How does infliximab work in rheumatoid arthritis?

Ravinder N Maini and Marc Feldmann

The Kennedy Institute of Rheumatology Division, Imperial College of Science Technology and Medicine, London, UK

Correspondence: Ravinder N. Maini, FRCP, The Kennedy Institute of Rheumatology Division, Imperial College of Science Technology and Medicine, 1 Aspenlea Road, London, W6 8LH, UK. Tel: +44(0)20 8383 4403; fax: +44(0)20 8748 3293; e-mail: r.maini@ic.ac.uk

Received: 31 October 2001
Accepted: 6 November 2001
Published: 27 March 2002

Arthritis Res 2002, **4 (suppl 2)**:S22-S28

(Print ISSN 1465-9905; Online ISSN 1465-9913)

Abstract

Since the initial characterization of tumor necrosis factor alpha (TNFα), it has become clear that TNFα has diverse biologic activity. The realization that TNFα plays a role in rheumatoid arthritis (RA) has led to the development of anti-TNF agents for the treatment of RA. Infliximab, a chimeric monoclonal antibody that specifically, and with high affinity, binds to TNFα and neutralizes the cytokine, is currently approved for the treatment of RA and Crohn's disease, another immune-inflammatory disorder. In addition to establishing the safety and efficacy of infliximab, clinical research has also provided insights into the complex cellular and cytokine-dependent pathways involved in the pathophysiology of RA, including evidence that supports TNFα involvement in cytokine regulation, cell recruitment, angiogenesis, and tissue destruction.

Keywords: infliximab, rheumatoid arthritis, signaling pathways, tumor necrosis factor

Introduction

Tumor necrosis factor alpha (TNFα) is the name given to a serum factor that was derived in 1975 from endotoxin-treated mice and found to be capable of inducing necrosis of a methylcholanthrene-induced murine sarcoma [1]. The molecular characterization of TNFα in the 1980s revealed that it is identical to cachectin, a previously described serum factor that was found to be responsible for weight loss and fever in experimental animal models [2,3]. The diverse biologic activities of TNFα soon became apparent. Aside from its tumoricidal property, it was recognized that, following injection into animals or humans, TNFα causes signs and symptoms of shock, including multi-organ damage via pro-inflammatory effects on vascular endothelium. The realization that TNFα may play a role in rheumatoid arthritis (RA) followed four demonstrations: firstly, its ability to degrade cartilage and bone *in vitro*; secondly, its arthritogenic properties in animal models; thirdly, its co-localization with TNF receptors in RA synovium and the pannus-cartilage junction; and fourthly, its pivotal role in regulating the production of interleukin (IL)-1 in cultured RA-derived synovial cells (a mixture of lymphoid cells, macrophages, dendritic cells, B cells, endothelial cells, and fibroblasts) [4,5].

Support for the role of TNFα in RA, and hence its promise as a therapeutic target candidate, came from the observation that the clinical signs and tissue damage of collagen-induced arthritis in mice were ameliorated by administration of a monoclonal anti-TNFα antibody [6]. In 1992, 20 patients with active RA despite treatment with disease-modifying antirheumatic drugs were the first to be treated with an anti-TNFα agent, infliximab (Remicade®, Centocor, Inc, Malvern, Pa). In this open-label clinical trial by our group at the Kennedy Institute of Rheumatology Division, the safety and marked anti-inflammatory effect of

ATTRACT = Anti-Tumor necrosis factor Trial in Rheumatoid Arthritis with Concomitant Therapy; CRP = C-reactive protein; IL = interleukin; MTX = methotrexate; RA = rheumatoid arthritis; TNFα = tumor necrosis factor alpha; VEGF = vascular endothelial growth factor.

intravenously administered infliximab was associated with a dramatic reduction in C-reactive protein (CRP) and erythrocyte sedimentation rate [7]. A multicenter, randomized, placebo-controlled trial in Europe quickly followed and confirmed the anti-inflammatory effect of a single intravenous infusion of infliximab [8]. However, most patients relapsed within 3 to 8 weeks demonstrating the requirement for repeat therapy [9]. The duration of benefit before relapse was related to the size of the drug dose (1 or 10 mg/kg).

The efficacy and optimal dose of infliximab, as well as an enhanced therapeutic efficacy when coadministered with methotrexate (MTX), was subsequently established in a follow-up, randomized, controlled clinical trial [10,11]. The consistency of a sustained therapeutic clinical response in long-term treatment (2 years) with infliximab plus MTX under double-blinded, placebo-controlled conditions has now been demonstrated in the international, multicenter Anti-Tumor necrosis factor Trial in Rheumatoid Arthritis with Concomitant Therapy (ATTRACT) [12–14]. Patients were randomized to receive either four dose schedules of infliximab plus weekly doses of MTX (median dose 15 mg/wk) or MTX alone. Serial radiographs performed at 24, 54, and 102 weeks of this trial have revealed retardation or arrest (and even improvement in 39% to 54% of patients) of both joint space narrowing (which equates with cartilage loss) and bone erosion. These results are in contrast to the progressive damage in the control group of patients who were treated with MTX alone [12,14]. These data are consistent with the hypothesis that TNFα plays a key role in the perpetuation of inflammation and destruction of cartilage and bone in RA.

Infliximab

Infliximab, a chimeric (mouse Fv1, human IgG1) monoclonal antibody, specifically binds to both soluble and membrane-bound TNFα with high affinity (Ka = 10^{10} M^{-1}), forming stable nondisassociating immune complexes [15]. The binding of infliximab to TNFα prevents the binding of TNFα to its receptors and blocks the initiation of the intracellular signaling that leads to gene transcription and subsequent biologic activity. The binding of infliximab to membrane-bound TNFα *in vitro* results in lysis of cell lines via a complement- or antibody-dependent cell cytotoxicity mechanism [16,17]. Whether this *in vitro* action has an *in vivo* correlate has not been confirmed. The similarity of clinical results observed for infliximab and etanercept, another anti-TNF agent, suggests that cell lysis may not be a necessary prerequisite, as etanercept does not exhibit similar cell lytic properties *in vitro*.

Treatment of RA patients with infliximab has provided an opportunity for clinical investigations that have illuminated aspects of its cellular and molecular bases of action and have provided insights into the pathogenesis of RA.

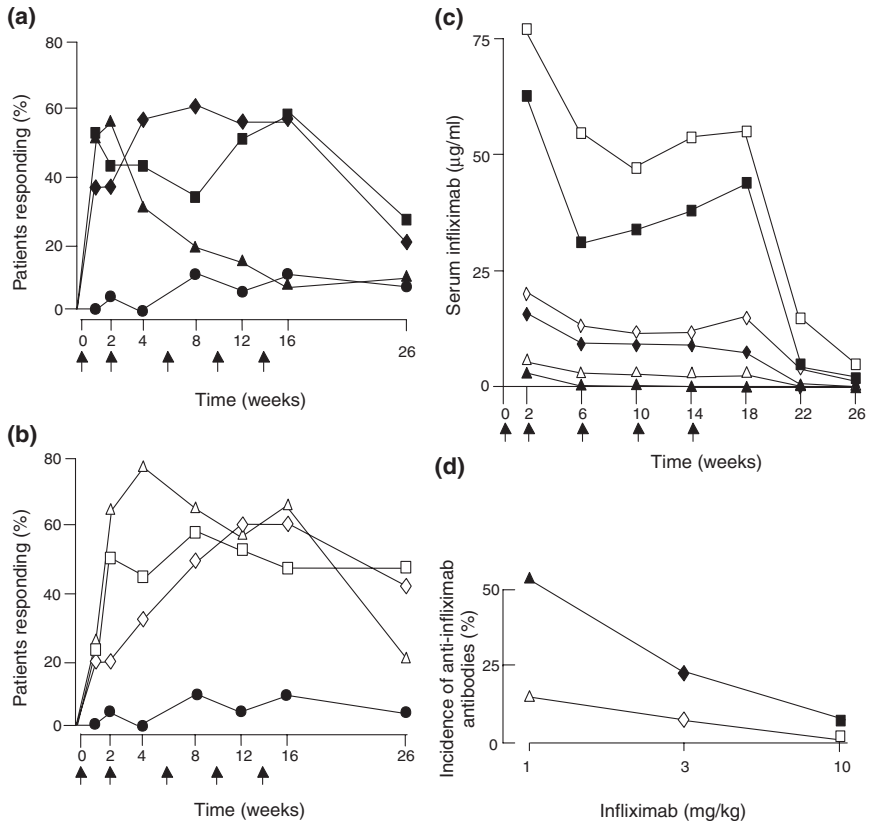
Pharmacokinetics and clinical response

Therapeutic response to infliximab correlates with the pharmacokinetics of infliximab and basal expression of TNFα in synovial tissue. Measurements of infliximab blood levels and TNFα expression in joints suggest that TNFα blockade at the site of production – mainly by cells of the macrophage lineage in the joint – is the key to its mode of action. There are close relationships between the dose of infliximab administered, infliximab blood concentrations, the durability of the clinical response, and eventual return of all clinical features following its clearance from blood [10,11]. Infliximab given repeatedly at a dose of 1 mg/kg was associated with a rapid loss of therapeutic response and accelerated clearance from the blood [10]. However, this study also demonstrated the synergy of infliximab when combined with MTX (Figure 1) [10], which is in part explained by a lowered incidence of anti-infliximab antibodies observed with combination therapy. These data indicate that the antibody affects the effector mechanisms and apparently does not terminate the more proximal events that drive the disease process. As approximately 60% to 70% of patients show an initial response to infliximab, it seems likely that, in a subset of the nonresponder population (defined by the ACR response), TNFα is not the key pivotal molecule regulating the cytokine network at that point of the disease course. However, a clear clinical and radiographic response has been noted in patients who do not demonstrate a response as measured by ACR 20 criteria. This conclusion is supported by the documentation of a correlation between a good clinical response to infliximab only when, prior to treatment, there is a significant level of expression of TNFα in synovial biopsies [18].

Infliximab regulates the cytokine network

It was noted in our first trial that, following the administration of infliximab, simultaneous reductions in CRP and IL-6 concentrations were observed in the blood [7]. This correlation was clearly demonstrated in a subsequent study, as was the rapid (within a few hours) reduction in serum IL-6 concentrations in infliximab-treated patients, but not in patients receiving placebo (Figure 2) [19]. As CRP production by hepatocytes is predominantly regulated by IL-6, the data are consistent with the conclusion that downregulation of IL-6 production in RA joints was a consequence of TNFα blockade. The dominant role of TNFα in the regulation of IL-6 in RA demonstrated *in vivo* was entirely consistent with the data obtained on the reduction of IL-6 production following the addition of anti-TNFα antibody to RA synovial membrane cell cultures *in vitro* [20]. The reduction of IL-1 synthesis in synovial tissue by an anti-TNFα antibody *in vitro* was the pivotal observation that led investigators to suspect the involvement of a cytokine cascade in RA [21]; however, it has been more difficult to verify these observations *in vivo*. Quantification of immunoreactive IL-1α and IL-1β by image analysis of synovial biopsies both before and 2 weeks after infliximab

Figure 1



Efficacy, pharmacokinetics, and antibody incidence of infliximab. Efficacy of infliximab as **(a)** monotherapy (closed symbols) and **(b)** in combination with methotrexate (open symbols) based on Paulus 20% criteria; infliximab 1 mg/kg (▲,△), infliximab 3 mg/kg (◆,◇), infliximab 10 mg/kg (■,□), and placebo plus methotrexate (●). Arrows indicate the infusion times of infliximab and placebo. **(c)** Pharmacokinetics of infliximab alone (closed symbols) or with methotrexate (open symbols); infliximab 1 mg/kg (▲,△) infliximab 3 mg/kg (◆,◇), and infliximab 10 mg/kg (■,□). **(d)** Incidence of anti-infliximab antibodies; infliximab alone (closed symbols) and infliximab plus methotrexate (open symbols). Panels a, b, and c, adapted with permission from Maini *et al*. [10]; panel d, data from Maini *et al*. [10]. Copyright 1999. American College of Rheumatology.

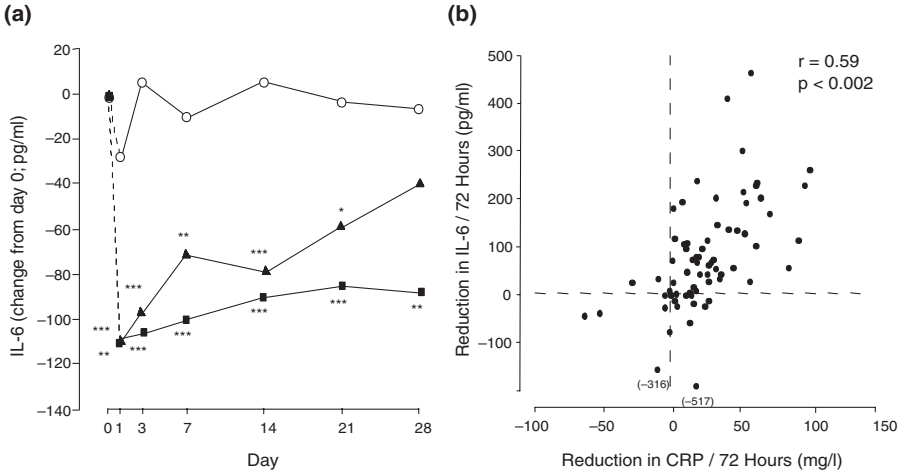
therapy revealed a reduction in, and linkage between, TNFα and IL-1 synthesis [18]. Serologic analysis of extremely low levels of IL-1 with different assays has provided conflicting results, with significant reductions observed in one laboratory and no consistent trend in another laboratory [19,22].

Following infliximab therapy, a reduction in serum concentration of IL-1ra (IL-1 receptor antagonist) and soluble TNF receptors has also provided evidence that two major anti-cytokines are regulated by TNFα (Figure 3) [19]. The simultaneous reduction in pro-inflammatory and anti-inflammatory molecules provides an interesting example of the dominance of TNFα in the cytokine network and a possible explanation for why anti-TNFα therapy does not restore a long-lasting remission but instead perpetuates the cytokine imbalance, and hence there is relapse of disease upon withdrawal of therapy.

Infliximab regulates cell recruitment

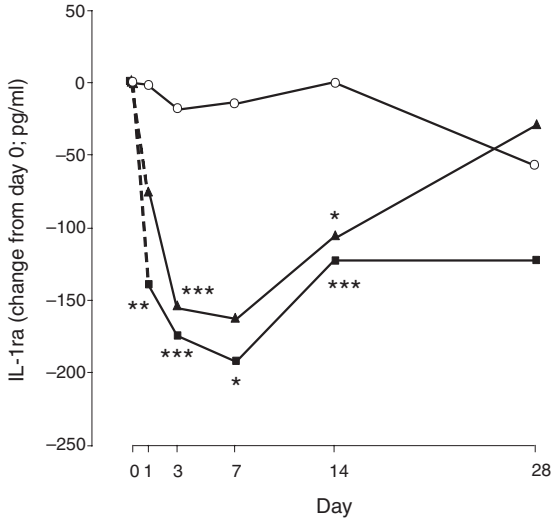
The marked reduction in the swelling and tenderness of joints following infliximab treatment was shown in an early study to be associated with a reduction in the cellularity of the synovium of RA patients [23]. In a detailed immunohistologic analysis of serial biopsies before and after infliximab, it was observed that a reduction in CD3+ and CD68+ cells was accompanied by a reduction in the adhesion molecules vascular cell adhesion molecule-1, intercellular adhesion molecule-1, and E-selectin [24]. Soluble E-selectin and soluble intercellular adhesion molecule-1 concentrations in blood were similarly reduced by infliximab, but not by placebo, and this reduction was closely correlated with an increase in circulating lymphocytes [25]. In further studies, it was found that the expression of the chemokines IL-8 and monocyte chemotactic protein-1 was also reduced in synovial biopsies within two weeks following infliximab therapy (Figure 4) [24,26,27]. These data

Figure 2



Effect of infliximab on IL-6 concentrations. **(a)** Circulating IL-6 concentrations in patients treated with a single infusion of infliximab or placebo on Day 0; infliximab 1 mg/kg (▲), infliximab 10 mg/kg (■), and placebo (○). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ compared with placebo. **(b)** The relationship between the reduction in circulating IL-6 by Day 3 and the reduction in C-reactive protein (CRP) over the same period ($r = 0.59$, $P < 0.002$). Adapted with permission from Charles *et al.* [19]. Copyright 1999. The American Association of Immunologists.

Figure 3



Effect of infliximab on circulating IL-1ra in patients treated with a single infusion of infliximab or placebo on Day 0. Infliximab 1 mg/kg (▲), infliximab 10 mg/kg (■), and placebo (○). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ compared with placebo. Adapted with permission from Charles *et al.* [19]. Copyright 1999. The American Association of Immunologists.

provided the evidence that anti-TNFα therapy regulates the expression of adhesion molecules and chemokines on rheumatoid vasculature. This led to the hypothesis that reversing the migration of circulating leukocytes into inflamed RA joints, and reversing their retention there, might be an important mechanism of action.

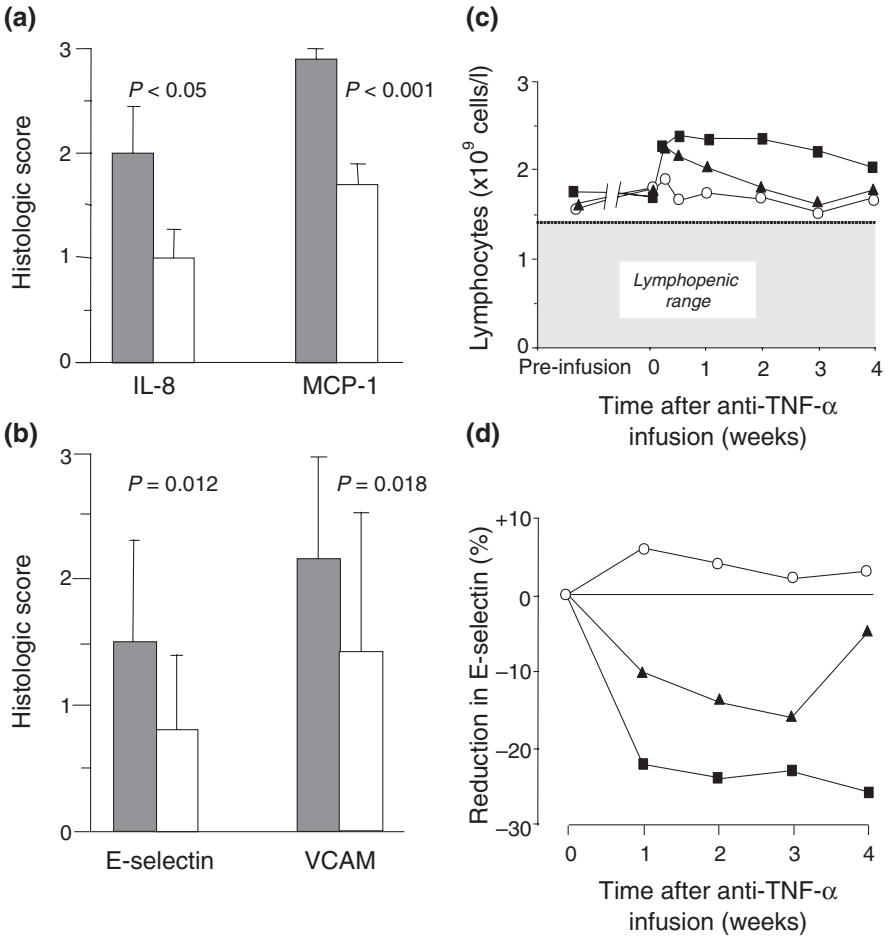
Direct evidence of a reduction in leukocyte recruitment in joints was obtained by gamma-camera imaging of ^{111}indium-labelled autologous polymorphonuclear cells in the hands and knees of RA patients before and after infliximab therapy (Figure 5) [26]. Because inflammatory disease is dependent on the influx of leukocytes, it is likely that this reduction in leukocyte trafficking is an important aspect of the mechanism of action of infliximab.

Infliximab regulates a major angiogenic factor and angiogenesis

From the early stages of disease, rheumatoid synovial inflammation is accompanied by a marked increase in angiogenesis. The increase in blood vessel density provides a conduit for the increased trafficking of blood-borne immune and inflammatory cells into joints. This increase in trafficking leads to the formation of vascular pannus tissue that invades and destroys cartilage and bone in the "bare area" of the attachment of synovium to subchondral bone.

The cytokine vascular endothelial growth factor (VEGF) is implicated in new blood vessel formation and is increased in the joints and blood of RA patients [25,28,29]. Infliximab therapy reduces circulating VEGF levels and the density of neovasculature in the synovium [25,30] (Figure 6). There is direct evidence of a reduction in the number of blood vessels in infliximab-treated patients. A reduction in angiogenesis may be relevant to our understanding of the anti-inflammatory and antidestructive properties of infliximab. In addition, although unproven, the exudative leakage of plasma mediated by VEGF may also be ameliorated by infliximab.

Figure 4



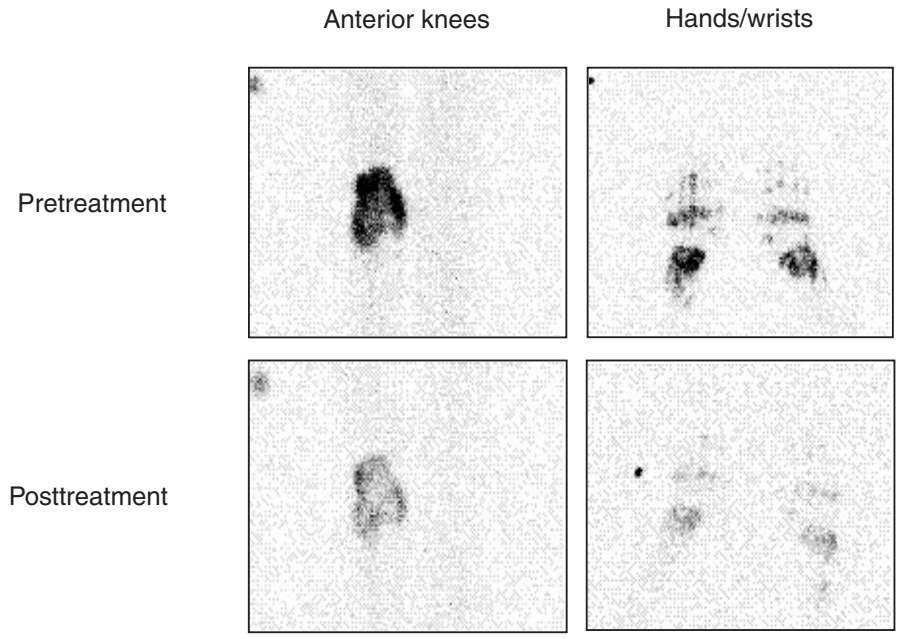
Histologic scores, expression of chemokines and adhesion molecules and lymphocyte counts before and after single infusion of infliximab. **(a)** and **(b)** Histologic scores of knee synovial biopsies before (shaded bar) and after (open bar) treatment by chemokine or adhesion molecule. **(c)** Circulating lymphocytes; infliximab 10 mg/kg (■), infliximab 1 mg/kg (▲), and placebo (○). **(d)** Change in serum E-selectin concentrations; infliximab 1mg/kg (▲), infliximab 10 mg/kg (■), and placebo (○). Panel a, data from Taylor PC *et al.* [26]; panel b, data from Tak PP *et al.* [24]; Panels c and d, adapted with permission from Paleolog *et al.* [25]. Copyright 1996, American College of Rheumatology.

Infliximab prevents cartilage catabolism and bone erosion

The most compelling evidence for the ability of anti-TNFα therapy to prevent cartilage loss and bone erosions following the onset of disease was obtained by histologic examination of joints in the collagen-induced arthritis mouse model of RA [6]. In this model, preservation of chondrocytes and cartilage matrix and the lack of pannus invasion of bone were notable features in response to treatment with infliximab. In RA patients in the ATTRACT trial, protection of cartilage and bone was observed – possibly with healing – as judged by comparison of baseline and 54-week radiographs of hands and feet in patients treated with infliximab [12,25]. This finding supports the conclusion that mechanisms of tissue destruction in RA are TNFα-dependent. Whether the coadministration of MTX with infliximab plays a part in the mechanism of action needs to be clarified. Because etanercept as monotherapy significantly slowed progression of bone erosions in early RA patients over one year compared with MTX monotherapy, the bone-protective action of anti-TNFα therapy is not in doubt [31]. A reduction in matrix metalloproteinase-1 and matrix metalloproteinase-3 following infliximab treatment has been documented, and although the cellular and molecular basis of anti-TNFα in this regard is not yet understood, this implies that a downregulation of matrix-degrading enzymes may be involved [32].

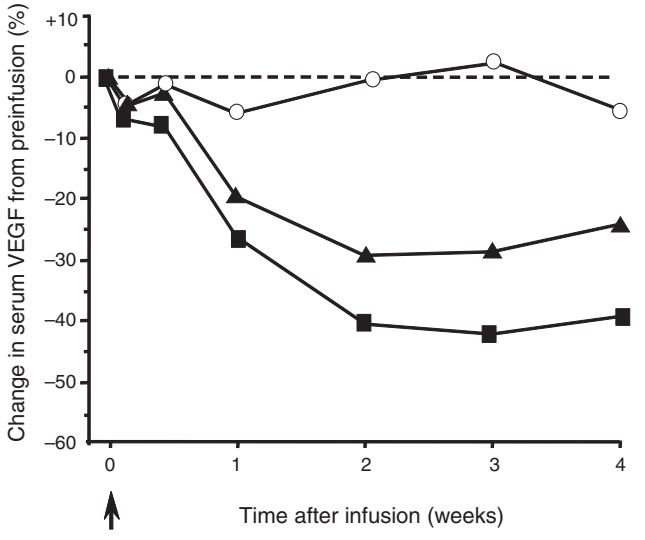
In animal models, IL-1 appears to play a critical role in cartilage destruction; it has been proposed that IL-1 may be a better therapeutic target in RA and that the joint protective effect of anti-TNFα therapy involves regulation of IL-1 production [33,34]. The activation and function of osteoclasts appear to involve not only IL-1 and TNFα, but also the

Figure 5



Gamma camera images of the knees and of the hands of a rheumatoid patient. Images were taken 22 hours after a bolus injection of autologous radiolabeled (^{111}Indium) granulocytes before and after a single 10 mg/kg intravenous bolus of infliximab. Adapted with permission from Taylor *et al*. [26]. Copyright 1999, American College of Rheumatology.

Figure 6



Serum VEGF concentrations at baseline and following a single infusion of infliximab in patients with active rheumatoid arthritis. Infliximab 1 mg/kg (▲), infliximab 10 mg/kg (■), and placebo (○). Adapted with permission from Paleolog *et al*. [29]. Copyright 1998, American College of Rheumatology.

receptor activator of NFκB ligand (RANKL), also known as TNF-related activation-induced cytokine (TRANCE), and the interaction of RANKL with RANK [35,36]. Further work is necessary to delineate the relative importance of these mechanisms.

Conclusion

Infliximab therapy for RA has illuminated the multiple pathways regulated by TNFα and its mechanism of action. These studies have begun to unravel the complex cellular and cytokine-dependent pathways that are involved and have provided a new therapeutic benchmark. The lessons we have learned will help to identify future research in developing the next generation of antirheumatic drugs with an improved efficacy and safety profile.

Acknowledgements

We wish to thank the Arthritis Research Council, which has supported our work through its grants to The Kennedy Institute of Rheumatology. We also thank Centocor, Inc. for providing additional support for the infliximab clinical studies.

References

1. Carswell EA, Old LJ, Kassel RL, Green S, Fiore N, Williamson B: **An endotoxin-induced serum factor that causes necrosis of tumors.** *Proc Natl Acad Sci USA* 1975, **72:**3666-3670.
2. Pennica D, Hayflick JS, Bringman TS, Pallandino MA, Goeddel DV: **Cloning and expression in Escherichia coli of the cDNA for murine tumor necrosis factor.** *Proc Natl Acad Sci USA* 1985, **82:**6060-6064.
3. Beutler B, Cerami A: **Cachectin and tumour necrosis factor as two sides of the same biological coin.** *Nature* 1986, **320:**584-588.
4. Feldmann M, Brennan FM, Maini RN: **Role of cytokines in rheumatoid arthritis.** *Annu Rev Immunol* 1996, **14:**397-440.

5. Maini RN, Feldmann M (Eds): *Pocket Reference to TNFα Antagonism and Rheumatoid Arthritis*. London: Science Press, Ltd; 2000.
6. 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:**9784-9788.
7. Elliott MJ, Maini RN, Feldmann M, Long-Fox A, Charles P, Katsikis P, Brennan FM, Walker J, Bijl H, Ghrayeb J, Woody JN: **Treatment of rheumatoid arthritis with chimeric monoclonal antibodies to tumor necrosis factor alpha.** *Arthritis Rheum* 1993, **36:**1681-1690.
8. Elliott MJ, Maini RN, Feldmann M, Kalden JR, Antoni C, Smolen JS, Leeb B, Breedveld FC, Macfarlane JD, Bijl H, Woody JN: **Randomised double-blind comparison of chimeric monoclonal antibody to tumour necrosis factor alpha (cA2) versus placebo in rheumatoid arthritis.** *Lancet* 1994, **344:**1105-1110.
9. Elliott MJ, Maini RN, Feldmann M, Long-Fox A, Charles P, Bijl H, Woody JN: **Repeated therapy with monoclonal antibody to tumour necrosis factor alpha (cA2) in patients with rheumatoid arthritis.** *Lancet* 1994, **344:**1125-1127.
10. Maini RN, Breedveld FC, Kalden JR, Smolen JS, Davis D, Macfarlane JD, Antoni C, Leeb B, Elliott MJ, Woody JN, Schaible TF, Feldmann M: **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:**1552-1563.
11. Maini RN, Elliott MJ, Long-Fox A, Feldmann M, Kalden JR, Antomio C, Smolen JS, Leeb B, Breedveld FC, Macfarlane JD, Bijl H, Woody JN: **Clinical response of rheumatoid arthritis to anti-TNFα (cA2) monoclonal antibody is related to administered dose and persistence of circulating antibody [abstract].** *Arthritis Rheum* 1995, **38(suppl 9):**S186.
12. Lipsky P, van der Heijde D, St. Clair W, Smolen J, Furst D, Kalden J, Weisman M, Breedveld F, Emery P, Keystone E, Harriman G, Maini R: **102-wk clinical & radiologic results from the ATTRACT trial: a 2 year, randomized, controlled, phase 3 trial of infliximab (Remicade®) in pts with active RA despite MTX [abstract].** *Arthritis Rheum* 2000, **43:**S269.
13. Maini R, St Clair EW, Breedveld F, Furst D, Kalden J, Weisman M, Smolen J, Emery P, Harriman G, Feldmann M, Lipsky P: **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:**1932-1939.
14. Lipsky PE, van der Heijde DM, St Clair EW, Furst DE, Breedveld FC, Kalden JR, Smolen JS, Weisman M, Emery P, Feldmann M, Harriman GR, Maini RN: **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:**1594-1602.
15. Knight DM, Trinh H, Le J, Siegel S, Shealy D, McDonough M, Scallon B, Moore MA, Vilcek J, Daddona P, Ghrayeb J: **Construction and initial characterization of a mouse-human chimeric anti-TNF antibody.** *Mol Immunol* 1993, **30:**1443-1453.
16. Siegel SA, Shealy DJ, Nakada MT, Le J, Woulfe DS, Probert L, Kollias G, Ghrayeb J, Vilcek J, Daddona PE: **The mouse/human chimeric monoclonal antibody cA2 neutralizes TNF in vitro and protects transgenic mice from cachexia and TNF lethality in vivo.** *Cytokine* 1995, **7:**15-25.
17. Barone D, Krantz C, Lambert D, Maggiora K, Mohler K: **Comparative analysis of the ability of etanercept and infliximab to lyse TNF-expressing cells in a complement dependent fashion [abstract].** *Arthritis Rheum* 1999, **42(suppl):**S90.
18. Ulfgren AK, Andersson U, Engstrom M, Klareskog L, Maini RN, Taylor PC: **Systemic anti-tumor necrosis factor alpha therapy in rheumatoid arthritis down-regulates synovial tumor necrosis factor alpha synthesis.** *Arthritis Rheum* 2000, **43:**2391-2396.
19. Charles P, Elliott MJ, Davis D, Potter A, Kalden JR, Antoni C, Breedveld FC, Smolen JS, Eberl G, deWoody K, Feldmann M, Maini RN: **Regulation of cytokines, cytokine inhibitors, and acute-phase proteins following anti-TNF-alpha therapy in rheumatoid arthritis.** *J Immunol* 1999, **163:**1521-1528.
20. Butler DM, Maini RN, Feldmann M, Brennan FM: **Modulation of proinflammatory cytokine release in rheumatoid synovial membrane cell cultures. Comparison of monoclonal anti TNF-alpha antibody with the interleukin-1 receptor antagonist.** *Eur Cytokine Netw* 1995, **6:**225-230.
21. Brennan FM, Chantry D, Jackson A, Maini R, Feldmann M: **Inhibitory effect of TNF alpha antibodies on synovial cell interleukin-1 production in rheumatoid arthritis.** *Lancet* 1989, **2:**244-247.
22. Lorenz HM, Antoni C, Valerius T, Repp R, Grunke M, Schwerdtner N, Nusslein H, Woody J, Kalden JR, Manger B: **In vivo blockade of TNF-alpha by intravenous infusion of a chimeric monoclonal TNF-alpha antibody in patients with rheumatoid arthritis. Short term cellular and molecular effects.** *J Immunol* 1996, **156:**1646-1653.
23. Maini RN, Elliott MJ, Brennan FM, Williams RO, Chu CQ, Paleolog E, Charles PJ, Taylor PC, Feldmann M: **Monoclonal anti-TNF alpha antibody as a probe of pathogenesis and therapy of rheumatoid disease.** *Immunol Rev* 1995, **144:**195-223.
24. Tak PP, Taylor PC, Breedveld FC, Smeets TJM, Daha MR, Kluin PM, Meinders AE, Maini RN: **Decrease in cellularity and expression of adhesion molecules by anti-tumor necrosis factor alpha monoclonal antibody treatment in patients with rheumatoid arthritis.** *Arthritis Rheum* 1996, **39:**1077-1081.
25. Paleolog EM, Young S, Stark AC, McCloskey RV, Feldmann M, Maini RN: **Modulation of angiogenic vascular endothelial growth factor by tumor necrosis factor alpha and interleukin-1 in rheumatoid arthritis.** *Arthritis Rheum* 1998, **41:**1258-1265.
26. Taylor PC, Peters AM, Paleolog E, Chapman PT, Elliott MJ, McCloskey R, Feldmann M, Maini RN: **Reduction of chemokine levels and leukocyte traffic to joints by tumor necrosis factor α blockade in patients with rheumatoid arthritis.** *Arthritis Rheum* 2000, **43:**38-47.
27. Paleolog EM, Hunt M, Elliott MJ, Feldmann M, Maini RN, Woody JN: **Deactivation of vascular endothelium by monoclonal anti-tumor necrosis factor alpha antibody in rheumatoid arthritis.** *Arthritis Rheum* 1996, **39**:1082-1091.
28. Fava RA, Olsen NJ, Spencer-Green G, Yeo KT, Yeo TK, Berse B, Jackman RW, Senger DR, Dvorak HF, Brown LF: **Vascular permeability factor/endothelial growth factor (VPF/VEGF): accumulation and expression in human synovial fluids and rheumatoid synovial tissue.** *J Exp Med* 1994, **180:**341-346.
29. Koch AE, Harlow LA, Haines GK, Amento EP, Unemori EN, Wong WL, Pope RM, Ferrara N: **Vascular endothelial growth factor. A cytokine modulating endothelial function in rheumatoid arthritis.** *J Immunol* 1994, **152:**4149-4156.
30. Taylor P, Patel S, Paleolog E, McCloskey R, Feldmann M, Maini RN: **Reduced synovial vascularity following TNFα blockade in RA. [abstract]** *Arthritis Rheum* 1998, **41(suppl 1):**S295.
31. Bathon JM, Martin RW, Fleischmann RM, Tesser JR, Schiff MH, Keystone EC, Genovese MC, Wasko MC, Moreland LW, Weaver AL, Markenson J, Finck BK: **A comparison of etanercept and methotrexate in patients with early rheumatoid arthritis.** *N Engl J Med* 2000, **343:**1586-1593.
32. Brennan FM, Browne KA, Green PA, Jaspar JM, Maini RN, Feldmann M: **Reduction of serum matrix metalloproteinase 1 and matrix metalloproteinase 3 in rheumatoid arthritis patients following anti-tumour necrosis factor-alpha (cA2) therapy.** *Br J Rheumatol* 1997, **36:**643-650.
33. van den Berg WB: **Uncoupling of inflammatory and destructive mechanisms in arthritis.** *Semin Arthritis Rheum* 2001, **20**:7-16.
34. Brennan FM: **A follow-up to "Anti-cytokine therapy in chronic destructive arthritis" by Wim B van den Berg.** *Arthritis Res* 2001, **3:**211-213.
35. Lader CS, Flanagan AM: **Prostaglandin E2, interleukin 1alpha, and tumor necrosis factor-alpha increase human osteoclast formation and bone resorption in vitro.** *Endocrinology* 1998, **139:**3157-3164.
36. Yasuda H, Shima N, Nakagawa N, Yamaguchi K, Kinosaki M, Mochizuki S, Tomoyasu A, Yano K, Goto M, Murakami A, Tsuda E, Morinaga T, Higashio K, Udagawa N, Takahashi N, Suda T: **Osteoclast differentiation factor is a ligand for osteoprotegerin/osteoclastogenesis-inhibitory factor and is identical to TRANCE/RANKL.** *Proc Natl Acad Sci USA* 1998, **95:**3597-3602.