

## CONCISE REPORT

# Up regulation of the production of tumour necrosis factor $\alpha$ and interferon $\gamma$ by T cells in ankylosing spondylitis during treatment with etanercept

J Zou, M Rudwaleit, J Brandt, A Thiel, J Braun, J Sieper

*Ann Rheum Dis* 2003;62:561–564

**Background:** Treatment of active ankylosing spondylitis (AS) with the recombinant, soluble tumour necrosis factor  $\alpha$  (TNF $\alpha$ ) receptor molecule etanercept has been shown to be clinically highly effective. The precise mechanism of action, however, is not known.

**Objective:** To assess the change in the cytokine secreting ability of CD4+ and CD8+ T cells and macrophages during etanercept treatment.

**Patients and methods:** Peripheral blood mononuclear cells from 10 patients with AS treated with 25 mg etanercept and 10 patients with AS treated with placebo were investigated during treatment given twice weekly subcutaneously. Production of cytokines by T cells was investigated after in vitro stimulation by flow cytometry.

**Results:** Twelve weeks of etanercept treatment induced a significant increase in the number of interferon  $\gamma$  (IFN $\gamma$ ) positive (14.2% (9.6–19.5%) before v 24.4% (13.4–36.4%) after) and TNF $\alpha$  positive CD4+ T cells ( $p=0.008$  for both cytokines) and IFN $\gamma$  positive (37.5% (19.0–45.4%) before v 52.9% (33.2–60.0%) after) and TNF $\alpha$  positive CD8+ T cells ( $p=0.008$  for both cytokines) upon phorbol myristate acetate/ionomycin stimulation, but not in the placebo group. Furthermore, etanercept treatment induced a significant increase in the number of IFN $\gamma$  positive CD8+ T cells ( $p=0.024$  at 12 weeks) and a non-significant increase of TNF $\alpha$  positive CD8+ T cells after in vitro stimulation with the aggrecan derived peptides.

**Conclusions:** Neutralisation of peripheral TNF $\alpha$  does not induce a down regulation of the ability of T cells to produce TNF $\alpha$  but rather an up regulation, possibly due to a counterregulatory mechanism.

In the past decade it has been shown that tumour necrosis factor alpha (TNF $\alpha$ ) is a major contributor to the pathogenesis of synovitis and joint destruction in rheumatoid arthritis (RA). To date, two forms of TNF inhibition therapy have been extensively investigated in RA. Both the TNF receptor-Fc fusion protein (TNFR:Fc, etanercept) and the chimeric anti-TNF $\alpha$  monoclonal antibody (infliximab) have been proved to be highly active for the treatment of RA<sup>1,2</sup> and psoriatic arthritis.<sup>3,4</sup>

We showed previously that TNF $\alpha$  is highly expressed in biopsy samples taken from sacroiliac joints of patients with ankylosing spondylitis (AS).<sup>5</sup> Encouraged by this finding and by the positive results of TNF blockade in other diseases, we showed recently that the disease activity index of patients with AS improved impressively when they were treated with the anti-TNF $\alpha$  monoclonal antibody infliximab.<sup>6</sup> Subsequently, in a placebo controlled trial a similar efficacy was shown for etanercept in the treatment of AS.<sup>7</sup>

Most recently, we also carried out a placebo controlled study with etanercept in 30 patients with AS. We obtained a similar good response<sup>8</sup> compared with the other etanercept studies in AS<sup>7</sup> and compared with our infliximab study in AS.<sup>6</sup> We took advantage of this trial and investigated longitudinally the T cell cytokine pattern during the etanercept study both in the etanercept and in the placebo group during treatment to examine the mechanism of etanercept treatment.

## PATIENTS AND METHODS

### Patients and design of the clinical study

In this placebo controlled double blind study 10 patients were randomly assigned to receive etanercept and 10 patients to placebo treatment. In the etanercept group patients were treated with 25 mg etanercept given twice weekly subcutaneously for 12 weeks, while patients of the placebo group were treated with placebo for six weeks and afterwards were switched to etanercept treatment for another six weeks.<sup>8</sup> Cytokines were investigated in both groups before, six weeks, and 12 weeks after treatment.

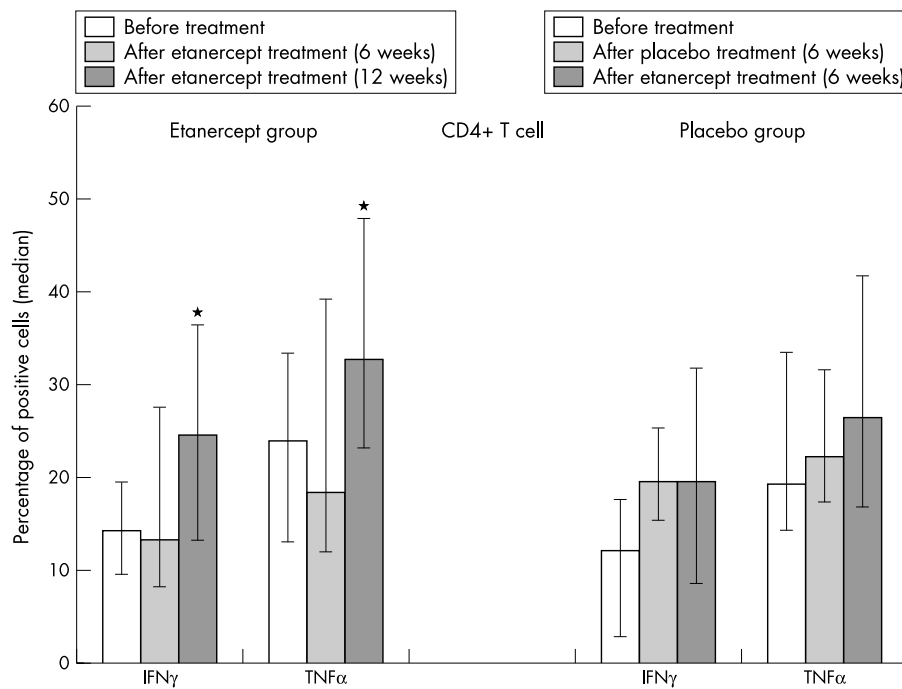
### In vitro stimulation of T cells by non-specific and specific antigen and analysis by flow cytometry

Peripheral blood mononuclear cells ( $1 \times 10^6$ ) were cultured for six hours either in the presence of 5 ng/ml phorbol myristate acetate (PMA; Sigma, St Louis, MO) plus 1 ng/ml ionomycin (Sigma, St Louis, MO), or stimulated with a pool of 46 overlapping 18-mer peptides (5  $\mu$ g/ml for each peptide) derived from the G1 domain of aggrecan in the presence of anti-CD28 (1  $\mu$ g/ml; Immunotech, Marseille, France), as described previously.<sup>9</sup> After this, cells were fixed and quadruply stained for surface markers CD3 (PE) and CD8 (Perc P) (Becton Dickinson, Heidelberg, Germany) and for two intracellular cytokines, either interferon  $\gamma$  (IFN $\gamma$ ; FITC)/interleukin (IL)4 (APC) or TNF $\alpha$  (FITC)/IL10 (APC) (Pharmingen, San Jose, CA). Positive cells were subsequently quantified by flow cytometry using a FACSCalibur from Becton Dickinson (San Jose, CA) with Cellquest software.<sup>9</sup>

### Statistical analysis

Because numbers in each groups were small the Wilcoxon test was used to compare cytokine production before and after treatment in the etanercept group, and before and after placebo or etanercept treatment in the placebo group. The medians and the 25th to 75th centiles are given. Differences were considered to be significant with a two tailed  $p$  value of  $<0.05$ .

**Abbreviations:** AS, ankylosing spondylitis; IFN $\gamma$ , interferon  $\gamma$ ; IL, interleukin; PMA, phorbol myristate acetate; RA, rheumatoid arthritis; TNF $\alpha$ , tumour necrosis factor  $\alpha$



**Figure 1** A significant increase in the production of IFN $\gamma$  and of TNF $\alpha$  by CD4+ T cells upon PMA/ionomycin stimulation was observed after 12 weeks of etanercept treatment in the etanercept group. \* $p=0.008$ , compared with before treatment, but no change in the cytokine production was detected after six weeks of etanercept treatment in the placebo group. The medians and ranges of non-specific cytokine production are indicated in the figure.

## RESULTS

### Etanercept treatment induces an increase in the number of IFN $\gamma$ and TNF $\alpha$ positive T cells after non-specific in vitro stimulation

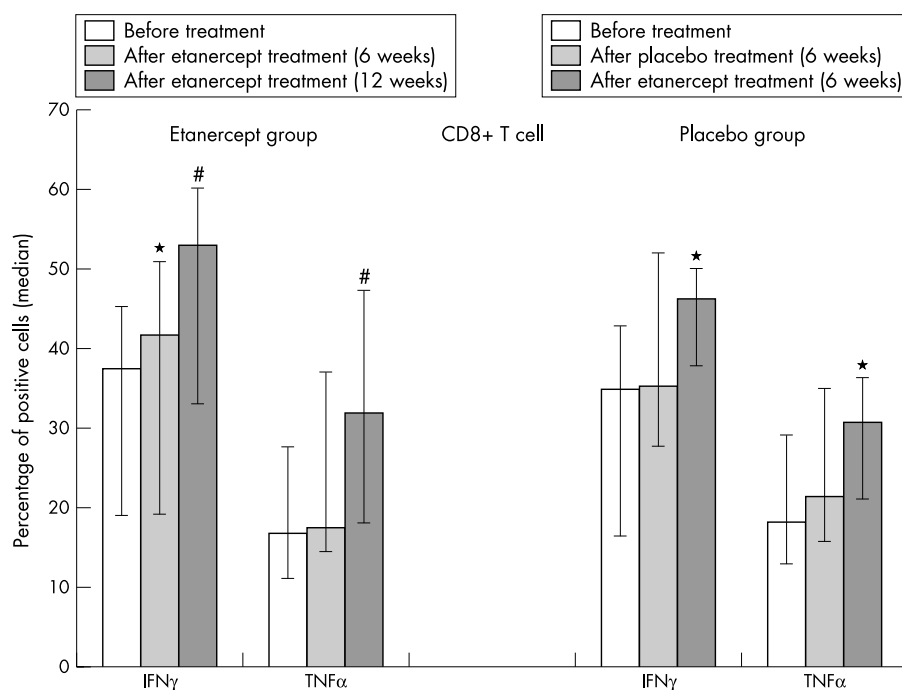
For the etanercept group, a significant change in the IFN or TNF $\alpha$  production by CD4+ T cells could be seen after 12 weeks of treatment but not yet after six weeks upon PMA/ionomycin stimulation at this time (fig 1).

In the CD8+ T cell population, six weeks of etanercept treatment induced a significant increase in the number of IFN $\gamma$  positive ( $p<0.05$ ) but not yet in the number of TNF $\alpha$  positive CD8+ T cells (fig 2). The increase became even clearer for cytokine production of CD8+ T cells after 12 weeks of etanercept treatment (fig 2).

In contrast, no change in the number of IFN or TNF $\alpha$  positive CD4+ and CD8+ T cells was seen during six weeks of placebo treatment upon PMA/ionomycin stimulation (figs 1 and 2). When these patients were switched to etanercept treatment, a similar increase in the cytokine production by CD8+ T cells was seen ( $p<0.05$  for difference between before and after etanercept treatment for both cytokines) (fig 2) but only a non-significant increase occurred for these cytokines secreted by CD4+ T cells (fig 1).

### Etanercept treatment induces an increase of IFN $\gamma$ production by CD8+ T cells after antigen-specific in vitro stimulation

After six weeks of etanercept treatment, a significant increase of IFN $\gamma$  positive but not of TNF $\alpha$  positive cells was found in the



**Figure 2** A significant increase in the production of IFN $\gamma$  and of TNF $\alpha$  by CD8+ T cells upon PMA/ionomycin stimulation is seen during etanercept treatment. No change in the cytokine production was detected after six weeks of treatment in the placebo group, but a significant increase in the production of IFN $\gamma$  and of TNF $\alpha$  by CD8+ T cells upon PMA/ionomycin stimulation was seen after the patients were switched to etanercept treatment. The median and range of non-specific cytokine production are indicated in the figure. \* $p<0.05$  compared with before treatment; # $p=0.008$  compared with before treatment.

**Table 1** IFN $\gamma$  production by CD8+ T cells after etanercept treatment

	IFN $\gamma$ /TNF $\alpha$ positive CD8+ T cells (%)	
	IFN $\gamma$	TNF $\alpha$
Etanercept group		
Before treatment	0.13 (0.02–0.21)	0.17 (0.03–0.46)
After etanercept treatment (6 weeks)	0.22 (0.06–0.29)*	0.24 (0.04–0.49)
After etanercept treatment (12 weeks)	0.23 (0.04–0.37)*	0.19 (0.04–0.3)
Placebo group		
Before treatment	0.19 (0.05–0.35)	0.14 (0.04–0.3)
After placebo treatment (6 weeks)	0.2 (0.03–0.38)	0.18 (0.03–0.22)
After etanercept treatment (6 weeks)	0.25 (0.04–0.48)*	0.23 (0.07–0.38)

Percentage of cytokine positive cells among the CD8+ T cell subpopulation upon in vitro stimulation with the overlapping peptides derived from the G1 domain of aggrecan: median (25th–75th centiles).  
\* $p < 0.05$ , compared with the value before treatment.

CD8+ subpopulation and a similar result was found after 12 weeks of treatment (table 1). There was no change in the number of IFN and TNF $\alpha$  positive CD4+ T cells after treatment upon in vitro stimulation with aggrecan G1 peptides.

No change was seen in the placebo group, but IFN $\gamma$  positive antigen-specific CD8+ T cells increased significantly after the patients were treated with placebo.

#### No change in IL4 or IL10 positive T cells during treatment

In both the etanercept and the placebo group, no significant changes of non-specific IL4 and IL10 secretion were seen during treatment (data not shown). G1 peptide-specific stimulation of T cells did not induce IL4 or IL10 secretion before or after treatment.

#### DISCUSSION

In this study we showed that treatment of patients with active AS with the soluble TNF receptor etanercept increases both TNF $\alpha$  secretion and IFN $\gamma$  secretion in the CD4+ T cell subpopulation after non-specific in vitro stimulation (at 12 weeks), but even more clearly in the CD8 subpopulation (at six and 12 weeks). We also observed an increase in the antigen-specific IFN $\gamma$  production of CD8+ T cells at six and 12 weeks, but no change occurred during placebo treatment. Interestingly, there was no clear effect on monocyte production of TNF $\alpha$  upon in vitro stimulation with lipopolysaccharide during treatment (data not shown).

It has been suggested that TNF $\alpha$  has an inhibitory effect on T cell function, which can be restored by TNF $\alpha$  blockade.<sup>10–12</sup> However, although our results are compatible with such an assumption, we showed that, in contrast with our study with etanercept, during treatment of patients with AS with infliximab both the non-specific and antigen-specific secretion of TNF $\alpha$  and IFN $\gamma$  by T cells is significantly down regulated, a finding which also correlated well with a good clinical response.<sup>6–13</sup> Thus, direct inhibition of TNF $\alpha$  by the TNF $\alpha$  blocking agents, and not restoration of immunosuppression induced by the disease, seems to be important. Although etanercept and infliximab seem to be similarly effective in the treatment of RA<sup>12</sup> and AS,<sup>6–8</sup> there are two important clinical differences: (a) infliximab is highly effective in Crohn's disease,<sup>14</sup> whereas etanercept is not<sup>15</sup>; (b) infliximab treatment seems to be associated with an increased rate of infection with *Mycobacterium tuberculosis*, whereas patients treated with etanercept seem to be less affected.<sup>16</sup> Whether a differential effect of the two drugs on T cell function can offer an explanation for this difference remains to be shown.

This small increase in the ability of the T cells to produce TNF $\alpha$  and IFN $\gamma$  was accompanied by a good clinical effect,<sup>8</sup> indicating that neutralisation of soluble TNF without affecting

T cell function is efficient. This increase might be explained by a counterregulatory effect on T cells.

#### ACKNOWLEDGEMENTS

We thank Peihua Wu, Martina Grolms, and all the doctors in the department of rheumatology for providing samples from patients and for technical assistance.

#### Authors' affiliations

**J Zou, M Rudwaleit, J Brandt, J Braun, J Sieper**, Rheumatology, Benjamin Franklin Klinikum, Deutsches RheumaForschungszentrum, Berlin, Germany

**A Thiel, J Sieper**, German Rheumatology Research Centre, Berlin, Germany

**J Braun**, Rheumazentrum Herne, Germany

Correspondence to: Professor J Sieper, Medical Department I, Rheumatology, University Hospital Benjamin Franklin, Hindenburgdamm 30, 12200 Berlin, Germany; hjsieper@zedat.fu-berlin.de

Accepted 9 December 2002

#### REFERENCES

- Lipsky PE, van der Heijde DM, St Clair EW, Furst DE, Breedveld FC, Kalden JR, *et al.* Infliximab and methotrexate in the treatment of rheumatoid arthritis. Anti-Tumor Necrosis Factor Trial in Rheumatoid Arthritis with Concomitant Therapy Study Group. *N Engl J Med* 2000;343:1594–602.
- Moreland LW, Baumgartner SW, Schiff MH, Tindall EA, Fleischmann RM, Weaver AL, *et al.* Treatment of rheumatoid arthritis with a recombinant human tumor necrosis factor receptor (p75)-Fc fusion protein. *N Engl J Med* 1997;337:141–7.
- Mease PJ, Goffe BS, Metz J, VanderStoep A, Finck B, Burge DJ. Etanercept in the treatment of psoriatic arthritis and psoriasis: a randomised trial. *Lancet*. 2000;356:385–90.
- Antoni C, Dechant C, Hanns-Martin Lorenz PD, Wendler J, Ogilvie A, Luefl M, *et al.* Open-label study of infliximab treatment for psoriatic arthritis: clinical and magnetic resonance imaging measurements of reduction of inflammation. *Arthritis Rheum* 2002;47:506–12.
- Braun J, Bollow M, Neure L, Seipelt E, Seyrekbasan F, Herbst H, *et al.* Use of immunohistologic and in situ hybridization techniques in the examination of sacroiliac joint biopsy specimens from patients with ankylosing spondylitis. *Arthritis Rheum* 1995;38:499–505.
- Braun J, Brandt J, Listing J, Zink A, Alten R, Krause A, *et al.* Treatment of active ankylosing spondylitis with infliximab—a double-blind placebo controlled multicenter trial. *Lancet* 2002;359:1187–9.
- Gorman JD, Sack KE, Davis JC. Etanercept in the treatment of ankylosing spondylitis: a randomized, double-blind, placebo-controlled study [abstract]. *Arthritis Rheum* 2000;43(suppl):S403.
- Brandt J, Kariouzov A, Listing J, Haibel H, Sørensen H, Grassnickel L, *et al.* Six month results of a double-blind placebo controlled treatment trial of etanercept in patients with active ankylosing spondylitis. *Arthritis Rheum* (in press).
- Thiel A, Wu P, Lauster R, Braun J, Radbruch A, Sieper J. Analysis of the antigen-specific T cell response in reactive arthritis by flow cytometry. *Arthritis Rheum* 2000;43:2834–42.
- Cope AP, Londei M, Chu NR, Cohen SB, Elliott MJ, Brennan FM, *et al.* Chronic exposure to tumor necrosis factor (TNF) in vitro impairs the activation of T cells through the T cell receptor/CD3 complex; reversal in vivo by anti-TNF antibodies in patients with rheumatoid arthritis. *J Clin Invest* 1994;94:749–60.
- Berg L, Lampa J, Rogberg S, van Vollenhoven R, Kloreskog L. Increased peripheral T cell reactivity to microbial antigens and collagen type II in

- rheumatoid arthritis after treatment with soluble TNF $\alpha$  receptors. *Ann Rheum Dis* 2001;60:133–9.
- 12 **Baeten D**, Van Damme N, Van den Bosch F, Kruithof E, De Vos M, Mielants H, et al. Impaired Th1 cytokine production in spondyloarthritis is restored by anti-TNF $\alpha$ . *Ann Rheum Dis* 2001;60:750–5.
  - 13 **Zou JX**, Rudwaleit M, Brandt J, Thiel A, Braun J, Sieper J. Downregulation of the non-specific and antigen-specific T cell cytokine response in ankylosing spondylitis during treatment with infliximab. *Arthritis Rheum* 2003;48:780–90.
  - 14 **Hanauer SB**, Feagan BG, Lichtenstein GR, Mayer LF, Schreiber S, Colombel JF, et al. Maintenance infliximab for Crohn's disease: the ACCENT I randomised trial. *Lancet* 2002;359:1541–9.
  - 15 **Sandborn WJ**, Hanauer SB, Katz S, Safdi M, Wolf DG, Baerg RD, et al. Etanercept for active Crohn's disease: a randomized, double-blind, placebo-controlled trial. *Gastroenterology* 2001;121:1088–94.
  - 16 **Keane J**, Gershon S, Wise RP, Mirabile-Levens E, Kasznica J, Schwieterman WD, et al. Tuberculosis associated with infliximab, a tumor necrosis factor alpha-neutralizing agent. *N Engl J Med* 2001;346:623–6.

### Clinical Evidence—Call for contributors

*Clinical Evidence* is a regularly updated evidence based journal available worldwide both as a paper version and on the internet. *Clinical Evidence* needs to recruit a number of new contributors. Contributors are health care professionals or epidemiologists with experience in evidence based medicine and the ability to write in a concise and structured way.

#### Currently, we are interested in finding contributors with an interest in the following clinical areas:

Altitude sickness; Autism; Basal cell carcinoma; Breast feeding; Carbon monoxide poisoning; Cervical cancer; Cystic fibrosis; Ectopic pregnancy; Grief/bereavement; Halitosis; Hodgkins disease; Infectious mononucleosis (glandular fever); Kidney stones; Malignant melanoma (metastatic); Mesothelioma; Myeloma; Ovarian cyst; Pancreatitis (acute); Pancreatitis (chronic); Polymyalgia rheumatica; Post-partum haemorrhage; Pulmonary embolism; Recurrent miscarriage; Repetitive strain injury; Scoliosis; Seasonal affective disorder; Squint; Systemic lupus erythematosus; Testicular cancer; Varicocele; Viral meningitis; Vitiligo

However, we are always looking for others, so do not let this list discourage you.

#### Being a contributor involves:

- Appraising the results of literature searches (performed by our Information Specialists) to identify high quality evidence for inclusion in the journal.
- Writing to a highly structured template (about 2000–3000 words), using evidence from selected studies, within 6–8 weeks of receiving the literature search results.
- Working with *Clinical Evidence* Editors to ensure that the text meets rigorous epidemiological and style standards.
- Updating the text every eight months to incorporate new evidence.
- Expanding the topic to include new questions once every 12–18 months.

If you would like to become a contributor for *Clinical Evidence* or require more information about what this involves please send your contact details and a copy of your CV, clearly stating the clinical area you are interested in, to Claire Folkes (cfolkes@bmjgroup.com).

### Call for peer reviewers

*Clinical Evidence* also needs to recruit a number of new peer reviewers specifically with an interest in the clinical areas stated above, and also others related to general practice. Peer reviewers are health care professionals or epidemiologists with experience in evidence based medicine. As a peer reviewer you would be asked for your views on the clinical relevance, validity, and accessibility of specific topics within the journal, and their usefulness to the intended audience (international generalists and health care professionals, possibly with limited statistical knowledge). Topics are usually 2000–3000 words in length and we would ask you to review between 2–5 topics per year. The peer review process takes place throughout the year, and our turnaround time for each review is ideally 10–14 days.

If you are interested in becoming a peer reviewer for *Clinical Evidence*, please complete the peer review questionnaire at [www.clinicalevidence.com](http://www.clinicalevidence.com) or contact Claire Folkes (cfolkes@bmjgroup.com).