Tumour necrosis factor blocking agents: a new therapeutic modality for inflammatory disorders

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ABSTRACT

Development of anti-tumour necrosis factor-α (anti-TNFα) treatment offers the potential to alter radically the course of inflammatory diseases such as rheumatoid arthritis and Crohn's disease using modalities directed against a specific inflammatory mediator. Controlled randomised trials in these diseases demonstrate clinical benefit associated with significant improvement in patients with severe active joint and intestinal disease, often when conventional therapies are unsuccessful. To date, anti-TNFα therapy has been well tolerated and shows a favourable safety profile. This review considers the nature of this therapy and current evidence of its clinical benefit and adverse effects.

KEY WORDS: Antibodies, monoclonal.
Etanercept. Infliximab.
Tumor necrosis factor.

Introduction

Cytokines play a central role in the human immune response and generally can be categorised as either pro-inflammatory or anti-inflammatory in action. Tumour necrosis factor-α (TNFα) is a pro-inflammatory cytokine, a key mediator of inflammation,[1] and the product of a single gene located on chromosome 6 in humans.

The major cell source of TNF is activated monocytes and macrophages, although antigen-stimulated lymphocytes can also secrete this protein.[2-4] Active soluble TNF is composed of three identical 17 kDa molecules that must bind two or more membrane-bound receptors to initiate intracellular signal transduction.[4,5]

Two distinct TNF receptors – TNFR1 (55 kDa) and TNFRII (75 kDa) – are encoded by separate genes.[1] These receptors are present on almost all cell types; however, activated cells shed surface receptors and these act as competitive natural inhibitors of soluble TNF.

It is evident that local TNFα effects can improve the host immune mechanism significantly by enhancing immune cell function and stimulation of inflammation. On the other hand, it may cause disease by mediating tissue injury, shock or catabolic illness.

TNFα is implicated in the pathogenesis of a range of diseases, including acquired immune deficiency syndrome (AIDS), cancer, septic shock syndrome, stroke, diabetes mellitus, lymphoma, leukaemia, inflammatory bowel diseases and rheumatoid arthritis.[1] Animal studies have demonstrated that TNFα plays a central role in host defence against tuberculosis.[6,7] In humans, therapeutic agents used to block the effect of TNFα are associated with reactivation of tuberculosis, suggesting that this cytokine has a key role in the control of latent infection.[8] Recent studies suggest that TNFα may be implicated in the pathophysiology of atherosclerosis[9] and in the progression of congestive heart failure.[10,11]

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Continued progress in our knowledge of the biology of TNFα and its receptors may allow the development of new therapeutic strategies for many of these diseases.

TNF role in the pathogenesis of rheumatoid arthritis

Rheumatoid arthritis (RA) is a chronic, progressive and systemic inflammatory disorder of undetermined aetiology, the main clinical manifestation of which is symmetrical polyarthritis (Figure 1). Current understanding indicates that inflammation and tissue destruction in rheumatoid synovium result from complex cell-cell interactions. These events are thought to be initiated by an interaction between antigen presenting cells and $CD4^+$ T cells (reviewed previously[12,13]).

Antigen presenting cells display Class II major histocompatability complex (MHC) molecules and peptide antigen(s) that bind to specific receptors on T cells. Although a number of cell types may act as antigen presenting cells, macrophages and dendritic cells are thought to play a key role in the pathogenesis of rheumatoid synovitis. Despite extensive studies carried out over the past 15 years, the nature of the stimulating antigenic peptide in RA remains unknown.

Whatever the initial antigenic stimulus, pro-inflammatory cytokines including interleukin (IL)-1β, IL-6 and TNFα play a pivotal role in all phases of RA. The role of TNF in arthritis has been shown in mice transgenic for human TNF, in which synovitis and destructive arthritis develop spontaneously.[14] Moreover, treatment of human RA synovial cultures with anti-TNF antibodies causes a significant reduction in pro-

inflammatory cytokines such as GM-CSF, IL-6 and IL-8.[15] Other studies have demonstrated that TNF is present in high concentrations in the synovium and synovial fluid from patients with RA[3,16] and that TNF levels in the synovium correlate with the degree of inflammation and bone erosion.[17,18]

In the synovium, TNF is produced by macrophages and synoviocytes and can trigger several important events that eventually lead to bone damage.[19] These include activation of endothelial cells, stimulation of fibroblast proliferation, induction of metalloproteinases and stimulation of production of other pro-inflammatory cytokines such as IL-1, IL-6 and GM-CSF. In addition, TNF stimulates osteoclasts indirectly via IL-1 production, leading to tissue damage.

Although up-regulation of anti-inflammatory mediators such as IL-10, IL-1 receptor antagonist and soluble TNF receptors have been reported in the synovium in RA, levels would appear to be inadequate to antagonise the effects of the pro-inflammatory cytokines.

TNF targeting strategies as a new treatment modality

Rheumatoid arthritis

Rheumatoid arthritis is a common disease and major source of morbidity (Figure 2). Current disease-modifying anti-rheumatic drugs do not appear to prevent the long-term joint damage associated with this disease. As the importance of TNF in RA became apparent, the intriguing possibility emerged that blocking TNF effects might improve disease symptoms and perhaps slow the inflammatory process.

One possible limitation of attempting to target a single cytokine is the overlap of the immune effects of IL-1, IL-6 and TNFα. Initially, blocking of TNFα effects was studied extensively in murine collagen-induced arthritis.[20-22] In these animal models, disease was prevented by the administration of anti-TNF antibodies. In addition, hamster monoclonal antibodies to murine TNFα administered over a 10-day period led to significant clinical and histological improvement in animals with collagen-induced arthritis. These results encouraged the initiation of clinical trials of anti-TNF therapy in humans.

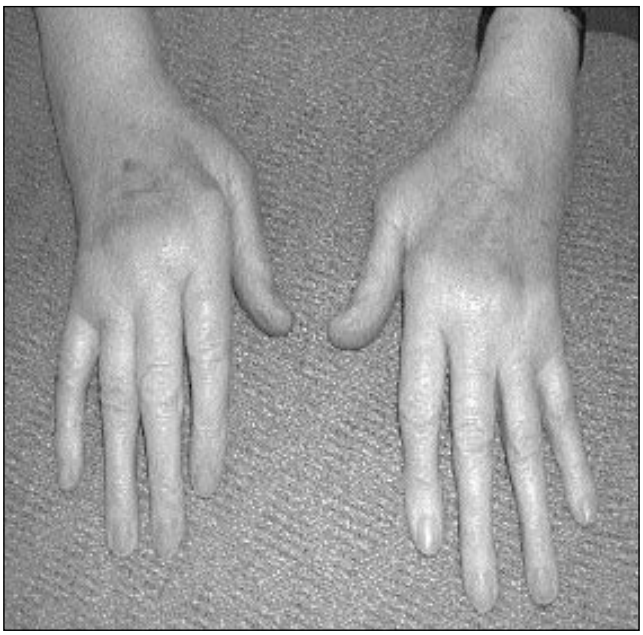
Initially, in humans, TNFα neutralisation was achieved using monoclonal antibodies. Infliximab is a chimaeric monoclonal antibody composed of a mouse variable-region binding site and a human Fc portion. It binds with high affinity and avidity to human soluble and membrane-bound TNFα.[23] Several important clinical trials have shown that infliximab is an effective treatment modality for RA.

An early study by Elliot *et al*.,[24] involving 20 patients with active RA, demonstrated that infusion of this antibody resulted in significant clinical improvement in joint pain, stiffness and swelling within 24 hours. In addition, reduced levels of C-reactive protein (CRP), serum amyloid A and IL-6 were noted during the study. These responses were sustained for the entire eight-week duration of the trial.

Subsequently, a double-blind, multicentre, randomised trial of a single infusion (1 or 10 mg/kg infliximab) compared with placebo in 73 patients with active RA was carried out.[25] Patients were evaluated according to Paulus 20 and Paulus 50 criteria – an amalgamation of clinical, observational and laboratory variables in response to therapy. There was considerable improvement in joint symptoms and marked reduction in inflammatory markers of disease activity, including CRP and erythrocyte sedimentation rate. Notably, the magnitude of response was impressive – exceeding 60% for patients on high-dose treatment and indicating a dose-response effect with infliximab in patients with RA.

Following the success of infliximab in these short-term studies, long-term clinical trials involving large numbers of patients were performed. Infliximab efficacy, both with and without methotrexate, was evaluated in a 26-week double-blind, placebo-controlled, multicentre trial involving 101 patients with active RA.[26] These patients, who had previously shown either incomplete response or flare-up of disease activity while receiving low-dose methotrexate, were randomised to one of seven groups (14/15 patients in each group). Patients received either intravenous infliximab at 1, 3, or 10 mg/kg, with or without methotrexate (7.5 mg/week), or intravenous placebo and methotrexate (7.5 mg/week) at

Fig. 1. Rheumatoid arthritis changes affecting hands and wrists.



Summary points - 1

Features of TNFα
• plays an important role in the pathogenesis of rheumatoid arthritis and Crohn's disease
• product of a single gene located on chromosome 6 in humans
• produced mainly by activated mononuclear phagocytes
• binds TNF receptors to initiate intracellular signal transduction
• systemic effects: activation of the acute-phase response stimulation of the production of IL-1 and IL-6 activation of the coagulation system suppression of bone marrow stem cell division endogenous pyrogen suppression of appetite induction of muscle wasting reduction of tissue prefusion

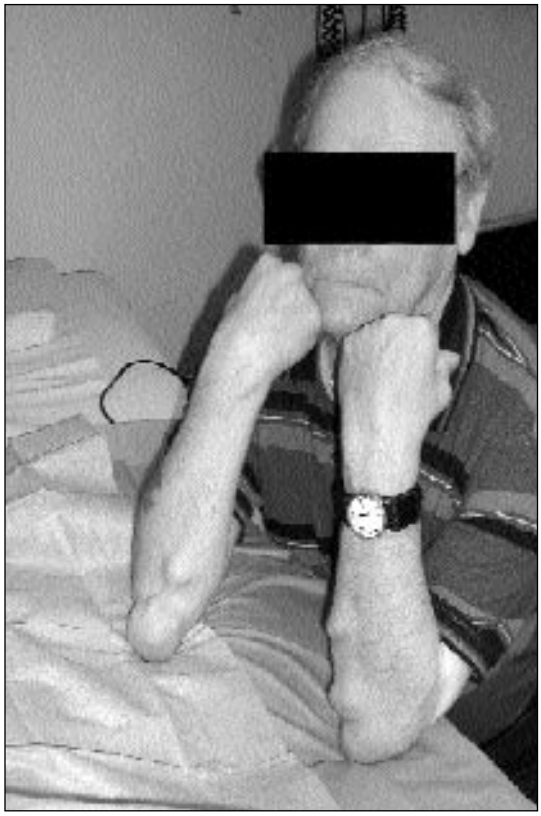


Fig. 2. Rheumatoid nodules (RA nodulosis) affecting both forearms.

weeks zero, 2, 6, 10 and 14, and were followed up to week 26.

Results demonstrated that approximately 60% of patients receiving 3 or 10 mg/kg infliximab, with or without methotrexate, achieved the Paulus 20 criteria for response to treatment, and showed reduction in swollen joint count and tender joint count to near remission levels. However, response was not well sustained once infliximab was stopped at week 14 in patients not receiving concomitant methotrexate treatment. Patients receiving placebo infusions and low-dose methotrexate continued to have active disease. Clearly, results of this study demonstrate that multiple infusions of high-dose infliximab were both effective and well tolerated.

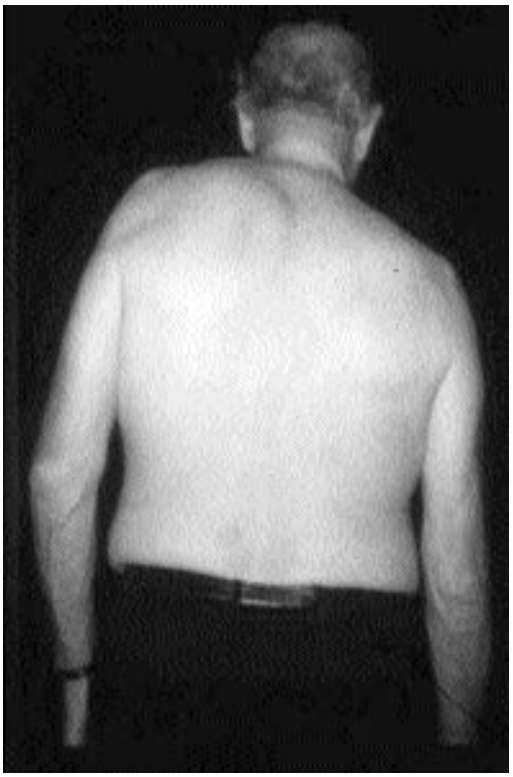
To date, ATTRACT[27] is the largest clinical trial of an anti-TNFα agent in RA. It is an international randomised, double-blind and placebo-controlled study of 428 patients with longstanding RA who failed to improve on at least three disease-modifying anti-rheumatic drugs. All had active disease despite treatment with methotrexate for at least three months. During the study, all patients continued to take methotrexate and were randomised to receive either 3 or 10 mg/kg infliximab (or placebo) at weeks zero, 2 and 6. Additional infusions of the same dose were given every four or eight weeks thereafter, together with a stable dose of methotrexate. Patients were assessed every four weeks, for 30 weeks.

The primary end-point of ATTRACT was to achieve the American College of Rheumatology (ACR) 20 response criteria, representing a 20% improvement from baseline at 30 weeks. Primary efficacy analysis, based on ACR 20 criteria, showed that 50-58% of patients in the infliximab/methotrexate group achieved a clinical response at 30 weeks, compared with just 20% in the methotrexate-only group.

Summary points - 2

TNFα blocking agents
• recommended for the treatment of active rheumatoid arthritis after an adequate trial of disease modifying anti-rheumatic drugs
• have been used successfully to treat patients with active and severe Crohn's disease
• have been shown to be efficacious in juvenile rheumatoid arthritis, psoriatic arthritis and ankylosing spondylitis
• currently being evaluated in the treatment of Wegener's granulomatosis, diffuse scleroderma, polymyositis, refractory sarcoidosis and heart failure
• should not be started or should be discontinued when serious infections occur
• further studies are required to determine the long-term efficacy and safety.

Fig. 3. Reduced flexion of the spine in a patient with ankylosing spondylitis.



Infliximab effects were achieved rapidly and sustained over 30 weeks. Differences in responses demonstrate the therapeutic benefit of infliximab in refractory RA. However, up to 50% of RA patients in this study did not show a significant response to this treatment modality.

Another approach used to block the effect of TNF in patients with RA is based on the discovery that membrane-associated TNF receptors exist in two soluble forms that retain ligand-binding capacity. Cloning of TNF receptor genes has allowed the development of recombinant soluble TNF receptor. Using this agent to block the effect of TNF has several important advantages over other therapeutic modalities used in RA. Firstly, it does not require mouse

amino acid sequences, thus reducing the antigenic potential and minimising the possibility of developing antibodies that might interfere with the therapeutic effect. Secondly, the agent is directed against active circulating TNF molecules and not against membrane-bound molecules, thus it neutralises TNF before it binds to its receptor.

Etanercept, a recently developed agent, consists of the extracellular portion of the human p75 TNF receptor, linked to the Fc portion of human IgG1.[28] It is highly specific and binds with high affinity to TNF. Moreover, the presence of the Fc portion of human IgG extends the half-life of etanercept five- to eight-fold *in vivo*.

Over several years, etanercept has been assessed in clinical trials involving over 500 patients with RA and juvenile RA. The initial study involved 180 patients with RA who failed to improve on up to four disease modifying anti-rheumatic drugs.[29] Patients received subcutaneous injections of placebo or etanercept (0.25, 2 or 16 mg/m^2) twice weekly for three months. At the end of the study, 75% of the patients in the group assigned 16 mg etanercept showed improvement of 20% or more in their symptoms, compared with 14% in the placebo group. However, patients relapsed two months after etanercept treatment ceased, suggesting the need for continuous treatment.

A further study examined the combination of methotrexate and etanercept versus methotrexate and placebo.[30] In this double-blind, placebo-controlled study, 89 adult patients with persistently active RA (despite treatment with methotrexate) were included. All patients received twice-weekly subcutaneous injections of either placebo or 25 mg etanercept, in addition to continuing methotrexate (12.5 or 25 mg per week). Results showed that 71% of patients in the etanercept/methotrexate group achieved 20% ACR response, compared with only 27% in the methotrexate-only group. Moreover, long-term (up to 24 months) improvement in disease activity was maintained in the majority of patients treated with etanercept.

Juvenile rheumatoid arthritis

Etanercept efficacy was evaluated in the treatment of juvenile RA – a disease characterised by chronic joint inflammation in children, which has many similarities to adult RA. A study of 69 children (age range: 4-17 years) who did not tolerate, or showed inadequate response to, methotrexate was carried out.[31] Patients received twice-weekly etanercept (0.4 mg/kg) subcutaneously for up to three months in the initial, open-label part of a multicentre trial. Those who responded were entered into a double-blind study and were randomly assigned to receive either placebo or etanercept for four months, or until flare-up of the disease occurred.

At the end of the open-label study, 51 patients (74%) had responded to etanercept treatment. In the double-blind study, 21 (81%) of the 26 patients who received placebo withdrew because of disease flare-up, compared with only seven (28%) of the 25 patients who received etanercept. Results showed that etanercept treatment led to significant improvement in patients with active polyarticular juvenile RA.

Encouraging results from these clinical trials led to approval of both etanercept and infliximab as new treatment modalities for RA and juvenile RA, and these drugs are now commercially available in Europe and the USA. A recently published consensus statement on the use of anti-TNF therapy stated that these agents should normally be used for the treatment of active RA or juvenile chronic arthritis after a full trial of an effective disease-modifying anti-rheumatic drug, such as methotrexate, proved to be inadequate.[32] These agents can be added to pre-existing treatment or, when appropriate, may replace previous disease-modifying anti-rheumatic drugs.

Psoriatic arthritis

Success with anti-TNF therapy in RA encouraged investigators to examine the effect of this new treatment modality in other inflammatory arthropathies. Up to 2% of patients with psoriasis develop psoriatic arthropathy – a chronic inflammatory disease that may affect the peripheral joints or the spine, and can be preceded by the skin disease by many years.

Etanercept efficacy was evaluated in a double-blind, placebo-controlled study involving 60 patients with psoriatic arthritis and psoriasis.[33] They were randomised to receive twice-weekly etanercept (25 mg) or placebo subcutaneously over a three-month period. At the end of the trial, 87% of patients treated with etanercept had significant symptomatic improvement, compared with just 23% in the placebo group. Moreover, some patients treated with etanercept showed 100% improvement in disease activity. The treatment was well tolerated and no serious adverse effects were recorded.

Ankylosing spondylitis

Ankylosing spondylitis is a chronic inflammatory disease of the spine and sacroiliac joints, which is strongly associated with HLA-B27. It is a progressive disease that causes severe restriction of movement and significant morbidity (Figure 3). In a recent 12-week placebo-controlled multicentre study,[34] 35 patients with active disease were randomly assigned to receive intravenous infliximab (5 mg/kg) and another 35 patients to receive placebo at weeks zero, 2, and 6.

Eighteen (53%) of 34 patients on infliximab showed regression of disease activity at week 12 of at least 50%, compared with three (9%) out of the 35 on placebo ($P<0.0001$). Moreover, function and quality of life also improved significantly in those on infliximab ($P<0.0001$). Although this study showed that infliximab is effective for treating patients with active ankylosing spondylitis, further long-term studies are needed before the true effect of anti-TNF therapy can be determined in this disease.

Inflammatory bowel disease

Crohn's disease

Several studies have shown that anti-TNFα therapy can be used successfully in the treatment of other inflammatory conditions such as inflammatory bowel disease. Crohn's disease is a chronic inflammatory disease, involving mainly the large and small bowel (Figure 4), in which anti-TNF therapy has demonstrated a significant effect on disease progression.

The first patient to be treated with infliximab was a young girl with severe disease that failed to respond to immunosuppressive treatment.[35] She showed significant symptomatic improvement after receiving two infusions of

the antibody (10 mg/kg) within 14 days. Stool consistency and frequency normalised, erythrocyte sedimentation rate decreased and intestinal ulceration disappeared. However, following initial favourable response, she relapsed and underwent several surgical procedures, and now has a permanent ileostomy.

The first controlled study to assess the effect of infliximab in Crohn's disease was a 12-week multicentre trial involving 108 patients with moderate to severe Crohn's disease that was resistant to treatment.[36] Patients were randomly assigned to receive a single intravenous infusion of either placebo or infliximab at 5 mg/kg, 10 mg/kg, or 20 mg/kg. Overall results showed that 33% of patients given the antibody went into remission, compared with only 4% in the placebo group.

Enterocutaneous fistulas are serious complications of Crohn's disease and are difficult to treat. In a study involving 94 adult patients with draining abdominal or perianal fistulas, treatment with infliximab led to closure for at least three months.[37] Infliximab was approved for clinical use in active Crohn's disease in the USA in 1998 and in Europe in 1999.[38]

More recently, the effect of CDP571 – a humanised antibody to TNF – was evaluated in patients with active Crohn's disease. In a double-blind, placebo-controlled trial, 169 patients with moderate to severe disease were investigated over 24 weeks.[39] Patients were initially randomised to a single dose of CDP571 (10 or 20 mg/kg) or placebo to assess dose response. Patients were then retreated with 10 mg/kg CDP571 or placebo every eight or 12 weeks to assess subsequent dosing intervals. At week 2, clinical response occurred in 45% of CDP571-treated patients, compared with 27% of patients in the placebo group ($P=0.023$).

Patients appeared to benefit from retreatment with CDP571 over 24 weeks, but not all of the results for secondary endpoints were statistically significant. The incidence of severe or serious adverse events was similar among all groups. Thus, CDP571 is safe and effective for treatment of patients with moderate to severe Crohn's disease, but additional studies are required to assess retreatment intervals.

Further indications for anti-TNF therapy

Findings of current studies indicate that TNF is a key inflammatory cytokine both in rheumatic diseases and in inflammatory bowel diseases. Success of anti-TNF therapy in this setting provides hope for other diseases in which TNF is thought to play a major role, and currently it is under examination in diffuse scleroderma,[40] polymyositis,[41] Wegener's granulomatosis[42] and refractory sarcoidosis.[43]

There is increasing evidence that cytokines in general and TNF in particular play an important role in cardiovascular disease.[10,11] This is not surprising since TNF modulates both cardiac contractility and peripheral resistance – the two most important haemodynamic determinants of cardiac function. Thus, increased levels of TNF, or of its soluble receptors, have been implicated in the pathophysiology of ischaemia-reperfusion injury, myocarditis, cardiac allograft and, more recently, in the progression of congestive heart failure. Thus, therapies directed against TNFα represent a novel approach to heart failure management.

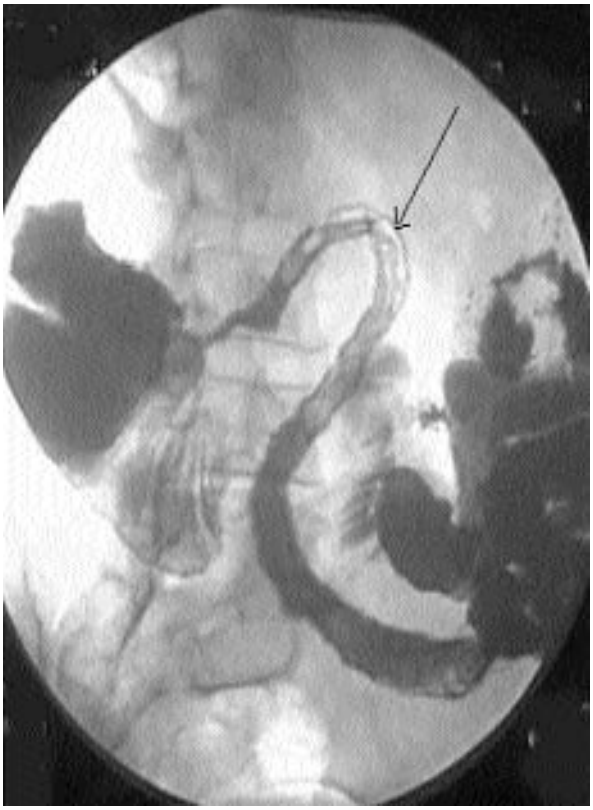


Fig. 4. Small intestinal enema showing ileal stricture (arrow) in a patient with Crohn's disease.

Anti-TNF strategies may target the mechanisms of immune activation, the intracellular pathways regulating TNF production, or the fate of TNF once released into the circulation. In a recent study to determine the effect of anti-TNFα therapy, a transgenic mouse strain (TNF1.6) with cardiac-specific over-expression of TNFα and congestive heart failure was studied.[44] Three groups of mice were examined: TNF1.6 mice treated with saline, wild-type mice treated with saline, and TNF1.6 mice treated with TNFα neutralising antibody (cV1q). Results showed that anti-TNF therapy might both preserve cardiac function and partially reverse pathological changes in congestive heart failure.

In humans, a recent double-blind, placebo-controlled study involving 47 patients with advanced heart failure showed that treatment with etanercept for three months resulted in a significant dose-dependent improvement in left ventricular structure and function.[45]

Results of studies investigating the effects of TNF blocking agents in other systemic diseases will be of interest because controlling TNF represents a new approach by which many of these diseases might be treated.

How safe is anti-TNF therapy?

Results of studies using infliximab in rheumatoid arthritis and Crohn's disease, at the doses described, suggest that it is safe and well tolerated. However, some adverse reactions have been reported,[46] with headache, nausea, sinusitis, rash and coughing being the most common. Incidence of infusion-related reactions were low and most were mild and did not increase in frequency or severity over time. Delayed-

type hypersensitivity reactions with arthralgia/myalgia, skin rash or fever occurring within two weeks of treatment were rarely reported.

Treatment with infliximab has been associated with the development of two types of antibodies in the recipient.[20,38,46] Human anti-chimaeric antibodies (HACA) have been detected in 13% of Crohn's disease patients and in 10% of RA patients. These were low-titre antibodies (< 1 in 40) and may have been associated with higher incidence of infusion reactions upon re-infusion. High-titre HACA neutralise infliximab and thus it is likely that therapeutic efficacy may be affected.

Anti-double stranded DNA (dsDNA) was another antibody detected in 9% and 16% of RA and Crohn's disease patients, respectively, following treatment. It occurred at variable low titres and rarely led to significant clinical complications. The mechanism by which infliximab induces anti-DNA production remains unclear.

Lupus was reported in less than 0.5% of patients treated with infliximab. None had renal involvement or major organ damage and all responded to infliximab withdrawal and medical treatment. More recently, infliximab has been associated with reversible cholestatic liver disease.[47] Although likely to be a rare adverse event, cholestatic liver injury should always be considered in patients receiving infliximab therapy who present with jaundice.

Data from several clinical trials indicate that etanercept has an even more favourable safety profile. The most commonly recognised adverse effects were injection site reactions – mild erythema, itch, swelling and pain.[48] These subsided with time and did not require discontinuation of treatment.

Serum antibodies to etanercept were detected at least once in 16% of treated RA patients; however, they were non-neutralising and not associated with any clinical sequel. Recently, development of systemic lupus erythematosus (SLE) symptoms in four female patients being treated with etanercept has been reported.[49] Etanercept was discontinued and the SLE-related symptoms promptly resolved.

Recent reports have demonstrated that both infliximab and etanercept are associated with tuberculosis. Although the number of patients exposed to both TNFα blocking agents were similar, only nine cases of tuberculosis in patients treated with etanercept have been reported to the Food and Drug Administration, compared with 70 cases in patients treated with infliximab.[8] This disparity may be related to the different mechanisms by which the two agents block TNFα effects;[50] however, the number of patients receiving infliximab rather than etanercept is larger in Europe than in other countries, and the majority of reports were from Europe.

There is no convincing evidence to suggest an increased incidence of malignancy with either drug. Effects of TNF blockade are unknown in patients with coexisting lymphoproliferative disorders, chronic infections (including human immunodeficiency virus infection and hepatitis B or C) or during pregnancy and lactation.

Overall, anti-TNF therapy seems well tolerated in patients with RA and Crohn's disease, when given as single or multiple doses. Short-term side effects are mild but long-term infectious and oncogenic adverse effects require further evaluation.

Conclusions

Anti-TNF therapy has revolutionised the treatment of inflammatory diseases by targeting a single pro-inflammatory cytokine. Controlled randomised clinical trials have demonstrated significant improvement in patients with severe active RA and Crohn's disease, often when conventional therapies have proved unsuccessful. The precise mechanism of action of anti-TNF therapy remains unclear; however, widespread anti-inflammatory activity suggests an effect on more than one branch of the immune system.

Anti-TNF therapy is expensive, and cost is a major limiting factor in the widespread use of this new treatment modality. For example, a combination of etanercept and methotrexate, treatment for six-months costs 38 times more in patients who achieve an ACR 20 outcome than methotrexate therapy alone.[51] Hence, anti-TNF agents should be used appropriately and by experienced physicians who are able to assess their efficacy and safety. Increased risk of reactivating tuberculosis means that screening and prophylaxis should be undertaken before commencing treatment with these agents.

Although short-term experience with anti-TNF therapy in RA and Crohn's disease appears favourable, further studies are needed in selected areas of toxicity and efficacy. Results of anti-TNF therapy trials in other diseases are awaited with interest. □

References

1 Tracey KJ. Tumour necrosis factor-alpha. In: Thomson A, ed. *The cytokine handbook*. London: Academic Press,1996: 289-304.

2 Pennica D, Nedwin GE, Hayflick JS *et al*. Human tumour necrosis factor: precursor structure, expression and homology to lymphotoxin. *Nature* 1984; **312**(5996): 724-9.

3 Chu CQ, Field M, Feldmann M, Maini RN. Localization of tumor necrosis factor alpha in synovial tissues and at the cartilage-pannus junction in patients with rheumatoid arthritis. *Arthritis Rheum* 199; **34**: 1125-32.

4 Bazzoni F, Beutler B. The tumor necrosis factor ligand and receptor families. *N Engl J Med* 1996; **334**: 1717-25.

5 Jones EY, Stuart DI, Walker N. Structure of tumour necrosis factor. *Nature* 1989; **338**(6212): 225-8.

6 Flynn JL, Goldstein MM, Chan J *et al*. Tumor necrosis factor-alpha is required in the protective immune response against *Mycobacterium tuberculosis* in mice. *Immunity* 1995; **2**: 561-72.

7 Turner J, Frank AA, Brooks JV, Marietta PM, Orme IM. Pentoxifylline treatment of mice with chronic pulmonary tuberculosis accelerates the development of destructive pathology. *Immunology* 2001; **102**: 248-53.

8 Keane J, Gershon S, Wise RP *et al*. Tuberculosis associated with infliximab, a tumor necrosis factor alpha-neutralizing agent. *N Engl J Med* 2001; **345**: 1098-104.

9 Skoog T, Dichtl W, Boquist S *et al*. Plasma tumour necrosis factor-alpha and early carotid atherosclerosis in healthy middle-aged men. *Eur Heart J* 2002; **23**: 376-83.

10 Ferrari R. The role of TNF in cardiovascular disease. *Pharmacol Res* 1999; **40**: 97-105.

11 Ceconi C, Curello S, Bachetti T, Corti A, Ferrari R. Tumor necrosis factor in congestive heart failure: a mechanism of disease for the new millennium? *Prog Cardiovasc Dis* 1998; **41** (Suppl 1): 25-30.

12 Arend WP. The pathophysiology and treatment of rheumatoid

arthritis. *Arthritis Rheum* 1997; **40**: 595-7.
13 Fox DA. The role of T cells in the immunopathogenesis of rheumatoid arthritis. *Arthritis Rheum* 1997; **40**: 598-609.
14 Keffer J, Probert L, Cazlaris H *et al*. Transgenic mice expressing human tumour necrosis factor: a predictive genetic model of arthritis. *EMBO J* 1991; **10**: 4025-31.
15 Feldmann M, Brennan FM, Maini RN. Role of cytokines in rheumatoid arthritis. *Annu Rev Immunol* 1996; **14**: 397-440.
16 Tetta C, Camussi G, Modena V, Di Vittorio C, Baglioni C. Tumour necrosis factor in serum and synovial fluid of patients with active and severe rheumatoid arthritis. *Ann Rheum Dis* 1990; **49**: 665-7.
17 Husby G, Williams RC Jr. Synovial localization of tumor necrosis factor in patients with rheumatoid arthritis. *J Autoimmun* 1988; **1**: 363-71.
18 Neidel J, Schulze M, Lindschau J. Association between degree of bone erosion and synovial fluid levels of tumor necrosis factor alpha in the knee joints of patients with rheumatoid arthritis. *Inflamm Res* 1995; **44**: 217-21.
19 Feldmann M, Maini RN. Anti-TNF alpha therapy of rheumatoid arthritis: what have we learned? *Annu Rev Immunol* 2001; **19**: 163-96.
20 Jorgensen C, Apparailly F, Sany J. Immunological evaluation of cytokine and anticytokine immunotherapy *in vivo*: what have we learnt? *Ann Rheum Dis* 1999; **58**: 136-141.
21 Williams RO, Feldmann M, Maini RN. Anti-tumor necrosis factor ameliorates joint disease in murine collagen-induced arthritis. *Proc Natl Acad Sci USA* 1992; **89**: 9784-8.
22 Piguet PF, Grau GE, Vesin C, Loetscher H, Gentz R, Lesslauer W. Evolution of collagen arthritis in mice is arrested by treatment with anti-tumour necrosis factor (TNF) antibody or a soluble TNF receptor. *Immunology* 1992; **77**: 510-4.
23 Knight DM, Trinh H, Le J *et al*. Construction and initial characterization of a mouse-human chimeric anti-TNF antibody. *Mol Immunol* 1993; **30**: 1443-53.
24 Elliott MJ, Maini RN, Feldmann M *et al*. Treatment of rheumatoid arthritis with chimeric monoclonal antibodies to tumor necrosis factor alpha. *Arthritis Rheum* 1993; 3**6**: 1681-90.
25 Elliott MJ, Maini RN, Feldmann M *et al*. Randomised double-blind comparison of chimeric monoclonal antibody to tumour necrosis factor alpha (cA2) versus placebo in rheumatoid arthritis. *Lancet* 1994; **344**: 1105-10.
26 Maini RN, Breedveld FC, Kalden JR *et al*. Therapeutic efficacy of multiple intravenous infusions of anti-tumor necrosis factor alpha monoclonal antibody combined with low-dose weekly methotrexate in rheumatoid arthritis. *Arthritis Rheum* 1998; **41**: 1552-63.
27 Maini R, St Clair EW, Breedveld F *et al*. Infliximab (chimeric anti-tumour necrosis factor alpha monoclonal antibody) versus placebo in rheumatoid arthritis patients receiving concomitant methotrexate: a randomised phase III trial. ATTRACT Study Group. *Lancet* 1999; **354**: 1932-9.
28 Mohler KM, Torrance DS, Smith CA *et al*. Soluble tumor necrosis factor (TNF) receptors are effective therapeutic agents in lethal endotoxemia and function simultaneously as both TNF carriers and TNF antagonists. *J Immunol* 1993; **151**: 1548-61.
29 Moreland LW, Baumgartner SW, Schiff MH *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.
30 Weinblatt ME, Kremer JM, Bankhurst AD *et al*. A trial of etanercept, a recombinant tumor necrosis factor receptor:Fc fusion protein, in patients with rheumatoid arthritis receiving methotrexate. *N Engl J Med* 1999; **340**: 253-9.
31 Lovell DJ, Giannini EH, Reiff A *et al*. Etanercept in children with polyarticular juvenile rheumatoid arthritis. Pediatric Rheumatology Collaborative Study Group. *N Engl J Med* 2000; **342**: 763-9.
32 Furst DE, Keystone EC, Breedveld FC *et al*. Updated consensus statement on tumour necrosis factor blocking agents for the treatment of rheumatoid arthritis and other rheumatic diseases (April 2001). *Ann Rheum Dis* 2001; Suppl 3: iii2-5.
33 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.
34 Braun J, Brandt J, Listing J *et al*. Treatment of active ankylosing spondylitis with infliximab: a randomised controlled multicentre trial. *Lancet* 2002; **359**: 1187-93.
35 Derkx B, Taminiau J, Radema S *et al*. Tumour necrosis factor antibody treatment in Crohn's disease. *Lancet* 1993; **342**: 173-4.
36 Targan SR, Hanauer SB, van Deventer SJ *et al*. A short-term study of chimeric monoclonal antibody cA2 to tumor necrosis factor alpha for Crohn's disease. Crohn's Disease cA2 Study Group. *N Engl J Med* 1997; **337**: 1029-35.
37 Present DH, Rutgeerts P, Targan S *et al*. Infliximab for the treatment of fistulas in patients with Crohn's disease. *N Engl J Med* 1999; **340**: 1398-405.
38 van Deventer SJ. Anti-TNF antibody treatment of Crohn's disease. *Ann Rheum Dis* 1999; **58** Suppl 1: I114-20.
39 Sandborn WJ, Feagan BG, Hanauer SB *et al*. An engineered human antibody to TNF (CDP571) for active Crohn's disease: a randomized double-blind placebo-controlled trial. *Gastroenterology* 2001; **120**: 1330-8.
40 Ellmann MH, MacDonald PA, Hayes FA. Etanercept as treatment for diffuse scleroderma: a pilot study. *Arthritis Rheum* 2000; **43** (9 Suppl): S392.
41 Saadeh CK. Etanercept is effective in the treatment of polymyositis/dermatomyositis which is refractory to conventional therapy including steroids and other disease-modifying agents. *Arthritis Rheum* 2000; **43** (9 Suppl): S193.
42 Spencer-Green G. Etanercept (Enbrel): update on therapeutic use. *Ann Rheum Dis 2000*; **59** Suppl 1: I46-9.
43 Baughman RP, Lower EE. Infliximab for refractory sarcoidosis. *Sarcoidosis Vasc Diffuse Lung Dis* 2001; **18**: 70-4.
44 Kadokami T, Frye C, Lemster B, Wagner CL, Feldman AM, McTiernan CF. Anti-tumor necrosis factor-alpha antibody limits heart failure in a transgenic model. *Circulation* 2001; **104**: 1094-7.
45 Bozkurt B, Torre-Amione G, Warren MS *et al*. Results of targeted anti-tumor necrosis factor therapy with etanercept (ENBREL) in patients with advanced heart failure. *Circulation* 2001; **103**: 1044-7.
46 Harriman G, Harper LK, Schaible TF. Summary of clinical trials in rheumatoid arthritis using infliximab, an anti-TNFα treatment. *Ann Rheum Dis* 1999; **58** Suppl 1: I61-4
47 Menghini VV, Arora AS. Infliximab-associated reversible cholestatic liver disease. *Mayo Clin Proc* 2001; **76**: 84-6.
48 Garrison L, McDonnell ND. Etanercept: therapeutic use in patients with rheumatoid arthritis. *Ann Rheum Dis* 1999; **58** Suppl 1: I65-9.
49 Shakoor N, Michalska M, Harris CA, Block JA. Drug-induced systemic lupus erythematosus associated with etanercept therapy. *Lancet* 2002; **359**: 579-80.
50 Scallon B, Cai A, Shealy D, Solowksi N, Song X, Wagner C. New comparisons of two types of TNFα antagonist approved for rheumatoid arthritis. *Arthritis Rheum* 2000; **43** (Suppl): S226.
51 Choi HK, Seeger JD, Kuntz KM.A. Cost-effectiveness analysis of treatment options for patients with methotrexate-resistant rheumatoid arthritis. *Arthritis Rheum* 2000; **43**: 2316-27.