
Immunological basis for the use of TNF α -blocking agents in ankylosing spondylitis and immunological changes during treatment

J.X. Zou¹, J. Braun², J. Sieper¹

¹Department of Rheumatology, Klinikum Benjamin Franklin, Free University, Berlin; ²Rheumazentrum Ruhrgebiet, Herne, Germany.

Jianxiang Zou, PhD, Fellow in Rheumatology; Jürgen Braun, MD, Professor in Rheumatology; Joachim Sieper, MD, Professor in Rheumatology.

Please address correspondence to: Joachim Sieper, MD, Medical Department I, Rheumatology, University Hospital Benjamin Franklin, Hindenburgdamm 30, 12200 Berlin, Germany.

E-mail: hjsieper@zedat.fu-berlin.de

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ABSTRACT

TNF α is expressed in high amounts at the site of inflammation in ankylosing spondylitis, which provided the basis to initiate treatment studies with TNF-blocking agents. We could show that the immunological effects of infliximab and etanercept differ in patients with AS, although the clinical effect was similarly good. While infliximab induced a downregulation of the production of the T-helper 1-cytokines IFN γ and TNF α , etanercept treatment triggered rather an upregulation of these cytokines secreted by T cells after in vitro stimulation.

Role of TNF in chronic inflammatory diseases

Tumour necrosis factor alpha (TNF) is a cytokine that is mainly produced by monocytes and macrophages and, to a lesser degree, by T cells. TNF mediates inflammation and is also supposed to have immunoregulatory activities. It has an effect on lymphocyte activation and fibroblast proliferation, on other cytokines, chemokines, prostaglandins, and metalloproteinases, and on the vasculature by promoting angiogenesis, upregulation of adhesion molecules, and transendothelial migration of leucocytes (1). It was shown that other proinflammatory cytokines such as IL-1 were inhibited if TNF was neutralized leading to the new concept that the proinflammatory cytokines were linked in a network with TNF at its apex (2, 3). Thus, it has been postulated that TNF has a central role in many immune mediated diseases.

Up to date, two forms of TNF inhibition therapy have been extensively investigated in rheumatoid arthritis (RA). Both the TNF receptor-Fc fusion protein (TNFR:Fc, etanercept) and anti-TNF monoclonal antibodies (in-

fliximab) have been proven to be highly active for the treatment of RA not only in reducing inflammation but also in stopping joint destruction (4, 5). While both infliximab and etanercept are also effective in psoriatic arthritis, in Crohn's disease, rather surprisingly, only infliximab induced clinical improvement but etanercept not (6, 7). However, until now it is not clear by which immunological mechanisms infliximab and etanercept induce their clinical effect.

TNF in ankylosing spondylitis

For both RA and Crohn's disease there has been evidence that T helper (h) 1 cytokines such as IFN and TNF are predominant (8) which might contribute to the initiation and the chronicity of these diseases and, thus, gave the rationale for treatment with TNF-blockers.

The situation is more complex with ankylosing spondylitis. We could show in peripheral blood that in B27-positive AS patients less TNF is produced by T cells compared to B27⁺ healthy controls (9). However, when T cell secretion was compared between patients with reactive arthritis, rheumatoid arthritis and healthy controls patients with RA showed a higher production of TNF and IFN compared to reactive arthritis but also, similar to the AS-patients reported above, a lower production of these Th1-cytokines than healthy controls (10). We also compared the presence or absence of an atopic disease between RA-patients, AS-patients and healthy controls. It has been proposed that allergies are less frequently found in Th1-diseases. Again, AS patients were more likely to have an allergic disease compared to RA patients but no difference was found in comparison to healthy controls (11). Furthermore, a certain poly-

morphism for the TNF -gene has been described which was present in AS-patients but not in healthy controls (12). However, no cytokine secretion data were investigated in these patients and, therefore, it is not known whether this TNF -polymorphism is associated with a lower TNF production or not. Other researchers found significantly higher TNF serum levels in AS patients compared with patients with non-inflammatory back pain, although the cytokine concentration did not correlate with laboratory and clinical parameters of disease activity (13). Most importantly, we could show recently that at the primary site of inflammation, the sacroiliac joint, high amounts of TNF messenger RNA (Fig. 1) (14) and protein (15) are present. Thus, although TNF does not seem to be elevated systemically, it is highly expressed locally. Taken together, these findings make a role for TNF in AS possible and suggest that anti-TNF agents could be an effective option for this disease.

Influence of anti-TNF therapy on biological parameters in ankylosing spondylitis

We conducted two placebo-controlled

studies, which are presented in more detail elsewhere in this supplement, treating AS-patients highly successfully either with infliximab or etanercept. These studies gave us the opportunity to investigate the cytokine secretion of T cells after antigen-specific and after non-specific stimulation *in vitro* during the 3 months treatment with infliximab versus placebo and during treatment with etanercept versus placebo. In the infliximab study, we could observe a clear decline of IFN - and TNF -secretion over 3 months after non-specific stimulation and after antigen-specific stimulation with the G1-domain of the cartilage proteoglycan aggrecan while no change of cytokine secretion could be observed in the placebo-group. However, a similar drop in the cytokine secretion by T cells occurred after placebo-patients were switched to infliximab (16).

In contrast, in the etanercept study a significant increase of TNF - and IFN -secretion was observed during treatment while again no changes were observed in the placebo group (unpublished observation). Figure 2 compares the effect of infliximab and of etanercept on cytokine secretion by T cells. Interestingly, when monocytes were

stimulated *in vitro* with LPS no change in the secretion of TNF was observed both during infliximab treatment and during etanercept treatment. Thus, infliximab seems to be effective, at least partly, through an inhibition of T-helper 1 function which lasts at least 6 weeks after the previous infusion while etanercept seems to work preferentially by catching soluble TNF without suppression of T cell function. The slight up-regulation of T cell function observed in these patients can probably be seen as a counter-regulation after neutralisation of peripheral TNF . While TNF produced by monocytes has been reported to go down immediately after an infliximab infusion (17) this does not seem to play a role several weeks after the last infusion despite the presence of a clinical effect.

Our data therefore indicate that the T cells might be a major target at least for infliximab. Our results also indicate that just neutralisation of TNF in the fluid phase cannot be the only explanation for infliximab because we found, in contrast to a treatment with etanercept, a long lasting suppression of T cell function. It has been proposed that infliximab could act by binding to membrane-associated TNF mediating lysis of activated macrophages and polymorphonuclear leucocytes via complement fixation or antibody-dependent cell cytotoxicity (18). We did not observe a significant change in the relative number of CD4+, CD8+, CD14+ and CD19+ cells in this study, which suggests that cytotoxicity is probably not involved in the therapeutic effect of infliximab. Rather an influence on the immunoregulation might play a role.

Although etanercept and infliximab seem to be similarly effective in the treatment of rheumatoid arthritis (4, 5) and ankylosing spondylitis (19, 20) there are two important clinical differences: (i) infliximab is highly effective in Crohn's disease while etanercept is not (6, 7); (ii) infliximab treatment is associated with a relatively high rate of infection with mycobacterium tuberculosis while etanercept is not (21). This seems to indicate that infliximab does not only block soluble TNF but also inhibits its production by T cells.

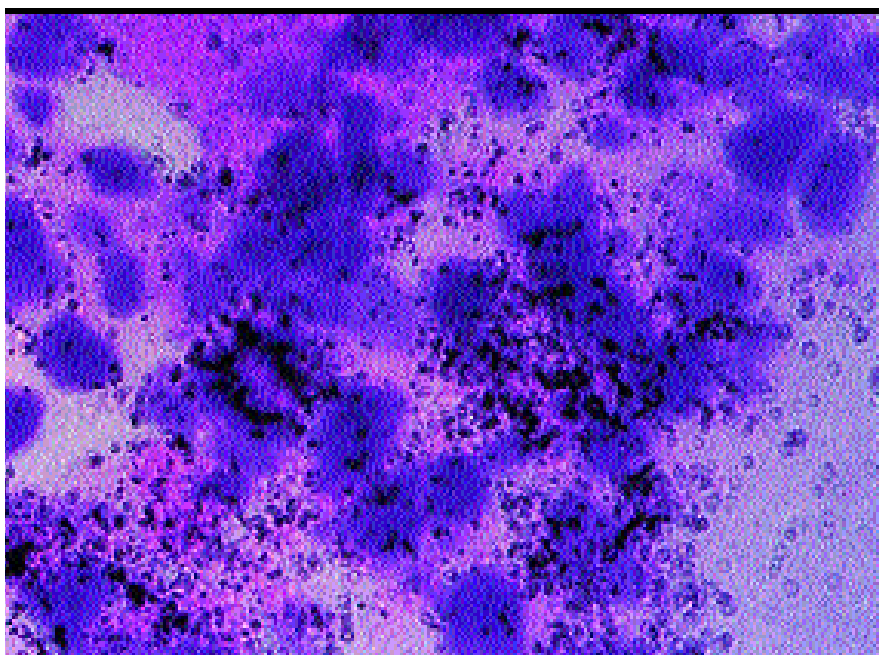


Fig. 1. Detection of TNF mRNA (black dots) in a biopsy from the sacroiliac joint in a patient with active ankylosing spondylitis. (Reproduced from ref. 14 with permission from John Wiley & Sons, Inc. ©1995 American College of Rheumatology)

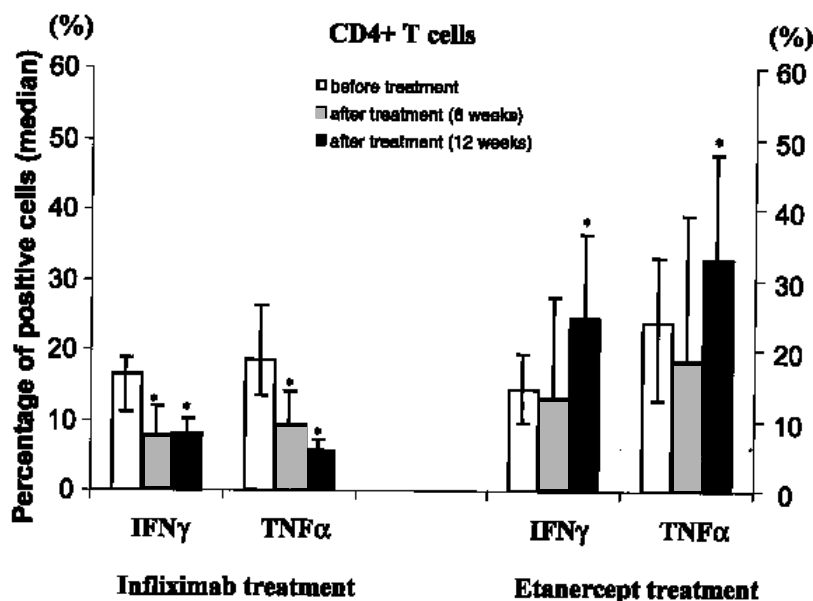


Fig. 2. Percentage of CD4+ T cells secreting IFN γ or TNF α after *in vitro* stimulation for 6 hours with PMA/ionomycin. CD4+ T cells being double positive for one cytokine plus the activation marker CD69 were quantified by flow cytometry. During treatment of patients with ankylosing spondylitis with infliximab the number of IFN γ - and TNF α -positive CD4+ T cells decreased significantly while, in contrast, during treatment with etanercept an increase can be observed. * $p < 0.05$ comparing with that before treatment.

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In contrast to our results, previous studies suggested that TNF α has an inhibitory effect on T cell function which can be restored by TNF α -blockade (22). One report with a similar study design as ours treating patients with various forms of spondyloarthropathies including AS patients with infliximab reported no change in the IFN γ -production by CD4+ T cells after 6 weeks, however, a significant increase of IFN γ -positive CD4+ T cells after 12 weeks (23). The reasons for the difference of these results compared to ours are not clear. The fact that we included a placebo group which showed no change, that the placebo group showed a similar drop in the cytokine production after these patients were treated with infliximab, that we observed a similar change in the IFN γ - and TNF α production, and that we found a reduction after both non-specific and antigen-specific stimulation *in vitro* are arguing in favor of our results. Thus, our results show a clear decrease in T helper 1 cell function during infliximab therapy.

In Crohn's disease treatment with infliximab caused also a clear reduction of IFN γ -production by T cells. It induced a sharp reduction in the number of IFN γ producing lamina propria mononuclear cells in gut biopsies (24) and in colonic T cell cultures derived from patients with Crohn's disease (25). Furthermore, it had been shown that TNF α increases the production of IFN γ by lamina propria MNC suggesting a direct link between the presence of TNF α and IFN γ -production (26). In this study such an association seemed to be specific for lamina propria MNC but not for PB MNC. Our study indicates that such a link is not specific for the gut.

We did not investigate T cell cytokine secretion after the first days following infusion and we can therefore not comment on this time point. It has been reported earlier that the number of IFN γ -secreting CD4+ T cells increases during the first 3 days in rheumatoid arthritis patients treated with infliximab (27). Nonetheless, during treatment over 3 months both the number of

CD4- and CD8-positive T cells producing TNF α and IFN γ was significantly reduced in patients with AS in our present study.

Rather surprisingly, we did not observe a change in the production of TNF α after *in vitro* stimulation of MNC with LPS, which preferentially stimulates monocytes, 6 weeks after start of treatment. One previous study conducted in Crohn's disease reported that TNF α secretion by monocytes decreased drastically in the first days after infusion of infliximab but increased steadily over the following 4 weeks (17). Thus, an inhibition of the TNF α -producing capacity of monocytes does not to be long lasting and does not correlate with the excellent clinical response we see after 6 weeks.

It is not known whether our results obtained during treatment studies with etanercept and infliximab in patients with ankylosing spondylitis is unique for AS or whether a similar finding is present in RA patients. Berg *et al.* reported that treatment of patients with RA with the soluble TNF α receptor etanercept may lead to a transient increase of the number of IFN γ + cells using the ELISPOT assay after 4 weeks but no change compared to baseline was observed after 8 weeks. They also described an increased peripheral T cell reactivity both to microbial antigens and to self antigens such as collagen II during treatment (28). This would be in line with the results presented by us. A similar study has not been performed during treatment of RA patients with infliximab.

Conclusion

In conclusion, TNF α is highly expressed locally at the site of inflammation in patients with ankylosing spondylitis, a finding which triggered treatment trials with the two TNF-blocking agents, infliximab and etanercept. Our data show that infliximab downregulates preferentially the T cell capacity in the production not only of TNF α but also of IFN γ , an effect which is still present at least 6 weeks after the last infusion. This lasting effect on the immunoregulation could explain not only its good clinical effect but also

some side effects. The observed reduction of the Th1-response is in line with the increased frequency of tuberculosis cases in patients treated with infliximab (21) because a Th1-response is crucial for fighting these intracellular microbes. In contrast, etanercept upregulates preferentially the T cell capacity in the production not only of TNF but also of IFN γ . These data indicate that the neutralization of soluble TNF is sufficient for its clinical effect and does not necessarily has an influence on TNF -production by T cells but can even, in the case of etanercept, induce an increased production on *in vitro* stimulation.

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