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REVIEW

Treatment of rheumatoid arthritis with tumour necrosis factor inhibitors

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Advances in our understanding of the key mediators of chronic inflammation and tissue damage characteristic of rheumatoid arthritis (RA) have resulted in the development of novel therapies primarily targeting pro-inflammatory cytokines. Inhibitors of tumour necrosis factor (TNF) are the most widely used of the biological therapies at present with five different agents currently available; four are based on monoclonal anti-TNF antibodies and a soluble TNF receptor-Fc fusion protein. Long-term use of these molecules has proven to be highly effective in the majority of patients; however, around one-third have a suboptimal response potentially leading to further cartilage and bone damage, furthermore these agents are expensive compared with conventional therapies such as methotrexate. Many recent studies have attempted to identify therapeutic response biomarkers of TNF inhibitors which could be used to improve therapeutic targeting. The presence of rheumatoid factor and anti-cyclic citullinated protein antibodies, present in around 65% of RA patients, are associated with a poorer response to anti-TNF agents. Poorer response is also associated with levels of C-reactive protein and cartilage degradation product at initiation of treatment. Intriguingly, genetic studies of variants of TNF and of genes encoding members of the Toll-like receptors, nuclear factor-kappa B and p38 mitogen-activated protein kinase signalling families have been associated with response to individual anti-TNF agents. Continued advances in technologies such as ultra high throughput sequencing and proteomics should facilitate the discovery of additional biomarkers of response to anti-TNF resulting in improved disease control and quality of life for RA patients and reduced costs for healthcare funders.

Abbreviations

DAS28, disease activity score in 28 joints; HACA, human anti-chimeric antibodies; LTα, lymphotoxin alpha; mTNF, membrane TNF; RA, rheumatoid arthritis; sTNF, soluble TNF; TNF, tumour necrosis factor

Introduction

Tumour necrosis factor (TNF) is a member of the TNF superfamily, which includes a number of structurally and functionally related trimeric molecules including lymphotoxin alpha (LTα), previously known as TNFβ, as well as the pro-apoptotic molecule Fas, the B cell regulatory molecule CD40 and receptor activator of nuclear factor kappa-b (Grewal, 2009). Cellular effects of this cytokine are diverse and include cell proliferation, differentiation and apoptosis (Gaur and Aggarwal, 2003). The biological functions of TNF relate to key roles in both innate and adaptive immunity. The TNF polypeptide is synthesized as a 26 kDa membrane-associated form (mTNF) which is proteolytically cleaved by a TNF-alpha converting enzyme to produce a 17 kDa soluble homotrimer (sTNF). Both mTNF and sTNF are biologically active. The effects of TNF are mediated by engagement of two receptors of the TNF receptor superfamily, p55/TNFRI and p75/TNFRII via distinct signalling pathways involving the transcription factor nuclear factor-kappa B (MacEwan, 2002). The elucidation of the role of TNF in rheumatoid arthritis (RA) provided a paradigm for the validation of cytokines as therapeutic targets in a range of immune-mediated inflammatory diseases subsequently. This validation began with the demonstration of excessive production of TNF at the site of inflammation both in animal models and human subjects (di Giovine *et al.*,

Table 1

Characteristics of different TNF inhibitors used in the treatment of RA

Structure	Infliximab Monoclonal antibody	Etanercept p75TNFR/Fc fusion	Adalimumab Monoclonal antibody	Certolizumab Pegylated mononclonal antibody	Golimumab Mononclonal antibody
Loading dosage required	Yes	No	No	Yes	No
Dosing					
1. Method	Intravenous	Subcutaneous	Subcutaneous	Subcutaneous	Subcutaneous
2. Frequency	8 weeks	Weekly	2 weeks	2 weeks	Monthly
Half-life in humans (days)	9.5	3	14	14	12
Target(s)	TNF	TNF & LT	TNF	TNF	TNF
Fully humanized	Partially murine	Yes	Yes	Yes	Yes

LT, lymphotoxin; TNF, tumour necrosis factor.

1988) and progressed to the efficacy of blockade of TNF shown in pivotal clinical trials in RA (Maini *et al.*, 1999) and other diseases. There are currently five TNF inhibitors licensed for the treatment of RA in Europe (Table 1). Infliximab, adalimumab and golimumab are TNF-specific monoclonal antibodies. Etanercept is a fusion protein comprising two TNFR2 extracellular domains fused to a single human IgG1Fc fragment containing the CH2 domain, the CH3 domain and hinge region, but not the CH1 domain. Certolizumab pegol is a TNF-specific Fab fragment bound to polyethylene glycol. All TNF inhibitors are capable of binding to and inhibiting the effects of TNF; however, during clinical use important differences in disease specificity, heterogeneity of response and possibly adverse events have emerged. All available agents seem to demonstrate equivalent efficacy in RA (Hochberg *et al.*, 2003; Kristensen *et al.*, 2007); however, there is a 30% rate of non-response. Non-responders to one agent may, however, respond to another (Gomez-Puerta *et al.*, 2004; Furst *et al.*, 2007). Both infliximab and adalimumab are more effective than etanercept in both psoriasis (Mease *et al.*, 2005; 2006), Crohn's disease (Peyrin-Biroulet *et al.*, 2008) and uveitis (Braun *et al.*, 2005; Tynjala *et al.*, 2007) and etanercept was ineffective in the treatment of primary vasculitis (Wegener's Granulomatosis Etanercept Trial (WGET) Research Group, 2005). Etanercept is associated with a lower risk of reactivation of tuberculosis which is the commonest severe side effect of anti-TNF therapy (Dixon *et al.*, 2010). It has been suggested that these differences may relate to differential ability of the various TNF blockers to break down granulomas (Wallis and Ehlers, 2005). Granuloma formation is dependent on close interaction between T cells and macrophages, in which TNF is believed to play a critical role. Such observations suggest there may be clinically significant differences between these agents. In this review, we will describe the pharmacological properties of the currently studied TNF inhibitors emphasizing biological differences, and suggest mechanisms that may be responsible for the heterogeneity of these clinical effects. Such insights may allow a more personalized approach to the use of these biological agents.

Pharmacokinetics and tissue absorption

All TNF inhibitors are recombinant proteins and are too large to be filtered at the glomerulus. The half-life of intravenously administered human IgG is between 30 and 40 days, but is rather more variable for specific antibodies (Schiff, 1986). The plasma half-life of intravenously administered infliximab is 9–12 days (Klotz *et al.*, 2007) although trough levels are likely to be more relevant to efficacy. Detectable trough levels of infliximab are associated with a better response to treatment of ulcerative colitis (Seow *et al.*, 2010) as well as RA (Krzysiek *et al.*, 2009; Radstake *et al.*, 2009) and ankylosing spondylitis (Krzysiek *et al.*, 2009). The different dosing regimes represent the optimal schedule for each agent to achieve trough levels. Despite infliximab having a half-life of 9–12 days, it is administered every 8 weeks, compared with adalimumab, which is administered every other week, but has a longer half-life of around 15 days. This is also the likely explanation for the observation that 50 mg of the fusion protein etanercept given once weekly has been shown to be as effective as 25 mg given twice weekly in RA.

Animal studies have demonstrated absorption of fluorescence-labelled anti-TNF agents to the site of inflammation in collagen-induced arthritis. Increased vascularity and endothelial permeability associated with inflammation might be expected to allow all three agents to penetrate the inflamed joint. Indeed, this is what was found; however, the distribution of Certolizumab to inflamed versus non-inflamed was higher than adalimumab or infliximab (Palframan *et al.*, 2009). Whether this difference is important clinically is not established.

Ligand–receptor interactions, specificity and valency

All five TNF blockers can bind sTNF and mTNF, but the fusion protein etanercept has additional specificity for both soluble

and membrane-associated LTα which is also able to engage both TNFR1 and TNFR2. The effects of combined blockade may confer a difference in efficacy or adverse effects if the balance between these two cytokines has a biological significance. Considerable variation in LTα binding capacity of etanercept measured in patients has been described (Gudbrandsdottir *et al.*, 2004a,b). Further differences exist between agents in the effects of engagement of mTNF. All agents are capable of blocking the interaction of mTNF with receptors on other cells; however, infliximab is able to cross-link mTNF and in some circumstances this can have agonistic effects on the target cell. These effects include suppression of T-cell proliferation, suppression of cytokine production and the induction of apoptosis. Etanercept, which is capable of binding a single homotrimer and therefore unable to cross-link mTNF, shares some but not all of these properties (Mitoma *et al.*, 2008).

A further consequence of differences in valency between TNF blockers relates to the ability to form drug-ligand complexes. All three monoclonal antibodies are bivalent and their specificity is directed to the monomeric subunit of the TNF homotrimer. Hence, a single homotrimer could be bound to up to three mAbs, and each mAb in turn could bind two homotrimers allowing the formation of large multimeric immune complexes. In contrast, both etanercept and certolizumab pegol are monovalent (Santora *et al.*, 2001; Scallon *et al.*, 2002). Possible consequences of the formation of such complexes include enhanced clearance due to phagocytosis (which may be responsible for the shorter observed half-life compared with IgG), enhanced immunogenicity or Fc-mediated effects (discussed below) (Scallon *et al.*, 2004), or adverse effects such type III hypersensitivity reactions.

Structure and effects of the Fc

All three monoclonals and etanercept contain the Fc fragment of human IgG1, while certolizumab pegol does not. The presence of Fc allows engagement of the drug or drug-ligand complexes with Fc receptors. Fc receptors are expressed on a variety of immune cells and have critical roles in regulating the effects of antibodies and immune complexes on immune cell function. The high-affinity FcgRI binds monomeric IgG, whereas the low-affinity Fcgamma RII and RIII require multimeric immune complexes. Subtypes of FcgammaRII and RIII have differing functions with some transducing stimulatory and some inhibitory signals (Ravetch and Bolland, 2001). The effects of engagement of Fcgamma receptors include enhanced phagocytosis (opsonization), enhancement of immunogenicity via more efficient antigen presentation, antibody dependent cytotoxicity, effects on cellular proliferation and differentiation, and production of cytokines. The engagement of Fc receptors by TNF blocking drugs and/or drug-ligand complexes has been demonstrated to induce a number of cellular effects *in vitro*. Complement-mediated cytotoxicity and antibody-dependent cytotoxicity induced by adalimumab and infliximab in an *in vitro* assay was greater than that induced by etanercept (Mitoma *et al.*, 2008).

Immunogenicity

As infliximab is a chimeric monoclonal antibody with mouse Fv region primary structure components, repeated administration would be expected to generate human anti-chimeric antibodies (HACA) has been observed. The prevalence of HACA varies between studies and in different diseases, but for RA ranges from 14% to 40%. The immunogenicity of infliximab is reduced by administration of higher doses (Ruperto *et al.*, 2007). The reason for this is not known, but may be due to a saturation phenomenon. Formation of HACA is also reduced by concomitant use of methotrexate, possibly due to immunomodulatory effects of the drug. While infliximab is the most immunogenic monoclonal, anti-idiotype antibodies can arise to monoclonals with a fully human primary structure (Bartelds *et al.*, 2007). The clinical significance of such antibodies will depend partly on whether they are neutralizing or not. In the case of infliximab, there are data suggesting that such HACA are indeed clinically relevant. The need for dose escalation of infliximab is associated with the presence of HACA (Haraoui *et al.*, 2006). Undetectable trough levels are associated with the presence of HACA and reduced efficacy in both ulcerative colitis (Seow *et al.*, 2010) and RA (Radstake *et al.*, 2009). A recent study reported that in RA subjects switching from infliximab to adalimumab, the presence of antibodies to infliximab was associated with a poorer response to adalimumab suggesting either cross-reaction of antibodies or shared immunogenicity (Bartelds *et al.*, 2010). The generation of antibodies to infliximab may also lead to the development of infusion reactions (Bendtzen *et al.*, 2006). Antibodies to etanercept are found at much lower frequency and are not associated with any clinically relevant effects (de Vries *et al.*, 2009). This may be because antibodies to etanercept might be expected to be specific for the hinge region, which would not affect the TNF binding domain.

Biomarkers of response to anti-TNF treatment of RA

Although the use of TNF inhibitors has revolutionized the treatment of aggressive RA, these agents are expensive and around 30% of patients fail to respond adequately. Improved therapeutic targeting of anti-TNF agents based on biomarker profiling has the potential to improve overall disease control leading to improve quality of life for the patient and reduced costs to healthcare providers (Table 2). In Britain RA patients treated with anti-TNF agents have been included in the British Society for Rheumatology Biologics Register. This is the largest world-wide prospective register of such patients in the world containing data on 19 000 patients. The primary aim of the register is to monitor the long-term safety profile of these agents; however, it includes 6 monthly response scores using the validated disease activity score in 28 joints (DAS28). Poorer response is associated with a higher baseline health assessment questionnaire score, female sex and smoking status (inflixmab but not etanercept) (Hyrich *et al.*, 2006). The search for biomarkers of response to anti-TNF agents is currently highly active. Most studies to date have explored the utility of genetics and autoantibody profiling to

Table 2

Recently identified biomarkers of response to anti-TNF treatment of RA

Type of biomarker	Associated clinical response	Reference
Genetic		
TNF	Response to infliximab but not etanercept associated with rs1800629 genotype	(Maxwell *et al.*, 2008)
TLR and NF-κB pathway genes	Response to etanercept but not infliximab associated with rs7744 (*MYD88*) and rs11591741 (*CHUK*) genotypes	(Potter *et al.*, 2010)
P38 MAPK pathway	Response to adalimumab and infliximab but not etanercept with rs1258012 (*MKNK1*), rs1286112 and rs1286078 (*RPS6KA5*) and rs2096525 (*MIF*)	(Coulthard *et al.*, 2010)
CD45	Response associated with rs10919563 in CCP antibody positive patients	(Cui *et al.*, 2010)
Autoantibodies		
RF and anti-CCP	Better response in autoantibody negative RA	(Potter *et al.*, 2009)
Gene expression		
mRNA levels	Poorer response to etanercept associated with down-regulation of expression of inflammatory transcripts in blood mononuclear cells after 72 h of treatment	(Koczan *et al.*, 2008)
Histopathology		
Synovial infiltrate	Lympocytic aggregates rather than diffuse infiltrate associated with improved response	(Klaasen *et al.*, 2009)
Systemic proteins		
C-reactive protein	Poorer response to infliximab associated with failure to suppress CRP after 2 weeks of treatment	(Buch *et al.*, 2005)
COMP	Low levels pretreatment associated with poorer response to adalimumab	(Morozzi *et al.*, 2007)
Anti-TNF antibodies		
Neutralizing antibodies	Non-response to adalimumab associated with the development of neutralizing antibodies	(Bartelds *et al.*, 2007)

CCP, cyclical citrullinated peptide; COMP, cartilage oligomeric matrix protein; CRP, c-reactive protein; MAPK, mitogen-activated protein kinase; NF-κB, nuclear factor-kappa B; RA, rheumatoid arthritis; RF, rheumatoid factor; TLR, toll-like receptor; TNF, tumour necrosis factor.

predict response to anti-TNF therapies; however, most of the studies have been underpowered or variable methods of assessing efficacy.

The advances in human genetics over the past decade including the characterization of human genetic variation allied with technological and bioinformatic advances have made genetics a particularly intensive area of study. In addition, the cost and ease of genotyping have fallen considerably in recent years. Genetic studies to date have used the candidate gene approach and a more comprehensive hypothesis free analysis using whole genome-associated design is awaited. The Biologics in Rheumatoid Arthritis Genetics and Genomics Study Syndicate was formed to facilitate well-powered studies using the British Society for Rheumatology Biologics Register database. Clinical efficacy was determined using the change in the DAS28 after 6 months of anti-TNF treatment. This is a validated composite measure of disease activity that includes variables such as total number of swollen and tender joints, patient visual analogue score and levels of the acute phase response such as C-reactive protein (van Gestel *et al.*, 1996). In a study of 908 patients, an association of homozygosity of the minor variant of TNF-308 (genotype frequency of 2%) was associated with non-response to etanercept but an excellent response to infliximab (Maxwell *et al.*, 2008). The Toll-like receptors and nuclear factor-kappa B signalling systems are two major regulators of the immune and inflammatory responses. Using the Biologics in Rheumatoid Arthritis Genetics and Genomics Study Syndicate population 187 single nucleotide polymorphisms in 24 genes within these two pathways were genotyped. Associations were demonstrated between response to etanercept but not infliximab and markers in *MYD88* (rs7744) and *CHUK* (rs11591741) genes (Potter *et al.*, 2010). Conversely in a study of 12 genes in the p38 mitogen-activated protein kinase pathway associations of markers within *MKNK1* (rs1258012), *RPS6KA5* (rs1286112 and rs1286078) and *MIF* (rs2096525) and response to anti-TNF monoclonal antibodies but not etanercept were detected (Coulthard *et al.*, 2010). The explanation for this finding may be related to the ability of anti-TNF monoclonal antibodies to transduce a reverse signal through mTNF. These studies indicate the potential of genetic biomarkers in helping to select the most appropriate anti-TNF agent for individual RA patients.

The role of autoantibodies in diagnosing RA is clearly established, and the presence of rheumatoid factor or anti-citrullinated protein antibodies also has prognostic value (Mewar *et al.*, 2006). Furthermore, the presence of these

autoantibodies is associated with a poorer response to anti-TNF agents, independent of disease activity (Potter *et al.*, 2009).

Although genetic markers and autoantibodies have been most extensively examined in relation to their ability to predict response to anti-TNF therapy for RA, a number of other therapeutic response biomarkers have been reported. Down-regulation of expression of a number of pro-inflammatory genes, including IL-1b, IL-8 and TNFAIP3, in peripheral blood mononuclear cells 72 h after the first dose of etanercept was associated with a good response during the first 3 months (Koczan *et al.*, 2008). The synovial infiltrate in RA varies between a diffuse cellular infiltrate or a more organized lymphocyte aggregate pattern which may include germinal centres and the latter has been associated with superior response to infliximab at 16 weeks (Klaasen *et al.*, 2009). Failure to suppress production of C-reative protein 2 weeks after starting infliximab was associated with a poor response after 12 weeks (Buch *et al.*, 2005). Low-serum levels of a cartilage turnover protein prior to starting adalimumab have been associated with a better response within the first 3 months (Morozzi *et al.*, 2007). The development of autoantibodies targeting individual anti-TNF agents has been proposed as a mechanism for non-response and antibodies against adalimumab usage was lower in these patients compared the group without antibodies and this could be the reason for the lower efficacy (Bartelds *et al.*, 2007).

Conclusion

The currently available biomarkers, however, have relatively limited clinical utility and large, sufficiently powered studies using validated outcome measures and state-of-the-art technologies such as ultra high throughput sequencing to determine both genetic variants and gene expression in relevant tissue and emerging proteomic approaches should lead to the identification of a more comprehensive biomarker panel that could be used in therapeutic targeting of these highly effective but expensive agents with resultant benefits to RA patients and healthcare funding agencies.

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Conflicts of interest

There are no conflicts of interest.

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