9.9)
Number of swollen joints	10.4 (5.5)	8.7 (4.7)	4.5 (2.8)	5.4 (3.4)
Patient's assessment of pain (cm)	6.6 (0.8)	5.7 (0.6)	6 (0.8)	6.1 (0.1)
Patient's global assessment of disease activity (cm)	7.4 (0.7)	6.2 (0.7)	5.8 (0.8) p<0.02*	6.8 (0.9)
Physician's global assessment of disease activity (cm)	6.2 (0.7)	6 (0.8)	5.2 (1)	5.8 (1)
HAQ	1.54 (0.21)	1.51 (0.27)	1.37 (0.3)	1.53 (0.33)

Where not specified differences between variables found at the time points of the study are not statistically significant.
*With respect to baseline (0); †with respect to second week.

linked IL18 assay standardised in our laboratory using antibody pairs and reagents provided from R & D Systems (Minneapolis, MN, USA) was used to measure IL18 serum concentrations. Briefly, an anti-IL18 monoclonal antibody was used to coat (2 μg/ml in phosphate buffered saline (PBS)) polystyrene ELISA plates (Maxisorb) which were then incubated overnight at room temperature. Plates were then blocked for two hours at room temperature with phosphate buffered saline (PBS)/bovine serum albumin (BSA) (1% v/w)/ sucrose (5% v/w). A solution made of TBS/BSA (0.1% v/w)/Tween 20 (0.05% v/v) was used to dilute standards (rhIL18, R&D Systems) and serum. After two hours of incubation and washing with PBS/Tween 20 (0.05% v/v) a secondary biotinylated antibody (R&D Systems; 250 ng/ml) was added and incubated for two hours at room temperature. After further washing, peroxidase conjugated streptavidin was added and incubated for 20 minutes at room temperature. The reaction was then developed with a solution of tetramethylbenzidine in the presence of H_2O_2, stopped with 4N sulphuric acid, and the optical density read at 450 nm.

Serum concentrations of IL13 and IL12 were measured by commercial ELISA kits (Bender Medsystems, Vienna, Austria) using the manufacturer's instructions. Briefly, polystyrene microplate wells (Sterilin) were coated overnight at 4°C with a murine monoclonal antibody directed against IL13 or IL12 (biologically active p70 heterodimer). A solution of PBS/Tween 20 (0.05% v/v) was used to wash each well between incubation steps. After a two hour incubation at room temperature with PBS/Tween 20 (0.05% v/v)/ BSA (0.5% v/w), a serum sample and murine monoclonal antibody-biotin conjugated (1:5000), directed against IL12 or murine monoclonal antibody-HRP conjugated (1:2000), directed against IL13, were added to the coated wells. After two hours at room temperature streptavidin-HRP was added to the IL12 assay only. After incubation a substrate solution (1:2 mixture of H_2O_2: e-tetramethylbenzidine) was added and after development the reaction was stopped by addition of 4N sulphuric acid. Optical density was read at 450 nm.

The main clinical and laboratory variables including erythrocyte sedimentation rate (ESR), C reactive protein (CRP), serum rheumatoid factor, tender and swollen joint count, patient's assessment of pain, patient's assessment of disease activity, physician's global assessment of disease activity, and Health Assessment Questionnaire (HAQ) were evaluated at baseline and after two, six, and 14 weeks. Statistical analysis was conducted with Fisher's exact test and continuous variables by Wilcoxon rank test for paired data.

RESULTS

Serum IL18, IL12, and IL13 concentrations and the main clinical and laboratory variables during infliximab therapy are shown in table 1. Two patients dropped out after the second

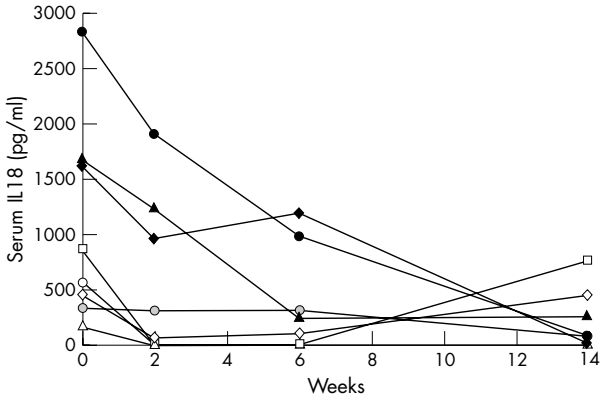

Figure 1 Serum IL18 concentrations of RA patients undergoing infliximab treatment for 14 weeks.

infusion owing to side effects. Serum IL18 was already markedly reduced after two weeks ($p<0.005$) in all the patients and remained significantly lower than baseline at six ($p<0.01$) and 14 ($p<0.01$) weeks (fig 1). The reduction of IL18 was not accompanied by a significant decrease in the concentrations of the other cytokines measured as no difference was found in serum concentrations of IL12 and IL13 at any time point during infliximab treatment (table 1).

American College of Rheumatology (ACR) 20 criteria for clinical remission at two, six, and 14 weeks were satisfied in 50%, 62.5%, and 12.5% of patients respectively. A significant reduction occurred in both ESR ($p<0.005$) and CRP ($p<0.02$) concentrations at two weeks compared with baseline, even though a slight but significant increase in ESR ($p<0.02$) and CRP ($p<0.03$) at six weeks compared with two weeks was also reported (table 1).

DISCUSSION

This preliminary study indicates that anti-TNFα treatment with infliximab resulted in a rapid and stable reduction in serum concentrations of IL18 in patients with RA. There was no significant reduction in serum concentrations of IL12 and IL13, a finding which may suggest that the effect of anti-TNFα treatment and the consequent reduction in IL18 do not parallel a significant modification of both Th1 and Th2 cytokines. Serum concentrations of IL12 as a result of the detection of the biologically active p70 heterodimer of this cytokine were compatible with those found in previous studies.[12 13] The slight differences are probably due to the characteristics of the populations studied. In our study serum concentrations of IL12 were not significantly affected by infliximab treatment. Moreover, we did not find a significant variation in IL13, a Th2 cytokine, the synthesis of which is induced by IL18 in cooperation with IL2 and suppressed by IFNγ. All these results suggest an interdependent regulation between IL18 and TNFα as also indicated by recent studies. It has been shown that TNFα stimulates IL18 production in synovial tissues in vitro[1] and the effect of endogenous IL18 on the development of collagen II induced arthritis and on TNFα production has recently been investigated.[14] Mice with an IL18 deficient DBA/1 background experienced a milder and significantly delayed collagen II induced arthritis which was characterised by reduced TNFα concentrations in serum and the spleen. These findings raise the possibility of a positive feedback loop between these two cytokines in vivo. Although our study does not show a direct effect on TNFα concentrations and activity, the significant reduction of IL18 serum concentrations may be accompanied by beneficial anti-inflammatory effects which are independent of TNFα blockade. Some recent investigations outlined several direct proinflammatory effects of this cytokine which may have a major impact in the pathogenesis of joint damage. In this respect, preliminary studies performed on rheumatoid synovia highlighted the ability of IL18 to increase the expression of the IL6 gene, and the production of cyclooxygenase-2 and stromelysin by chondrocytes. The exposure of normal cartilage to IL18 induces release of glycosaminoglycans.[15] Another property which is in keeping with the inflammatory action of this cytokine is its ability to enhance the production of CC and CXC chemokines, among which is IL8, by human leucocytes.[16] Moreover, a recent investigation provided evidence that IL18 increases the production of osteoprotegerin by stromal osteoblasts and thereby limits osteoclastogenesis.[17] The effect that reduction of IL18 activity due to the treatment with infliximab has on osteoclastogenesis awaits further investigation.

In conclusion, this study indicates a rapid and persistent decrease in serum concentrations of IL18 in all the patients in response to infliximab treatment, which seems to be independent of an indirect IFNγ mediated effect. Down regulation of IL18 may result in reduced TNFα production, so interrupting the positive feedback between the two cytokines, a likely monokine amplification network occurring in RA. However, IL18 down regulation could by itself amplify the anti-inflammatory effects derived from TNFα blockade. The development of IL18 specific blocking agents amenable to clinical application could eventually confirm this in vivo finding, so providing a rationale for a combined anticytokine treatment for RA.

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