

EXTENDED REPORTS

Effects of treatment with a fully human anti-tumour necrosis factor α monoclonal antibody on the local and systemic homeostasis of interleukin 1 and TNF α in patients with rheumatoid arthritis

P Barrera, L A B Joosten, A A den Broeder, L B A van de Putte, P L C M van Riel, W B van den Berg

Abstract

Objectives—To study the short term effects of a single dose of D2E7, a fully human anti-tumour necrosis factor (TNF α) monoclonal antibody (mAb), on the local and systemic homeostasis of interleukin 1 β (IL1 β) and TNF α in patients with rheumatoid arthritis (RA).

Methods—All patients with RA enrolled in a phase I, single dose, placebo controlled study with D2E7 in our centre were studied. Systemic cytokine levels, acute phase reactants, and leucocyte counts were studied at days 0, 1, and 14 after the first administration of anti-TNF mAb (n=39) or placebo (n=11). The cellularity and the expression of IL1 and TNF α in synovial tissue were studied in knee biopsy specimens obtained at baseline and at day 14 in 25 consenting patients.

Results—A single dose of anti-TNF mAb induced a rapid clinical improvement, a decrease in acute phase reaction, and increased lymphocyte counts in patients with active RA. The protein levels of IL1 β in the circulation were low and remained unchanged, but the systemic levels of IL1 β mRNA (p=0.002) and the concentrations of IL1 receptor antagonist (IL1ra) and IL6 (p=0.0001) had already dropped within 24 hours and this persisted up to day 14. Systemic levels of TNF α mRNA were low and remained unchanged, though total TNF α (free and bound) in the circulation increased after D2E7, probably reflecting the presence of TNF-antiTNF mAb complexes (p<0.005, at days 1 and 14). Both TNF receptors dropped below baseline levels at day 14 (p<0.005). Despite clinical improvement of arthritis, no consistent immunohistological changes were seen two weeks after anti-TNF administration. Endothelial staining for IL1 β tended to decrease in treated patients (p=0.06) but not in responders. The staining for IL1 β and TNF α in sublining layers and vessels

was mutually correlated ($r_s=0.47$ and 0.58 respectively, p<0.0005) and the microscopic scores for inflammation correlated with sublining TNF α and IL1 β scores ($r_s=0.65$ and 0.54 respectively, p<0.0001), though none of these showed significant changes during the study.

Conclusions—Blocking TNF α in RA results in down regulation of IL1 β mRNA at the systemic level and in reduction of the endogenous antagonists for IL1 and TNF and of other cytokines related to the acute phase response, such as IL6, within days. At the synovial level, anti-TNF treatment does not modulate IL1 β and TNF α in the short term. The synovial expression of these cytokines does not reflect clinical response to TNF neutralisation.

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In recent years, several approaches aimed at specific neutralisation of proinflammatory cytokines, such as interleukin 1 (IL1) and tumour necrosis factor (TNF), have proved successful in chronic inflammatory diseases, such as rheumatoid arthritis (RA)^{1–4} and Crohn's disease.^{5,6}

In patients with RA, TNF has been targeted using monoclonal antibodies (mAb), either chimeric^{2,3} or humanised,⁴ and TNF receptor-fusion proteins.^{7,8} Some of these TNF antagonists have shown their efficacy in multicentre, placebo controlled trials and have been recently approved by the FDA. A potential drawback of some TNF blocking agents is the development of human antichimeric antibodies, which may hamper or shorten the long term therapeutic effects. This complication could be overcome using mAb devoid of murine regions.

D2E7 (Knoll-BASF, Germany) is a fully human IgG1 anti-TNF α mAb with high specificity for recombinant and natural TNF α , and it is generated with phage display techniques.⁹ Studies in more than 150 patients

Department of Rheumatology, University Hospital, Nijmegen, The Netherlands

P Barrera
L A B Joosten
A A den Broeder
L B A van de Putte
P L C M van Riel
W B van den Berg

Correspondence to:
Dr P Barrera, Department of Rheumatology, University Hospital Nijmegen, PO Box 9101, 6500 HB Nijmegen, The Netherlands
p.barrera@reuma.azn.nl

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show that repeated intravenous or subcutaneous administration of D2E7 is safe and results in rapid clinical improvement in patients with active RA.¹⁰⁻¹³

Several studies with another IgG1 anti-TNF mAb have clearly shown that blocking TNF reduces the acute phase reaction and decreases the local and systemic levels of adhesion molecule in patients with RA.¹⁴⁻¹⁷ In vitro studies have also shown that neutralisation of TNF reduces the production of IL1 in synovial cultures.¹⁸ Whether such treatments also down regulate the synovial expression of IL1 and TNF in RA has not yet been fully elucidated. Observations in small numbers of patients suggest that this might be the case.^{14 19 20} In the present study we investigated the short term effects of the first dose of D2E7 or placebo on the homeostasis of the two main proinflammatory cytokines IL1 and TNF at the systemic and the synovial level.

Patients and methods

PATIENTS

Patients with RA according to the American College of Rheumatology criteria²¹ and with active disease, defined by a disease activity score (DAS)²² >3.2, who were enrolled in a double blind, multicentre clinical trial with anti-TNF α monoclonal antibody (D2E7) at our centre, were studied. Disease modifying antirheumatic drug treatment was withheld and non-steroidal anti-inflammatory drugs or low dose oral steroids (<10 mg/day), or both, were kept constant in a three week wash out period before and during the study. Patients were randomly assigned to receive 0.5, 1, 3, 5, or 10 mg/kg of D2E7 and each dose group included two patients treated with placebo, who received active drug at six weeks if they still fulfilled the entry criteria.^{11 13}

D2E7 or placebo was given as a slow intravenous infusion over three to five minutes. The preparation consists of a non-pyrogenic solution of 25 mg/ml D2E7 mAb in 1.2%

mannitol, 0.12% citric acid, 0.02% sodium citrate. The placebo consists of the same ingredients, except that D2E7 is excluded.

Results of the multicentre study including 120 patients in three centres show that clinical response is already apparent within 24 hours and maximal between one and two weeks after D2E7 administration.^{11 13} The clinical effect is maximal at a dose of 1 mg/kg and shows a plateau in the dose-response curve thereafter.^{11 13} Therefore, for the aims of this study, we focused on the short term effects seen during the first two weeks after infusion of D2E7/placebo and subdivided the patients into three dose groups consisting of placebo (n=11), 0.5 mg/kg (n=8), and 1-10 mg/kg D2E7 (n=31).

CONCENTRATIONS AND GENE EXPRESSION OF CYTOKINES IN PERIPHERAL BLOOD

Blood samples were drawn at 8-9 am on days 0, 1, and 14 after infusion in endotoxin-free Vacutainer tubes with 15% EDTA-K₃ (Becton and Dickinson, Rutherford, NJ). Samples were directly centrifuged (2250 g, 10 minutes and 15 000 g, 5 minutes) to assess circulating cytokine concentrations in platelet-free plasma. Aliquots were stored at -20°C until assay.

IL1 β was measured using a high sensitivity enzyme linked immunosorbent assay (ELISA; sensitivity 0.1 pg/ml; Quantikine HS, R&D, Minneapolis, USA) according to the manufacturer's instructions. Interleukin 1 receptor antagonist (IL1ra) was measured by radioimmunoassay (sensitivity 40 pg/ml; interassay and intra-assay variation <10%, respectively). IL6 and both soluble TNF receptors (sTNFR) were measured by ELISA (sensitivity 8 pg/ml and 0.1 ng/ml respectively) as previously described.^{23 24} Total TNF α was measured by ELISA (sensitivity 10 pg/ml). The latter had been validated in spiking experiments showing adequate TNF α recovery and no hampering by therapeutic concentrations of D2E7 (range 25-250 mg/l).¹¹

Table 1 Baseline characteristics and response percentages in this study according to the dose group

	Placebo (n=11)	0.5 mg/kg (n=8)	1-10 mg/kg (n=31)	p Value
Age, mean (SD) (years)	60 (8)	53 (18)	57 (14)	
Female (%)	73	75	55	
RF* positivity (%)	100	100	97	
Disease duration, median (p25-p75) (years)	13 (4-23)	8 (4-15)	10 (5-19)	
Previous DMARDs*, mean (SD) (n)	4.5 (1.6)	4.5 (2.6)	5 (1.7)	
Prednisone (%)	72	38	52	
DAS*, mean (SD)	4.8 (1.1)	5.2 (1.3)	5.2 (1)	
Ritchie articular index, mean (SD)	22 (13)	25 (11)	26 (11)	
Swollen joint count, mean (SD)	18 (5)	18 (5)	18 (6)	
ESR*, median (p25-p75) (mm/1st h)	24 (7-39)	15 (9-38)	36 (23-55)	0.05
CRP*, median (p25-p75) (mg/l)	23 (6-90)	22 (12-42)	63 (33-115)	0.05
Haemoglobin, mean (SD) (mmol/l)	7.6 (0.9)	7.4 (1.2)	7.2 (1.0)	
Leucocytes, median (p25-p75) ($\times 10^9/l$)	8.1 (6.3-10.5)	7.5 (6.5-8.9)	7.2 (5.9-10.4)	
Polymorphonuclear cells	5.77 (4.76-8.12)	5.07 (4.55-6.51)	4.78 (4.05-8.16)	
Lymphocytes	1.16 (0.85-1.60)	1.37 (1.08-2.26)	1.14 (0.85-1.31)	
Monocytes	0.52 (0.40-0.58)	0.42 (0.38-0.49)	0.46 (0.38-0.63)	
Platelets, mean (SD) ($\times 10^9/l$)	329 (48)	306 (65)	377 (130)	
Responders† at (%)				
2 Weeks	9	37.5	65‡	
12 Weeks	—	50	76.6	
24 Weeks	—	87.5	70	

*RF = rheumatoid factor; DMARDs = disease modifying antirheumatic drugs; DAS = disease activity score; ESR = erythrocyte sedimentation rate; CRP = C reactive protein.

†Response according to the EULAR criteria for moderate and good response.

‡16% of the patients showed clinical response already at 24 hours after 1-10 mg/kg D2E7 administration.

Aliquots of 500 μ l blood for RNA isolation were mixed with guanidinium isothiocyanate at a ratio of 1:1 and stored at -70°C . Isolation of whole blood mRNA and reverse transcriptase polymerase chain reaction (RT-PCR) analysis were performed as previously described by Netea *et al.*²⁵ Briefly, aliquots of 0.5 μ g total RNA were diluted in 20 μ l RT buffer (50 mM Tris-HCl pH 8.3, 75 mM KCl, 3 mM MgCl_2) containing 10 mM dithiothreitol, 5 μ M random hexamers, 250 μ M dNTPs, 20 U RNasin, and 200 M MLV RT. RT reaction was performed for 10 minutes at 20°C , 45 minutes

at 42°C , and 10 minutes at 95°C in a Mastercycler 5330 (Eppendorf, Hamburg, Germany). PCR reaction mixtures consisted of 3 μ l cDNA in 50 μ l PCR buffer (20 mM Tris-HCl pH 8.4, 50 mM KCl, 1.5 mM MgCl_2 , 0.001% gelatin) containing 100 μ M dNTPs, 1.25 U Taq polymerase, and 0.3 μ M of each primer.²⁵ A total of 29 PCR cycles (denaturation 30 seconds at 92°C , annealing 30 seconds at 55°C , and extension 90 seconds at 72°C) were performed for IL1 β mRNA and TNF α mRNA, and 24 cycles were performed for β_2 microglobulin. PCR products underwent electrophoresis on 2% agarose gel and were stained with ethidium bromide. Gels were scanned on a densitometer and analysed using the Molecular Analyst software. Results are expressed as a ratio of IL1 β or TNF α mRNA to the housekeeping gene.

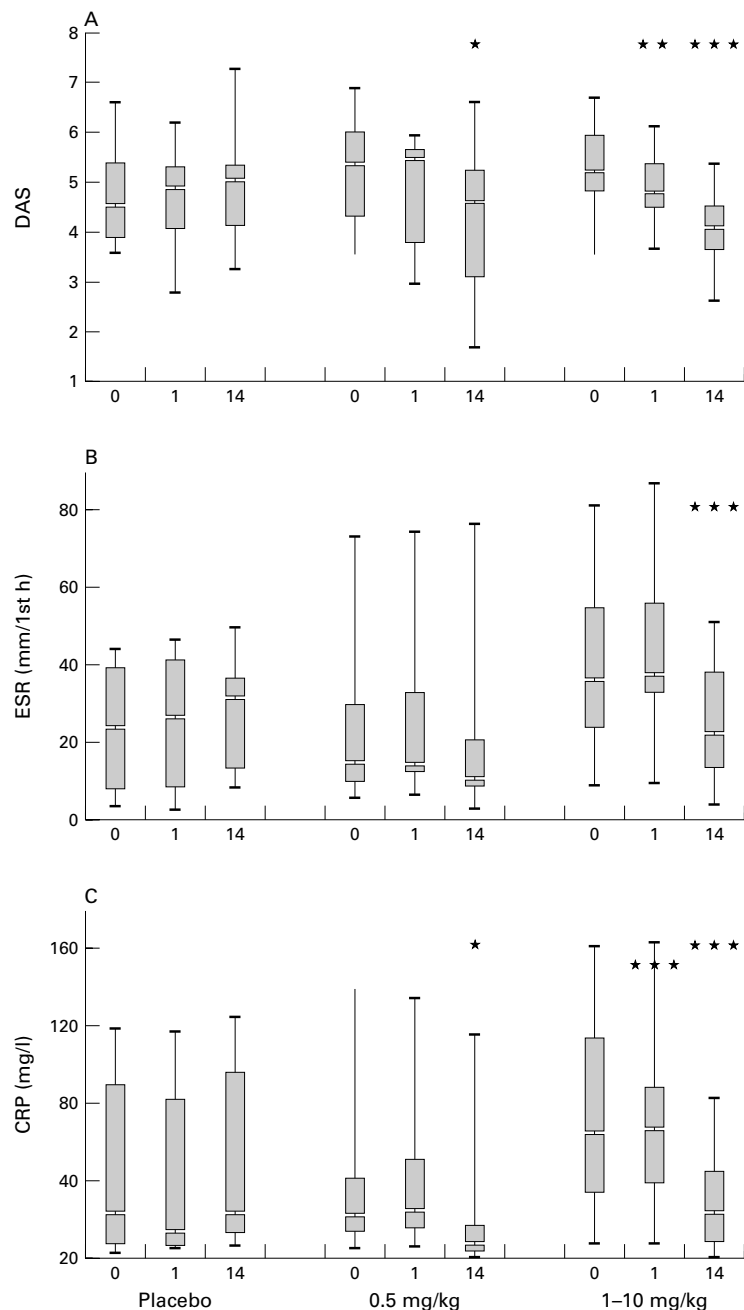


Figure 1 (A) Disease activity score (DAS), (B) erythrocyte sedimentation rate (ESR), and (C) C reactive protein (CRP) in patients receiving a single dose of placebo or 0.5 mg/kg or 1–10 mg/kg anti-tumour necrosis factor monoclonal antibody. Boxes represent the 25th, 50th, and 75th centiles; vertical lines indicate the 5th and 95th centiles. Measurements on days 0, 1, and 14 after infusion (x axis). Comparisons versus baseline shown as * $p < 0.05$, ** $p < 0.005$, and *** $p < 0.0005$.

SYNOVIAL BIOPSIES AND IMMUNOHISTOCHEMISTRY

Percutaneous synovial biopsy specimens of the knee were obtained with a Parker Pearson needle at baseline and 14 days after the first dose of D2E7/placebo in all consenting patients. Biopsy was preceded by knee joint examination for tenderness (0=none, 1=response on questioning, 2=spontaneous response, 3=withdrawal) and swelling (0=none, 1=thickening without loss of bony contours; bulge sign, 2=loss of bony contours; palpable but not tightly distending effusions, 3=bulging synovial proliferation; tightly distending effusions). Macroscopic knee joint scores were calculated by adding up the tenderness and swelling scores. An average of 30 biopsy specimens was obtained at each occasion and immediately fixed in 10% formalin and embedded in paraffin. Serial 7 μ M microtome sections were mounted on superfrost slides and either stained with haematoxylin and eosin (H&E) or used for histochemical staining as previously described.^{26,27} For each marker, all sections were stained in the same run to minimise interassay variations. Slides were incubated with anti-TNF α (IgG1, Monosan, Uden, The Netherlands) or anti-IL1 β mAb (IgM, 12E9, Oncogene Science, Manhasset, NY, USA). This primary step was followed by incubation with normal horse serum and with biotinylated horse antimurine IgG. Slices were stained with avidin peroxidase (Elite kit, Vector, Burlingame, CA), developed with diaminobenzidine and counterstained with haematoxylin for three minutes. Controls consisted of (a) irrelevant primary isotype-specific IgG1 and IgM antibodies obtained from normal horse serum, and (b) omission of the secondary antibodies.

All areas of each section were randomly analysed by two "blinded" observers using semi-quantitative five point scales. H&E sections were scored for the presence of lymphocytes, plasma cells, and polymorphonuclear cells with a scale ranging from 0 (no or minimal infiltration) to 4 (abundant inflammatory infiltrate), the lining hyperplasia was also scored (0=1–2 layers, 1=3–4 layers, 2=5–6 layers, and 3=more than 6 layers). An "inflammation score" was

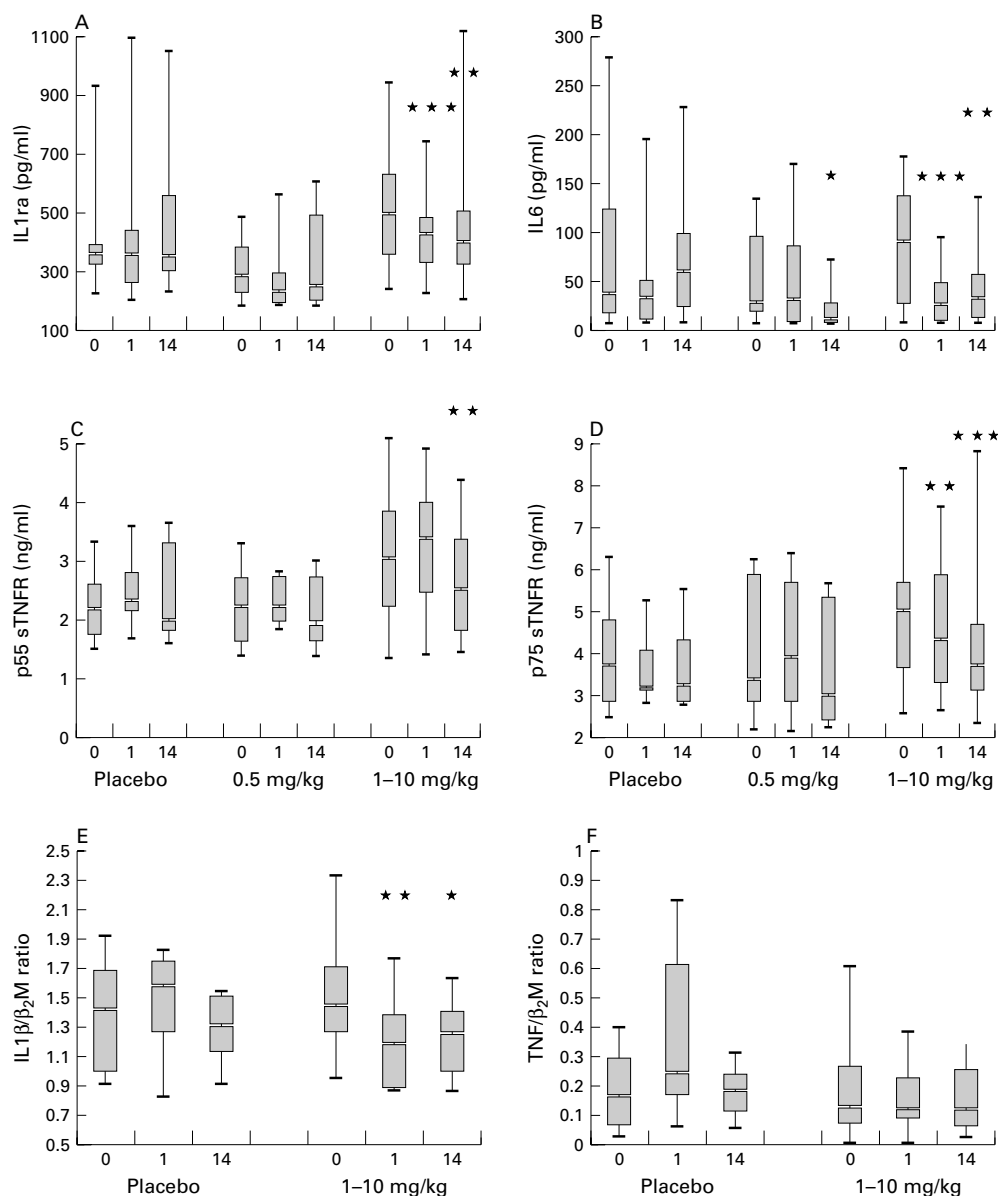


Figure 2 Circulating concentrations of (A) interleukin 1 receptor antagonist (IL1ra), (B) IL6, (C) 55th centile (p55), and (D) 75th centile (p75) soluble tumour necrosis factor receptors (sTNFR). (E) IL1 β and (F) TNFa mRNA in whole blood normalised for the presence of β_2 microglobulin (β_2 M). Boxes represent 25th, 50th, and 75th centiles; vertical lines indicate the 5th and 95th centiles. Measurements on days 0, 1, and 14 after infusion (x axis). Comparisons versus baseline shown as * p <0.5, ** p <0.005, and *** p <0.0005.

calculated by adding these four components (range 0–15), as previously described by Tak *et al.*¹⁴ The staining for IL1 and TNF in lining, sublining, and vessels was also scored semi-quantitatively on a five point scale (0 to 4). Differences in readings of one point were taken as the average, differences exceeding one point were resolved by mutual agreement.

STATISTICAL ANALYSIS

Analysis was performed using the SAS statistical package (SAS 6.04 PC version). Data are expressed as means (SD) or as median (25th (p25)–75th (p75) centile) if appropriate. Within-group comparisons were analysed by paired Student's *t* or Mann Whitney rank sum test. Baseline comparisons between groups were performed using one way analysis of variance or Kruskal-Wallis tests. Correlations are expressed using Spearman's rank correlation coefficient.

Results

CLINICAL RESULTS, ACUTE PHASE REACTION, AND LEUCOCYTE SUBSETS

Table 1 summarises the baseline characteristics of the patients included in the present study. Patients were subdivided into groups receiving placebo, 0.5 mg/kg D2E7, and 1–10 mg/kg D2E7 because, as previously mentioned, the dose-response curve in the multicentre study (n=120) showed a plateau at doses of 1 mg/kg D2E7.^{11 13}

Administration of D2E7, but not placebo, rapidly reduced disease activity as measured by the DAS, a composite disease activity score²² (fig 1A), and its individual components, including swollen and tender joint counts, patient well-being (data not shown), and erythrocyte sedimentation rate (ESR; fig 1B). In the group treated with 1–10 mg/kg D2E7, the decrease in

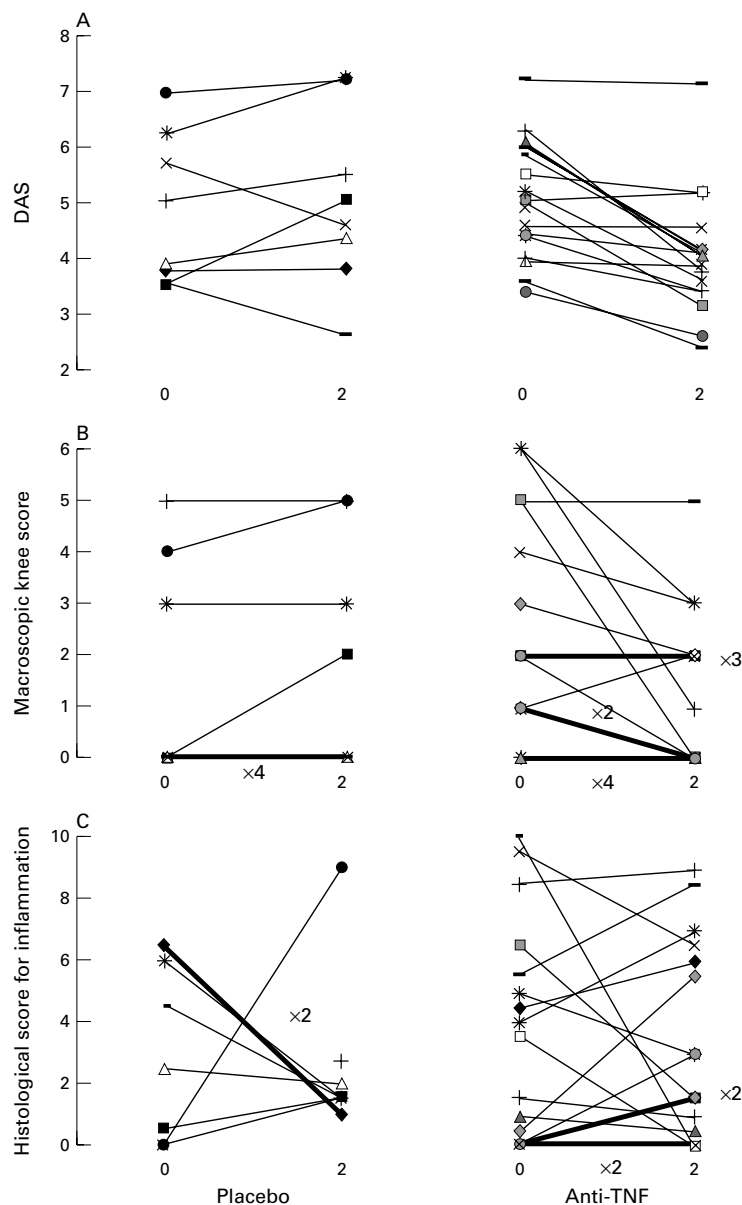


Figure 3 (A) Disease activity score (DAS), (B) pain and swelling scores, and (C) histological scores for inflammation in 25 patients undergoing serial biopsies. Time in weeks and placebo ($n=8$) or anti-tumour necrosis factor (anti-TNF) treatment ($n=17$) shown in the x axis. Scores for more than one patient (\times exact number) shown as bold lines.

DAS was already significant within 24 hours after the infusion (mean (SD) 4.87 (0.79) *v* 5.25 (0.95); $p<0.002$) and reached a nadir at week 2 (4.06 (0.85), $p<0.0001$). The 0.5 mg/kg group showed a drop in DAS values significant at week 2 ($p<0.01$). At this time point, 20/31 (65%) and 3/8 (38%) of the patients treated with 1–10 and 0.5 mg/kg D2E7, respectively, fulfilled the EULAR criteria for clinical response²⁸ in contrast with only 1/11 (9%) patients receiving placebo. Five (16%) patients in the 1–10 mg/kg group showed clinical response already 24 hours after infusion.

Baseline acute phase reaction parameters, such as the ESR, C reactive protein (CRP), and platelet counts tended to be higher in the 1–10 mg/kg group (table 1) and were also significantly decreased two weeks after administration of anti-TNF mAb but unchanged or

increased in the placebo group (figs 1B and C). The 0.5 mg/kg group, which included fewer patients ($n=8$), also showed a clear decrease in ESR and CRP after two weeks.

Baseline white blood cell and leucocyte subset counts were similar in all groups (table 1). Total white blood cell counts did not change during the study (data not shown), but a rise in day 1 lymphocyte counts was seen in all groups, being more marked in patients receiving 1–10 mg/kg D2E7 (median 14.5% before infusion *v* 22% at day 1; $p<0.01$). In this group only, the lymphocyte counts were still higher than baseline at day 14. Conversely, there was a drop in day 1 polymorphonuclear cell counts in all groups, which only reached significance in the 1–10 mg/kg group at day 1 (median 77% at baseline *v* 66.5% at day 1; $p=0.02$).

SYSTEMIC CYTOKINE MEASUREMENTS

Baseline IL1 β and TNF α levels were below or around the detection limit of the radioimmunoassays currently used in our laboratory and similar to those found in healthy subjects (data not shown). A high sensitivity ELISA (Quantikine HS, R&D, Minneapolis, USA) showed that IL1 β protein levels were low and did not change significantly after the first dose of D2E7 or placebo (median levels at baseline, day 1, and day 14 were 0.55, 0.4, and 0.5 pg/ml after 1–10 mg/kg D2E7 and remained at 0.6 pg/ml before and after placebo administration).

Mean baseline TNF α levels (free and bound) measured by ELISA did not change after infusion of placebo but increased after D2E7 administration (41 pg/ml at baseline *v* 76 and 63 pg/ml at days 1 ($p<0.0001$) and 14 ($p<0.01$), respectively), which may reflect the formation of circulating TNF-anti-TNF complexes.

To complement the data at the protein level, systemic IL1 β and TNF α mRNA levels in whole blood were measured in the placebo and 1–10 mg/kg D2E7 group by RT-PCR. Baseline IL1 β / β_2 microglobulin ratios in patients with RA were raised compared with those in healthy controls (median (range) 1.45 (0.57–2.95) *v* 0.35 (<0.10–0.69)). As shown in fig 2, IL1 β mRNA expression decreased within 24 hours after D2E7 administration ($p=0.002$) and remained lower than baseline at day 14 ($p=0.007$), whereas no significant changes occurred after placebo. Systemic TNF α mRNA levels were lower than those of IL1 β , overlapped largely with those found in healthy controls (median (range) 0.13 (<0.10–0.85) in RA *v* <0.10 (<0.10–0.30) in controls), and remained unchanged during the study. Systemic levels of IL1 β and TNF α mRNA were not correlated (r_s (95%CI)=0.13 (–0.18 to 0.43)).

The concentrations of IL1ra and IL6 decreased sharply after D2E7 administration ($p<0.0001$ and <0.005 at days 1 and 14, respectively, in the 1–10 mg/kg group). Both sTNFR types decreased also after treatment. In patients receiving 1–10 mg/kg D2E7, the p75 levels dropped from median baseline values of 5 ng/ml to 4.3 ($p<0.005$) and 3.7 ng/ml

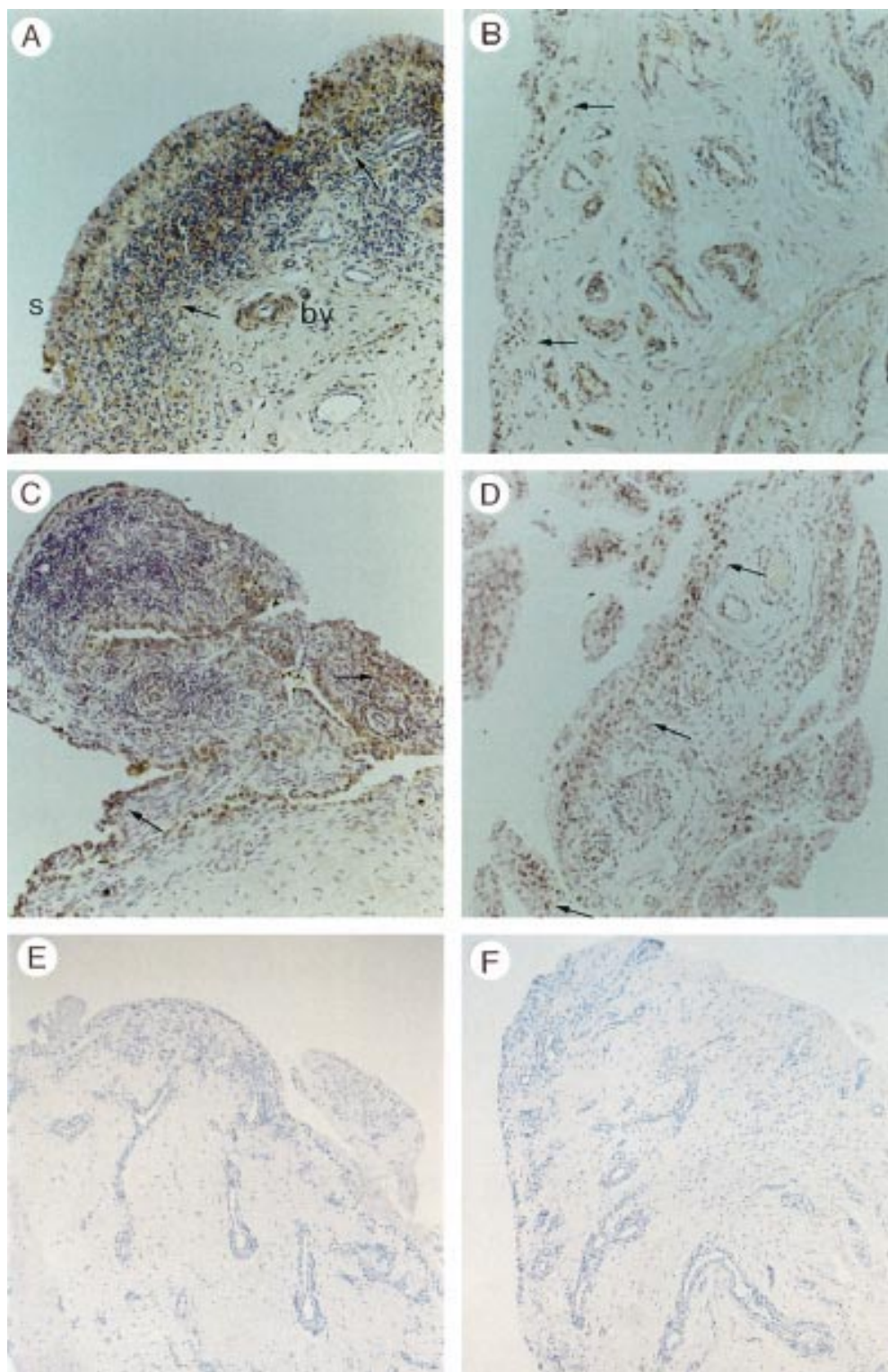


Figure 4 Immunohistochemical staining in the same patient at baseline ((A) tumour necrosis factor α (TNF α), (B) interleukin 1 β (IL1 β)) and 14 days after ((C) TNF α , (D) IL1 β) the first dose of anti-TNF α . Note the unchanged expression of cytokines in the synovial lining (s, arrows), sublining, and blood vessels (bv). No IL1 β or TNF α staining was seen in controls with (E) an irrelevant primary antibody and (F) when the secondary antibody was omitted. Original magnification $\times 200$.

($p=0.0001$) after 1 and 14 days respectively and the p55 sTNFR levels fell from median baseline values of 3.1 ng/ml to 2.5 ng/ml at day 14 ($p<0.002$) (fig 2).

Positive correlations between acute phase reactants and the endogenous antagonists of IL1 and TNF were found: CRP measurements

correlated with the levels of IL1ra ($r_s=0.47$; $p<0.001$), p55 sTNFR ($r_s=0.66$; $p<0.0001$) and IL6 ($r_s=0.65$; $p<0.0001$), whereas ESR levels correlated with p75 sTNFR ($r_s=0.51$; $p<0.002$). In contrast, neither TNF α nor IL1 β , at protein or mRNA level, were related to acute phase parameters.

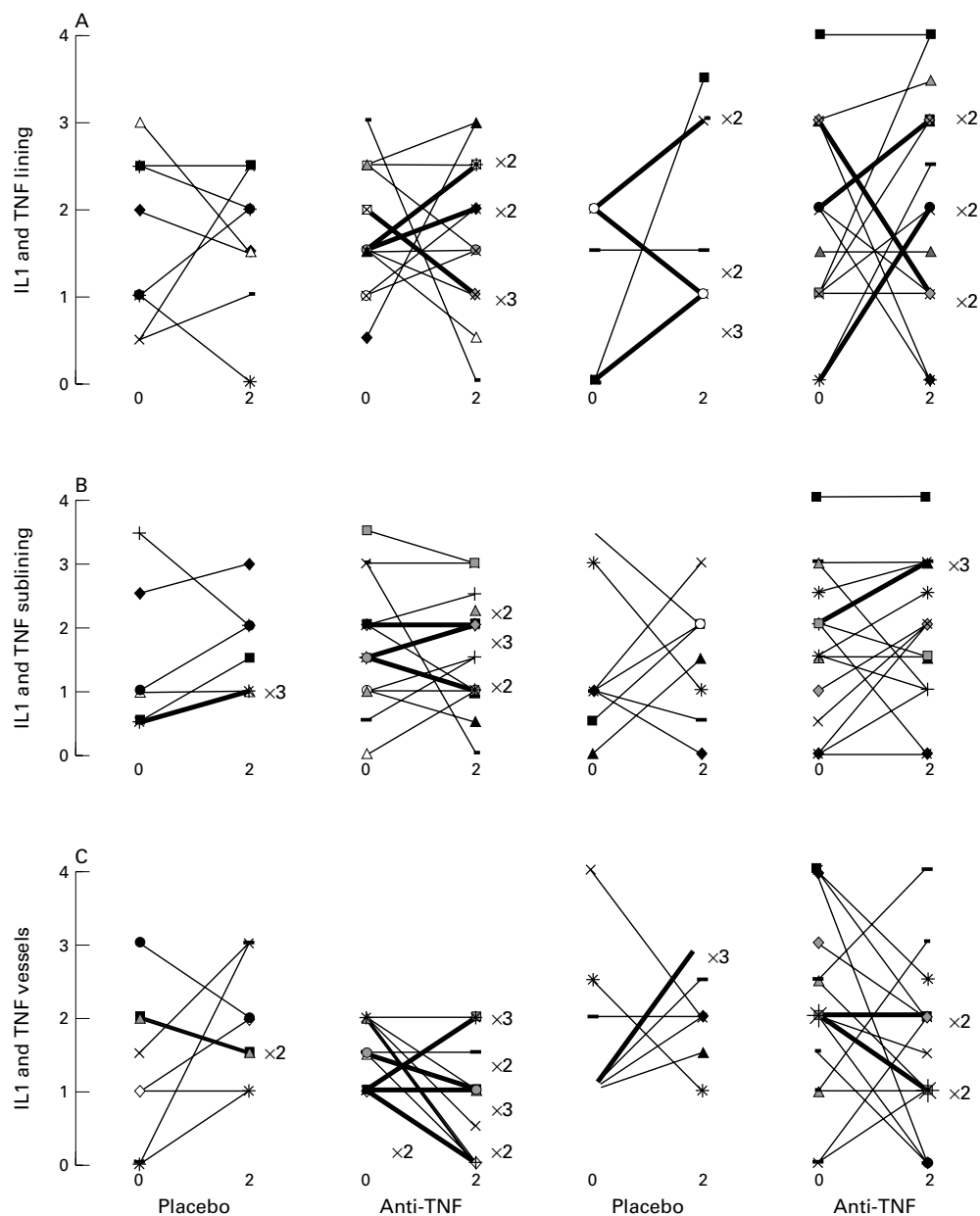


Figure 5 Semiquantitative scores for interleukin 1 β (IL1 β ; left columns) and tumour necrosis factor α (TNF α ; right columns). Staining in (A) lining, (B) sublining, and (C) vessels in biopsy specimens taken at baseline and day 14 after administration of placebo (n=8) or anti-TNF treatment (n=17). Scores for more than one patient (\times exact number) shown as bold lines.

IMMUNOHISTOLOGICAL FINDINGS

A total of 25 patients underwent synovial biopsies at baseline and 14 days after infusion of placebo (n=8) or 1–10 mg/kg D2E7 (n=17). At this time in the treated group, the DAS had dropped from 5.0 (1.0) to 4.0 (1.1) ($p < 0.0005$), and 11 patients fulfilled the EULAR criteria for clinical response.²⁸ Conversely, DAS scores remained unchanged or increased (4.8 (1.3) at baseline *v* 5.1 (1.6) after 14 days) in the placebo group, where only one patient fulfilled the EULAR response criteria²⁸ (fig 3A). The macroscopic joint scores for swelling and pain at the biopsied joint were unchanged two weeks after placebo but decreased in treated patients ($p = 0.02$; fig 3B). The microscopic scores for inflammation¹⁴ showed a more scattered pattern and no

significant changes in either group (fig 3C). Baseline microscopic inflammation scores were weakly correlated with the macroscopic scores for pain and swelling ($r_s = 0.3$; $p < 0.05$).

TNF α and IL1 β staining were seen in all layers of most biopsy specimens. The scores for both cytokines in the lining, sublining, and especially in vessels were mutually correlated ($r_s = 0.43$; $p = 0.002$ for the lining and $r_s = 0.47$ and 0.58 ; $p < 0.0005$ for the sublining and vessels, respectively). Nevertheless, some differences were found in the staining patterns of both cytokines: TNF showed a more dense and intense staining in sublining layers, lymphocyte aggregates, and vessels than IL1, which tended to be more diffuse (fig 4). The microscopic scores for inflammation were positively correlated with the scores for TNF

and IL1 in the sublining ($r_s=0.65$ and 0.54 respectively; $p<0.0001$) and to a lesser extent with the TNF staining in the lining ($r_s=0.38$; $p=0.005$).

Figure 5 shows the individual synovial scores for IL1 and TNF after administration of anti-TNF mAb or placebo. As shown, there was a marked interindividual and intra-individual variation in the synovial staining for these cytokines. Decreases greater than one point in the scores for IL1 β or TNF α were rare among placebo treated patients. On the other hand, both increased and decreased scores for IL1 β and TNF α were detected in the lining and sublining of patients treated with anti-TNF. With the exception of a trend towards a decrease in the IL1 β staining in vessels among the treated patients ($p<0.06$), no significant changes were seen.

Additionally, analysis was performed according to the clinical response achieved at day 14 after infusion. At this time point, a total of 12 patients (all but one in the group treated with anti-TNF) were responders according to the EULAR criteria²⁸ (DAS decreased from 4.9 (1.1) to 3.4 (0.6); $p<0.0001$), whereas 13 patients, including seven who had received placebo, were non-responders (DAS 5.1 (1.2) *v* 5.2 (1.2)). Macroscopic knee joint score for swelling and pain dropped from 2 (2.3) to 0.8 (1.1) ($p<0.05$) among responders but remained unchanged (2.2 (2.0) *v* 2.2 (2.0)) in the non-responders.

In contrast with the clinical improvement observed in responders, the microscopic inflammation scores (H&E) and the staining for IL1 β or TNF α in lining, sublining, and vessels were not significantly changed from baseline compared with day 14 (data not shown). Subgroup analysis taking into account the individual dose of D2E7 given gave the same results.

Discussion

Our study shows that in patients with active RA, a single dose of human anti-TNF antibody is clinically effective and results in rapid down regulation of IL1 β mRNA at the systemic level. Such effect was seen in unstimulated conditions, using a sensitive PCR, normalised for the presence of β_2 microglobulin, which corrects for fluctuations in peripheral blood mononuclear cell (PBMC) counts. The inhibition of IL1 by anti-TNF mAb treatment occurred at the transcriptional level and seemed rather specific. TNF neutralisation did not alter the levels of TNF α message in the circulation, and no change in IL1 β or TNF α mRNA expression was seen in the placebo group.

The production of both IL1 and TNF is transcriptionally regulated^{29,30} and both cytokines share many proinflammatory effects. Therefore, a decrease in IL1 message might play a part in at least some of the downstream changes seen after treatment with this and other anti-TNF mAb. These include inhibition of IL6 and acute phase reaction, chemokines and nitric oxide production, and a decrease in angiogenesis, endothelial activation, and cartilage breakdown markers^{16,17,31}

In our study, baseline mRNA levels of IL1 β in whole blood were raised in patients with RA compared with normal controls. Raised levels of mRNA for IL1 and other proinflammatory cytokines such as IL8 have also been measured in isolated PBMC of patients with RA,^{32,33} and these may reflect an increased activation status of PBMC in the circulation. Systemic TNF mRNA levels were lower, and did not correlate with those of IL1 mRNA, which is in line with the earlier reported differential regulation of IL1 and TNF in PBMC^{30,32} and in whole blood.²⁹

Inhibition of the synthesis of IL1 β after blocking TNF has been shown to occur in rheumatoid synovial cultures.^{18,34} Direct evidence for such an effect in patients with RA has been hard to find because, as we and others have shown,¹⁷ protein levels of IL1 β in the circulation are mostly low or undetectable.

Indirect evidence for an effect of blocking TNF on the homeostasis of IL1 can be sought in the effects of such treatment on the concentrations of its natural antagonist IL1ra. This and other studies³⁵ have shown that the levels of ILra are raised in patients with RA and positively correlated with acute phase reaction parameters and with IL6 levels. It has been proposed that in RA, the raised levels of IL1ra may reflect an increased production or activity of IL1, or both.³⁵ Treatment with D2E7 diminished the levels of IL1ra and IL6 with similar kinetics, and such effects have also been reported after the administration of cA2, a chimeric anti-TNF monoclonal antibody, in patients with RA.^{15,36}

The fully human anti-TNF α antibody used in this study is of the same isotype and is as clinically efficacious and has other systemic effects in common with the chimeric IgG1 anti-TNF α mAb, infliximab. One of those is the reduction of circulating levels of both soluble TNF receptors after treatment. This has led to the hypothesis that anti-TNF mAb act mainly through neutralisation of TNF because this cytokine is a major regulator of TNFR release.¹⁷ However, decreases in circulating sTNFR levels are not specific for anti-TNF mAb treatment and probably mirror the acute phase reaction.²⁴ Another common effect of both anti-TNF antibodies was a transient rise in lymphocyte counts and a reciprocal drop in granulocyte counts,¹⁷ which has not been reported after blocking TNF with TNFR:Fc fusion proteins. Whether this rise in lymphocytes is due to a deactivation of the vascular endothelium, as proposed,³⁷ or is a non-specific phenomenon, as suggested by the similar trends seen in the placebo group, remains to be elucidated.

At the target organ level, significant reductions of pain and swelling at the biopsied joint occurred concomitantly with the systemic improvement seen two weeks after D2E7 but not after placebo. The improvement in the macroscopic inflammation scores was also evident when analysed according to the clinical response. The microscopic inflammation scores (H&E) and the staining for IL1 β and TNF α in lining, sublining, and vessels showed

large intra-individual variations. Except for a trend towards a decrease in the endothelial staining for IL1 β among treated patients we found no consistent changes two weeks after the start of anti-TNF treatment.

Previously, Tak *et al* reported a reduction in T cells and in the expression of adhesion molecules in RA synovial tissue after TNF neutralisation.^{14, 20} It has also been suggested that the synovial expression of IL1 β and TNF α decreases four weeks after an initial dose of anti-TNF using other TNF blocking strategies. This was either reported in a limited number of patients¹⁴ or in abstract form, or both,^{19, 20} which precludes firm conclusions.

Our results are in line with recent observations in experimental arthritis, which also show that anti-TNF treatment significantly reduces joint swelling³⁸ but leaves the production of IL1 in the synovium unaltered.³⁹ That the local production and expression of IL1 during arthritis is not driven by TNF has been elegantly demonstrated in TNF knockout mice.³⁹ After induction of arthritis in the latter, there is a lack of joint swelling and a marked reduction in the late synovial infiltrate but continuing local production of IL1. Interestingly, the amelioration in swelling and infiltrate in these mice is abolished if the membrane-bound TNF is reintroduced into the model.⁴⁰

Taken together, our results confirm that TNF neutralisation with anti-TNF mAb in RA is very effective in controlling clinical inflammation and decreasing the acute phase reaction in patients with active RA. Blocking TNF inhibits IL1 β at the transcriptional level in the circulation and this may be implicated in its mechanism of action. Our study further confirms that the effect of anti-TNF mAb is not due to up regulation of the endogenous antagonists for IL1 and TNF because these decrease during treatment mirroring the acute phase reaction.

TNF neutralisation has a rapid clinical effect and our goal was to analyse short term systemic and local changes in the homeostasis of IL1 and TNF. In the short term, blocking TNF did not modify the local expression of IL1 β or TNF α or the histological inflammation scores in the joint. Local changes, therefore, do not precede, or occur concomitantly, with systemic improvement. Whether such changes occur in a delayed fashion is now subject to further studies.

Our results corroborate the discordance in macroscopic and microscopic signs of synovial inflammation. Moreover, they suggest that the synovial expression of TNF and IL1 does not relate to concomitant signs of synovitis. Whether the former is predictive for future joint damage has not yet been elucidated. None the less, in view of the independent regulation of these cytokines, future therapeutic efforts which combine neutralisation of IL1 and TNF are warranted in patients with RA.

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