# Role of TNFα in Ankylosing Spondylitis and other chronic inflammatory diseases

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Tumour necrosis factor alpha (TNFα) is a cytokine that is mainly produced by monocytes and macrophages and, to a lesser degree, by T cells. TNFα 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 TNFα was neutralized leading to the new concept that the proinflammatory cytokines were linked in a network with TNFα at its apex (2, 3). Thus, it has been postulated that TNFα 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 (infliximab) 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.

For both RA and Crohn's disease there has been evidence that T helper (h) 1 cytokines such as IFNγ and TNFα are predominant (8) which might contribute to the initiation and the chronicity of these diseases and, thus, gave the rationale for treatment with TNFα-blockers. The situation is more complex with ankylosing spondylitis (AS). Peripheral blood T cells of B27-positive AS patients produce less TNFα as compared to B27+ healthy controls (9). However, when the T cell secretion was compared between patients with reactive arthritis (ReA), RA and healthy controls, patients with RA showed a higher production of TNFα and IFNγ compared to ReA. However, ReA patients showed, similar to AS patients, a lower production of these Th1-cytokines than healthy controls (10). In a large epidemiological study, the presence or absence of an atopic disease was compared between RA and AS patients and healthy controls. Allergies have been reported to occur less frequently 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 polymorphism for the TNFα-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 TNFα-polymorphism is associated with a lower TNFα production. Other researchers found signigicantly higher TNFα serum levels in AS patients compared with patients with noninflammatory back pain, although the cytokine concentration did not correlate with laboratory and clinical parameters of disease activity (13). Most importantly, it was recently shown that at the primary site of inflammation in AS, in the sacroiliac joint, high amounts of TNFα messenger RNA (14) and protein (15) are present. Thus, although TNFα does not seem to be elevated systemically, it is highly expressed locally. Taken together, these findings make a role for TNFα in AS possible and support the role of anti-TNFα agents in the treatment of AS and other spondyloarthritides.

As shown in several placebo-controlled studies now, AS patients can be successfully treated with infliximab, etanercept and presumably also adalimumab. These studies gave the opportunity to investigate the cytokine secretion of T cells after antigen-specific and after non-specific stimulation in vitro during the three months treatment with infliximab versus placebo and during treatment with etanercept versus placebo. In the infliximab study, a clear decline of IFNγ- and TNFα-secretion was observed 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 TNFα- and IFNγ-secretion was observed during treatment, while no changes were observed in the placebo group (17). Interestingly, when monocytes were stimulated in vitro with LPS no change in the secretion of TNFα 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 TNFα 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 TNFα. While TNFα produced by monocytes has been reported to go down immediately after an infliximab infusion (18) this does not seem to play a role several weeks after the last infusion despite the presence of a clinical effect.

These data therefore indicate that the T cells might be a major target at least for infliximab. The results also indicate that just neutralisation of TNFα 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 TNFα mediating lysis of activated macrophages and polymorphonuclear leucocytes via complement fixation or antibody-dependent cell cytotoxicity (19). 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 RA (4, 5) and AS (20,21) 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 (22). This seems to indicate that infliximab does not only block soluble TNFα but also inhibits its production by T cells.

Previous studies from other authors have suggested that TNFα has an inhibitory effect on T cell function which can be restored by TNFα-blockade (23). One report with a similar study design treating patients with various forms of SpA including AS patients with infliximab reported no change in the IFNγ-production by CD4 + T cells after 6 weeks, however, a significant increase of IFNγ-positive CD4+ T cells after 12 weeks (24). The reasons for these different results are not clear. The fact that a placebo group was included in the studies which showed no change, and that the placebo group showed a similar drop in the cytokine production after these patients were treated with infliximab, and that a similar change in the IFNγ- and TNFα production was observed, and that a reduction after both non-specific and antigen-specific stimulation in vitro was found are argueing in favor of the earlier presented data which show a decrease in T helper 1 cell function during infliximab therapy.

In Crohn's disease treatment with infliximab caused also a clear reduction of IFNγ-production by T cells. It induced a sharp reduction in the number of IFNγ producing lamina propria mononuclear cells in gut biopsies (25) and in colonic T cell cultures derived from patients with Crohn's disease (26). Furthermore, it had been shown that TNFα increases the production of IFNγ by lamina propria MNC suggesting a direct link between the presence of TNFα and IFNγ-production (27). In this study such an association seemed to be specific for lamina propria MNC but not for PB MNC. Other studies indicated that such a link is not specific for the gut.

The T cell cytokine secretion was not examined after the first days following infusion which may explain the difference to earlier data in which the number of IFNg-secreting CD4+ T cells was reported to increase during the first 3 days in patients treated with infliximab (28). Nonetheless, during treatment over 3 months both the number of CD4- and CD8-positive T cells producing TNFα and IFNg was significantly reduced in patients with AS in the large study (16).

Rather surprisingly, no change in the production of TNFα after in vitro stimulation of MNC with LPS, which preferentially stimulates monocytes, was observed 6 weeks after start of treatment. One previous study conducted in Crohn's disease reported that TNFα secretion by monocytes decreased drastically in the first days after infusion of infliximab but increased steadily over the following 4 weeks (29). Thus, an inhibition of the TNFα-producing capacitiy of monocytes does not to be long lasting and does not correlate with the excellent clinical response seen after 6 weeks.

It is not known whether the results obtained during treatment studies with etanercept and infliximab in patients with AS are unique for AS or whether a similar finding may apply to RA patients. Berg et al reported that treatment of patients with RA with the soluble TNFa receptor

etanercept may lead to a transient increase of the number of IFNγ+ cells using the ELISPOT assay after 4 weeks but no change compared to baseline was observed after 8 weeks. These authors also described an increased peripheral T cell reactivity both to microbial antigens and to self antigens such as collagen II during treatment (30). 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.

In conclusion, TNFα 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. There are data that show that infliximab downregulates preferentially the T cell capacitiy in the production not only of TNFα but also of IFNγ - an effect which is still present at least 6 weeks after the last infusion. This lasting effect on the immunoregulation could explain not only the good clinical effect but also some side effects. The observed reduction of the Th1-response is in line with the somewhat increased frequency of tuberculosis 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 capacitiy in the production not only of TNFα but also of IFNγ_. _These data indicate that the neutralization of soluble TNFα is sufficient for its clinical effect and does not necessarily have an influence on TNFα prodcution by T cells, but can, even in the case of etanercept, induce an increased production on in vitro stimulation.

All data support the view of a major role of TNFα in the pathogenesis of AS. However, the definite genetic load including the HLA B27 association cannot be explained by this feature to date.

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