True infliximab resistance in rheumatoid arthritis; a role for lymphotoxin-alpha?
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Title: True infliximab resistance in rheumatoid arthritis; a role for lymphotoxin-alpha?

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Abstract

Introduction: The combination of methotrexate and the anti-tumour necrosis factor alpha (TNF-alpha) antibody infliximab is a very effective treatment of rheumatoid arthritis (RA). However a proportion of patients are not responsive to this therapy. Inefficacy may represent a TNF-alpha independent disease or insufficient drug at the site of action.

Case Report: This case report describes a patient with RA resistant to repeated high-dose infliximab infusions and intra-articular infliximab into an inflamed knee. No beneficial clinical effect was observed. Pre-injection arthroscopic biopsy of the study knee demonstrated TNF-alpha staining but also confirmed presence of lymphotoxin-alpha (LT-alpha or TNF-beta) on immunohistochemistry. Subsequent treatment with etanercept (which blocks LT-alpha as well as TNF-alpha) resulted in clinical remission of disease.

Conclusion: This case suggests that resistance to TNF blockade may occur when TNF-alpha is not the dominant inflammatory cytokine and implicates LT-alpha as possessing a pathogenic role in RA.

Keywords: Rheumatoid arthritis, anti-TNF, infliximab, lymphotoxin-alpha
The combination of methotrexate and the anti-tumour necrosis factor alpha (TNF-alpha) monoclonal antibody infliximab has been proven very beneficial in the treatment of rheumatoid arthritis (RA). However, approximately 35% of patients do not achieve good clinical improvement [1]. The reason(s) for this lack of clinical response to TNF-alpha blockade are unclear. Possible reasons include inadequate levels of drug reaching the primary site of disease or pathophysiological subsets of RA, where the predominant cytokine driving inflammation is not TNF-alpha. We report on a patient who failed to respond to both high-dose infliximab infusions and intra-articular (IA) infliximab but responded to the alternative TNF-alpha blocking drug etanercept. This case offers new insight into the causes for anti-TNF-alpha non-response.

**Case Report**

A 55-year old woman presented with a four-month history of polyarthritis predominately involving hands, wrists, knees and feet. There was a history of hypothyroidism and she was a cigarette smoker with no family history of RA. Her medications were diclofenac 75mg slow release formulation twice daily, thyroxine 50 micrograms daily and hormone replacement therapy (oestradiol 2mg and norethisterone acetate 1 mg). She had 300 minutes of early morning stiffness (EMS) and on examination had a tender joint count (TJC) of 33 and a swollen joint count (SJC) of 9. Conventional radiography of hands and feet demonstrated peri-articular soft tissue swelling and osteopenia but no radiographic erosions. Magnetic resonance imaging of her metacarpophalangeal joints confirmed synovitis. She was rheumatoid factor positive, HLA-DR4 positive, had a C-reactive protein (CRP) level of 48mg/dl and normal thyroid function tests. On the basis of above she was diagnosed as having RA.
After giving informed consent, the patient was entered into a research ethics committee approved study employing high dose anti-TNF blockade in early, poor prognosis RA patients [2]. In accordance with the study protocol she was commenced on methotrexate 7.5mg once weekly, folic acid 5mg twice weekly and infusions of infliximab 10mg/kg at week 0, 2, 6, 10 then 8-weekly. By week 12 she had no improvement with EMS 300 minutes, TJC 26, SJC 13 and CRP of 41mg/dl. In an attempt to induce significant response the patient underwent a further series of four high-dose infliximab infusions at similar time intervals. Over this six-month period the methotrexate dose was increased from 7.5mg to 20mg once weekly. After six months of therapy she had EMS 300 minutes, TJC 20, SJC 16 and CRP 125. Since she was clearly having no response she was withdrawn from the study.

At withdrawal, her left knee was aspirated and injected with 80mg of methylprednisolone and she was given 120mg methylprednisolone intramuscularly. Her left knee had an excellent clinical response to the IA corticosteroid. Despite this she then continued to have very active disease in most of her other joints. As her right knee continued to be painful and swollen she underwent an arthroscopy in March 2000. This demonstrated typical RA findings of moderate synovial membrane villous formation and marked vascularity. Five weeks following this procedure her arthroscopic portals were healed but she had no change in her disease state, including persistent symptoms and signs in her right knee. In order to address the issues concerning TNF-alpha resistance, IA infliximab was given. Previous reports have demonstrated the effectiveness of IA TNF-blocking agents both locally and systemically [3]. The right knee was injected with 70mg (in 7ml solution) of infliximab. The patient was reviewed on a daily basis over the next five days and no adverse event was recorded. However, the patient reported no change in pain in the right knee and there was no change in the effusion present. At day 5 the knee was aspirated and 80mg of IA methylprednisolone was injected. The knee had an excellent clinical response. With the patient continuing to have very active disease, sulphasalazine and hydroxychloroquine were added to her second line therapy (sulphasalazine dose increased to 1g daily
over 2 weeks). The following month she came off sulphasalasine due to gastrointestinal side effects. Her MTX dose was consequently increased to 25mg once weekly (over 4 weeks).

The patient continued to show unacceptable level of disease activity. Consequently, hydroxychloroquine was stopped and the patient was commenced on etanercept subcutaneously 25 mg twice weekly. By week 12 of etanercept treatment a dramatic symptomatic and clinical response was observed. She had no EMS and on examination TJC 2, SJC 1, CRP normalised. A 'good' EULAR and ACR 50 responses were achieved. By 9 months her ACR response had improved to ACR70. She has continued to be in clinical remission for over two and a half years.

Synovial membrane biopsies obtained from arthroscopy were embedded in optimum cutting temperature (OCT) on cold hexane, snap frozen and stored at –80°C. Cryostat sections (3µ and 4µ thick) were mounted on superfrost slides (Surgipath) and dried overnight at 37°C. Sections were fixed for 20 minutes in acetone before incubating with monoclonal antibodies anti-TNF-alpha (monoclonal mouse IgG1 clone 28401 R&D Systems) and anti-lymphotoxin-alpha (LT-alpha) (monoclonal mouse IgG1 clone 5802.21 R&D Systems) for 1 hour. A standard staining procedure using ChemMate (DAKO) was used. Endogenous peroxidase activity was blocked for 10 minutes. Secondary biotinylated antibody was added and incubated for 30 minutes followed by addition of horseradish peroxidase-conjugated avidin-biotin complex for another 30 minutes. 3,3'-diaminobenzidine (DAB) was used to develop colour and was terminated with serial washings at 10-15 minutes. The slides were counterstained in haematoxylin (Sigma), dehydrated in ethanol and xylene and then mounted.

Arthroscopic inspection revealed typical RA synovitis, with villous proliferation. Histology confirmed dense cellular infiltration. Expression of TNF-alpha was seen in the lining and sub-lining layers. Staining for LT-alpha however was also positive again in both the lining and sub-lining layers.
(illustrated in fig.1). The positive control used was another patient with rheumatoid arthritis with no previous biologic exposure.

Discussion

This report presents a patient with recent onset RA who deteriorated on repeated high-dose intravenous infusions, flared after IA infliximab but who showed a dramatic response (achieving remission) to etanercept, an alternative TNF-alpha-blocking therapy. Infliximab is a chimeric monoclonal antibody composed of the variable region of mouse anti-human TNF-alpha antibody fused to human IgG1. Infliximab binds and neutralises both soluble and membrane-bound TNF-alpha and can lyse cells bearing the latter via complement activation or antibody-dependent cell-mediated cytotoxicity. Etanercept is a dimeric TNFReceptor:IgG1 fusion protein that neutralises both TNF-alpha and (unlike infliximab) LT-alpha, but does not possess the cytotoxic effect in vitro. The contrasting effects of etanercept following infliximab raises various questions regarding the complex issue of non-response.

A number of factors can be considered in this respect, the first being when the response and non-response is the product of the current scoring tools used. Both the total lack of response on infliximab and degree of response observed on etanercept were dramatic and at the extreme; the differences could not be explained by an artefact of measurement. Low serum drug levels could also contribute to a diminished response but would explain neither failure of response nor the flare of disease as seen here. Furthermore the administration of an intravenous, high dose drug with a loading regimen is likely to ensure appropriate serum levels avoiding potential pharmacokinetic issues. Another reason cited for infliximab non-response is the formation of human anti-chimeric antibodies (HACAs). Again we do not believe our case represents this primarily for two reasons. The first is that with HACAs a clinical response is initially seen which is later lost. As stated above our patient failed to show any signs of
improvement on high-dose infliximab at any time point including immediately post first infusion.

Second the knee joint was treated with intra-articular infliximab. The infliximab dose given would overwhelm any HACAs present, yet this injection produced a flare of disease. In the absence of any of these explanations seeming likely this raises the issue of whether other cytokines within the complex network could possess a pathogenic role. The presence of LT-α staining in the pre-injection synovial biopsy of our patient may be relevant to explaining the subsequent outcome.

Evidence suggests that chronic inflammation has many of the characteristics of lymphoid organ neogenesis, and that LT-alpha appears to play a crucial role in both. LT-alpha is a member of the TNF family, also known as TNF-beta [4]. It binds to TNFR1 (p55) and TNFR2 (p75). Knockout mice models have demonstrated LT-alpha’s crucial role in lymphoid organ development [6,7]. LT-alpha however also exhibits pro-inflammatory effects and induces inflammation in in vivo studies of transgenic mice, confirming earlier in vitro studies [8,9].

This is the first report of such diametrically opposing responses to infliximab and etanercept. The detection of synovial LT-alpha (as well as TNF-alpha) expression raises the interesting possibility of LT-alpha playing at least a partial role in disease drive and pathogenesis. To add further weight to this concept further synovial studies correlating LT-alpha and TNF-alpha expression with response and non-response to infliximab are required. The case however epitomizes the phenomenon of true resistance to infliximab prompting the need for further investigation.

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References


**Figure 1** Panels (A) and (D) are the negative controls for the study patient and positive control respectively. Panels (B) and (E) demonstrate TNF-alpha expression detected with anti-TNF-alpha monoclonal mouse IgG1 clone 28401 in the study patient and positive control respectively. Panels (C) and (F) demonstrate LT-alpha expression detected with anti-LT-alpha monoclonal mouse IgG1 clone 5802.21 in the study patient and positive control respectively.