Anti-TNF Antibodies: Lessons from the Past, Roadmap for the Future

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Abstract Tumor necrosis factor alpha (TNF) is an important cell-signaling component of the immune system. Since its discovery over 20 years ago, much has been learned about its functions under normal and disease conditions. Nonclinical studies suggested a role for TNF in chronic immune-mediated inflammatory diseases, such as rheumatoid arthritis, Crohn's disease, and psoriasis, and therefore neutralizing monoclonal antibodies specific to human TNF were developed for clinical evaluation. Treatment with anti-TNF monoclonal antibodies (infliximab, adalimumab, and certolizumab pegol) has been shown to provide substantial benefit to patients through reductions in both localized and systemic expression of markers associated with inflammation. In addition, there are beneficial effects of anti-TNF treatment on markers of bone and cartilage turnover. Further exploration of changes in these markers and their correlation with clinical measures of efficacy will be required to allow accurate prediction of those patients most in need of these treatments. Both the clinical and commercial experience with these anti-TNF antibodies provide a wealth of information regarding their pharmacological effects in humans.

1 Introduction

Tumor necrosis factor-alpha (TNF) is a proinflammatory cytokine, and is a known mediator of chronic immune-mediated inflammatory diseases, such as rheumatoid arthritis (RA), Crohn's disease (CD), and psoriasis. TNF is expressed as a transmembrane precursor that undergoes proteolytic processing to form a soluble trimer. The binding of both the membrane-bound and soluble forms of TNF to its receptors, TNFR1 and TNFR2, initiates the expression of several other proinflammatory cytokines (eg., interleukin [IL]-1, IL-6, and interferon [IFN]-γ), cell adhesion molecules (eg., intracellular adhesion molecule [ICAM]-1), and general inflammatory markers. Several animal and disease models have provided the foundation for the development of anti-TNF antibodies as treatments for chronic inflammatory diseases.

Two TNF-specific monoclonal antibodies, infliximab (Remicade®) and adalimumab (Humira®), have been approved for patient use (Table 1). In addition, the TNF-specific, pegylated Fab′ antibody fragment certolizumab pegol (Cimzia®) has reached the final phase of clinical development (Table 1), and other TNF antagonist therapies are also approved or under development. The goal of therapy with anti-TNF antibodies is to reduce the levels of TNF in the circulation to ameliorate the clinical signs of disease, without causing systemic immunosuppression in the patient.

Here, we will discuss the role of TNF (and its receptors) in chronic inflammatory diseases, including the results of several animal and disease models. In addition, we describe the development of three anti-TNF antibodies (infliximab, adalimumab, and certolizumab pegol), and their pharmacokinetic and pharmacodynamic properties.

Table 1 Anti-TNF monoclonal antibodies

	Infliximab (Remicade®, Centocor)	Adalimumab (Humira®, Abbott)	Certolizumab-pegol (CDP-870/Cimzia®, UCB)
Description	Human/mouse chimeric IgG1, kappa	Human IgG1, kappa	Human Fab′ fragment pegylated
Cell line for manufacture	Mouse myeloma	Chinese hamster ovary	*Escherichia coli*
Route of administration	Intravenous	Subcutaneous	Subcutaneous
Half-life (days)	7.7–9.5	10–20	14
Current/approved dosing	3–10 mg kg^{-1} at 0, 2, and 6 weeks and then every 8 weeks	40 mg weekly or every other week	400 mg every 4 weeks
Indications	Approved for: CD, RA, AS, PsA, UC, Ps	Approved for: CD, RA, AS, PsA	In Phase III studies for: CD, RA
Changes in biomarkers	CRP, inflammatory cytokines, anti-CCP, RF, MMPs, bone and cartilage markers, regulatory T cells	CRP, inflammatory cytokines, anti-CCP, RF, MMPs	CRP

CD, Crohn's disease; RA, rheumatoid arthritis; AS, ankylosing spondylitis; PsA, psoriatic arthritis; UC, ulcerative colitis; Ps, psoriasis; CRP, C-reactive protein; CCP, cyclic citrullinated peptide; RF, rheumatoid factor; MMPs, matrix metalloproteinases

Another anti-TNF biologic therapy, etanercept (Enbrel®), is a fusion protein of two TNFR2 receptor extracellular domains and the Fc portion of human IgG, and this agent has also been approved for the treatment of several immune-mediated inflammatory diseases. However, because this product is not an anti-TNF monoclonal antibody, it is not discussed in this review.

2 Lessons from TNF Characterization and Receptor Activation

An endotoxin-induced serum factor originally described by Old and colleagues (Carswell et al. 1975) demonstrated a remarkable ability to lyse specific murine tumor cells, and this biologic activity is reflected in the name for this protein–tumor necrosis factor, or TNF. When TNF was purified to homogeneity (Aggarwal et al. 1985), and the gene encoding TNF was cloned (Pennica et al. 1984), it was soon recognized to be identical to cachectin, a protein that suppressed anabolic enzymes in adipocytes (Beutler et al. 1985a). Two specific receptors were also identified: TNFR1 (also known as p55 or CD120a) and TNFR2 (also known as p75 or CD120b). TNF and its receptors became the prototypes for the TNF ligand superfamily and the corresponding TNF receptor superfamily (Aggarwal 2003), whose members are integral to the control of cell differentiation, proliferation, and apoptosis necessary for mammalian development, in particular immune function and hematopoeisis. In contrast, uncontrolled excessive production of TNF can lead

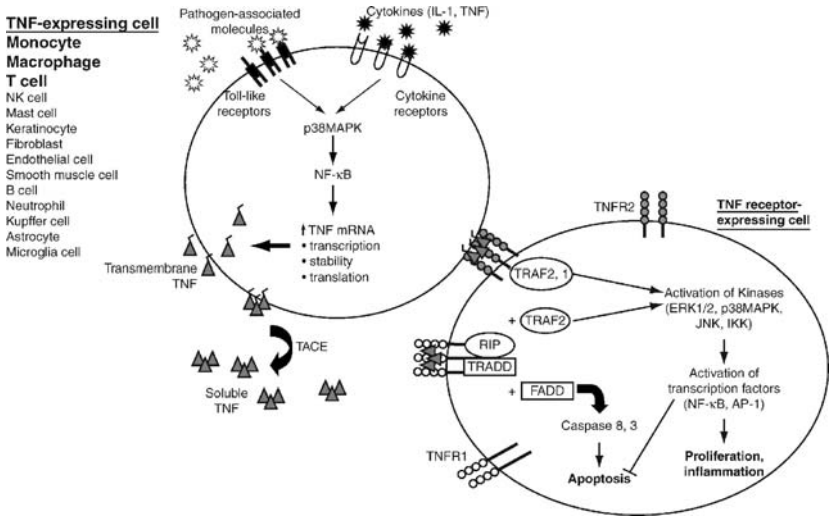

Fig. 1 Mechanisms for TNF expression and signaling. TNF, tumor necrosis factor; IL-1, interleukin-1; NF-κB, nuclear factor kappa B; TACE, TNF-alpha converting enzyme; TNFR1, TNF receptor 1; TNFR2, TNF receptor 2; TRADD, TNF receptor-associated death domain; TRAF2, TNF receptor-associated factor 2; RIP, receptor-interacting protein; FADD, Fas-associated death domain; AP-1, activator protein-1; ERK1/2, extracellular signal-related kinase 1/2; p38 MAPK, p38 mitogen-activated protein kinase; JNK, c-Jun N-terminal kinase; IKK, I kappaB kinase

to the chronic inflammation (Feldmann et al. 2004) that is the hallmark of diseases such as RA, CD, and psoriasis.

TNF is secreted primarily by activated monocytes, macrophages, and T cells, although a number of other cell sources have also been described (Fig. 1). Activation of Toll-like receptors by a variety of pathogen-associated molecules, or the activation of cytokine receptors such as IL-1, stimulates nuclear factor kappa B (NF-κB) transcription factors that increase TNF gene transcription, as well as genes encoding trans-acting factors that significantly increase TNF mRNA stability (Seko et al. 2006), and rapidly increase TNF protein secretion. TNF is expressed as a 26 kDa type II membrane-bound protein that self-associates into the bioactive homotrimer (Black et al. 1997; Moss et al. 1997), and is rapidly released by the protease TNF-alpha converting enzyme (TACE, also known as ADAM17). Both the transmembrane (Smith and Baglioni 1987) and soluble trimeric forms of TNF (Perez et al. 1990) can activate the TNF receptors. The extracellular domains of TNFR1 and TNFR2 bind to the cleft between TNF subunits, which causes the clustering of the receptor cytoplasmic domains and initiates signaling (Bazzoni and Beutler 1996).

The existence of two receptors contributes to the diversity of processes involving TNF. TNFR1 is constitutively expressed on virtually all nucleated cells, while expression of TNFR2 is limited to immune cells and endothelial cells (Aggarwal 2003). In addition to tissue distribution, there are clear differences in the signaling pathways activated by these two receptors. Activation of TNFR1 can have several outcomes, depending upon the expression of various accessory proteins present in

the specific cell types. The cytoplasmic domain of TNFR1 includes a death domain motif that can form signaling complexes that directly activate caspase-3 and caspase-8 and initiate apoptosis (Ashkenazi and Dixit 1998). After TNF binding, the death domain recruits accessory proteins such as TNF receptor-associated death domain (TRADD), receptor-interacting protein (RIP), and TNF receptor-associated factor 2 (TRAF2). At this point, the presence of other accessory proteins in the cell determines whether signaling will initiate or inhibit apoptosis. Further recruitment of the Fas-associated death domain (FADD) leads to the binding and activation of procaspase-8, which in turn activates caspase-3 and induces apoptosis.

Alternatively, TRAF2 can recruit cellular inhibitors of apoptosis (cIAP-1 and cIAP-2) and activate signaling pathways leading to nuclear translocation of the antiapoptotic transcription factors NF-κB and activator protein-1 (AP-1), which regulate the expression of genes necessary to block apoptosis and to increase cell proliferation and proinflammatory proteins (Baud and Karin 2001). The NF-κB pathway alone activates over 200 proinflammatory genes (Kumar et al. 2004). The control of the molecular switch that determines whether the cell will undergo apoptosis or proliferation is not well understood. Several reports suggest that internalization of the TNF/TNFR1 signaling complex is necessary for activation of apoptosis (Micheau and Tschopp 2003; Schutze et al. 1999), while the clustering of the TNF/TNFR1 signaling complex in lipid rafts leads to activation of the NF-κB and AP-1 pathways (Legler et al. 2003). Additional studies will be necessary to understand these important mechanistic features of the TNF/TNFR1 signaling complex.

TNFR2 does appear to have specific signaling functions in T cells that lack the TNFR1 receptor (Grell et al. 1998). Through interactions with TRAF2 (Rothe et al. 1994), TNF binding to TNFR2 can also activate the NF-κB and AP-1 pathways. Although TNFR2 lacks the death domain motif found on TNFR1, it can mediate apoptosis through a currently unknown pathway (Haridas et al. 1998). TNFR2 preferentially binds to the transmembrane form of TNF (Grell et al. 1995); this binding, therefore, requires cell–cell contact, which may provide greater control over the activation of TNFR2.

3 Lessons from In Vitro Studies

The availability of purified TNF, and antibodies to TNF, led to an escalation in studies using various cell lines. The effect of TNF on cell survival was evaluated with a variety of tumor and normal cell lines (Sugarman et al. 1985). Although TNF did kill some tumor cell lines, it behaved as a growth factor for the majority of cell lines, as well as for diploid human fibroblasts. Normal fibroblasts expressed IL-1 and IL-6 following exposure to TNF (Zhang et al. 1990), and synovial fibroblasts from patients with RA produced a wide variety of cytokines, chemokines, and growth factors (Koch et al. 1995). The addition of anti-TNF antibody to the cultured synovial fibroblasts specifically reduced the expression of IL-1, IL-6, IL-8, and granulocyte-macrophage colony stimulating factor (GM-CSF), and provided the first clue that TNF might be a primary regulator of proinflammatory cytokines

(Feldmann and Maini 2001). The addition of TNF to cultured human endothelial cells induced the expression of adhesion proteins and a procoagulant factor identified later as tissue factor (Bevilacqua and Gimbrone 1987). An antibody specific for TNF was shown to reduce the expression of E-selectin, ICAM-1, and vascular cell adhesion molecule (VCAM)-1 by endothelial cells, even when added 4–10 h after the TNF challenge (Nakada et al. 1998). Changes in human epithelial cells treated with TNF include reduced transepithelial resistance and increased permeability of the epithelial barrier. The effects were concentration-dependent, reversible, and inhibited by anti-TNF antibody (Mullin and Snock 1990). TNF has been shown to enhance antigen-stimulated human B cell proliferation and differentiation in the presence of IL-2, independent of similar activities mediated by IL-1 (Jelinek and Lipsky 1987). Primary activation of T cells by TNF induced expression of TNF receptors. The addition of TNF to activated T cells also increased the expression of HLA-DR antigens and high affinity IL-2 receptors, and was effective as a costimulator of IL-2-dependent IFN-γ production (Scheurich et al. 1987).

The addition of anti-TNF antibody to murine calvarial cells suppresses secretion of IL-6 and differentiation into bone-resorbing osteoclasts. IL-6 production in response to exogenous IL-6 or parathyroid hormone was also blocked by anti-TNF antibody (Passeri et al. 1994). The role of TNF in the maturation of osteoclast progenitor cells has recently been reviewed (Boyce et al. 2006). Cultured human chondrocytes treated with TNF show increased levels of caseinase activity and prostaglandin E2, as well as increased resorption of human articular cartilage (Bunning and Russell 1989).

It is clear from these examples that TNF is capable of modulating the survival and activity of many different cell types. As discussed later in this chapter, these in vitro effects of TNF are reflected in the systemic pharmacodynamic changes seen in both animal models and in patients, and this underscores the value of these types of studies in developing a clear understanding of the biological disease processes.

4 Lessons from Animal Models

4.1 TNF-Deficient Mice

Several laboratories have generated TNF-deficient mouse strains and reported on the phenotype of these animals (Korner et al. 1997; Marino et al. 1997; Pasparakis et al. 1996). Although these mice are viable, fertile, and have no gross structural or morphological defects, they do exhibit altered cellular organization in lymph nodes and Peyer's patches following challenge with antigen or pathogens. Specifically, primary B cell follicles are absent from the spleen, no organized follicular dendritic cell networks or germinal centers can be found, and there is a lack of granuloma formation. Phagocytic and T cell responses appear normal while humoral responses to T cell-dependent antigens are reduced. Compared with wild-type mice, the TNF-deficient mice are resistant to lipopolysaccharide challenge following D-galactosamine treatment, but are more susceptible to infectious agents

such as *Listeria monocytogenes*, *Candida albicans*, and *Cryptosporidum parvum.* A similar increased susceptibility to infection with *Mycobacterium tuberculosis* has been reported in anti-TNF treated mice (Flynn et al. 1995).

4.2 Tumorigenesis

Despite its name, TNF does not induce cell death in the majority of evaluated tumor cell lines (Sugarman et al. 1985). Nonetheless, treatment with TNF has been studied in a number of tumor models and in patients (Burke 1999). Systemic toxicity (hypertension and organ failure) has limited its clinical use to isolated limb perfusion for regionally advanced melanomas and soft tissue sarcomas of the limbs, in combination with an effective chemotherapeutic compound, such as the alkylating agent melphalan (Lejeune et al. 2006). Conversely, several authors have recently presented convincing arguments that TNF behaves as a tumor promoter within the context of the tumor microenvironment (Karin and Greten 2005; Szlosarek et al. 2006). The induction of nitric oxide and angiogenic factors, induction of matrix metalloproteinases (MMPs), enhancement of tumor cell motility, as well as a role as an autocrine survival and growth factor, are all tumor-promoting properties described for TNF. Overexpression of TNF by a Chinese hamster ovary cell line conferred invasive properties on tumor xenografts, while treatment with neutralizing anti-TNF antibodies blocked metastasis (Malik et al. 1990). In TNF-deficient mice (Moore et al. 1999) or wild-type mice treated with anti-TNF antibodies (Scott et al. 2003), significant reductions in carcinogen-induced skin tumors were observed compared with control animals.

NF-κB has also been described as a key link between inflammation and cancer. The spontaneous development of cholestatic hepatitis followed by hepatocellular carcinoma in Mdr2-deficient mice was accompanied by chronic expression of TNF in adjacent inflammatory and endothelial cells, and persistent activation of NF-κB. Late stage tumor development was significantly reduced by switching off the NF-κB signaling pathway or blocking TNF with neutralizing antibodies (Pikarsky et al. 2004).

4.3 Sepsis

Administration of TNF to rats resulted in hypotension, metabolic acidosis, acute pulmonary inflammation, and death within hours, similar to symptoms caused by bacterial endotoxin (Tracey et al. 1986). Treatment with a polyclonal antibody specific for TNF protected mice from a subsequent lethal challenge of endotoxin (Beutler et al. 1985b). The protective effect of TNF-neutralizing antibodies has been seen in a variety of sepsis animal models, including nonhuman primates (Bodmer et al. 1993). However, clinical trials with anti-TNF monoclonal antibodies were uniformly disappointing (Freeman and Natanson 2000) and are no longer being actively pursued.

4.4 Arthritis

Cartilage explants treated with recombinant human TNF show evidence of tissue destruction as indicated by enhanced resorption and inhibition of proteoglycan synthesis (Saklatvala 1986). Further data demonstrating the direct role of TNF in inflammatory arthritis came from the laboratory of George Kollias, which developed genetically engineered mice that constitutively express human TNF (Keffer et al. 1991). These mice developed clinical and histological changes characteristic of RA, and prophylactic treatment with anti-TNF antibody significantly inhibited disease activity. Interestingly, arthritic disease was also seen in mice that overexpressed a modified *TNF* gene that lacked the protease cleavage site, and therefore only expressed the transmembrane form of TNF. This result suggests that production of transmembrane TNF alone, which presumably would be restricted to local tissues and signaling by cell–cell contact, was sufficient for development and progression of arthritis in this model (Georgopoulos et al. 1996). Additional studies in the human TNF transgenic mouse model demonstrated that the features and symptoms of existing arthritic disease were reversed by anti-TNF antibody treatment. Amelioration of disease was associated with reduced arthritic scores and reversal of existing structural damage, including synovitis and periosteal bone erosions evident on histology. Repair of cartilage was age-dependent, as reversal of proteoglycan degradation was observed only in younger mice (Shealy et al. 2002).

Three laboratories corroborated these observations by showing that anti-TNF antibodies could reduce disease activity in the standard collagen-induced arthritis model (Piguet et al. 1992; Thorbecke et al. 1992; Williams et al. 1992). Anti-TNF treatment was effective when administered both prior to disease onset, and after significant disease was evident (Williams et al. 1992). Together, these studies provided a strong rationale for the initiation of clinical trials with anti-TNF monoclonal antibodies in patients with RA.

4.5 Inflammatory Bowel Disease

A wide variety of animal models that mimic inflammatory bowel disease in humans have been developed, including spontaneous or chemically-induced disease in normal mice, immune-mediated models, and disease in genetically-modified mice (Pizarro et al. 2003). The cotton top tamarin develops a spontaneous colitis with attributes that parallel ulcerative colitis in humans. When treated with an anti-TNF antibody, these animals showed a rapid improvement in body weight, fecal matter consistency, and rectal biopsy pathology (Watkins et al. 1997). Treatment with anti-TNF antibodies was also effective in a mouse model of chronic colitis induced by 2,4,6-trinitrobenzene sulfonic acid (TNBS) (Neurath et al. 1997). Macrophage-enriched lamina propria cells isolated from anti-TNF-treated mice produced considerably less IL-1 and IL-6 in culture. TNF-deficient mice challenged with TNBS

did not develop disease, while introduction of a mouse TNF transgene in these mice was sufficient to render them sensitive to TNBS-induced colitis.

Mice deficient in IL-10 develop a chronic colitis of the colon with transmural involvement. When anti-TNF treatment was initiated in 4-week-old mice, disease activity was significantly suppressed compared with untreated animals, as indicated by reduced disease scores, histological analysis of the gut, and reduced levels of soluble TNFR2 and IL-1β in stool samples (Scheinin et al. 2003). Adoptive transfer of the CD45RBhi subset of CD4+ cells into immune-deficient mice has also been shown to result in intestinal inflammation. Anti-TNF treatment during the first 4 weeks following cell transfer had little effect, but continued treatment reduced the severity of colitis compared with untreated control animals (Powrie et al. 1994).

A recently described mouse strain derived from the SAMP1/Yit mouse has been shown to develop chronic ileitis, similar to CD, with perianal fistulas. A single injection of anti-TNF antibody rapidly suppressed the degree of intestinal inflammation and epithelial cell damage compared with control mice (Marini et al. 2003). Effects associated with inhibition of TNF included increased apoptosis of lamina propria mononuclear cells, while conversely suppressing the apoptosis of intestinal epithelial cells.

The collective data from this wide variety of intestinal inflammation models are substantial, and clearly supported further evaluation of the use of anti-TNF antibodies in patients with inflammatory bowel disease.

4.6 Psoriasis

The most definitive research on TNF neutralization in a psoriasis model was a xenotransplantation model in which human pre-psoriatic skin was engrafted onto immune-deficient mice (Boyman et al. 2004). Psoriatic skin lesions developed spontaneously on human skin grafts in AGR129 mice that lacked type I and type II IFN receptors and the recombination-activating gene 2. Approximately 6–8 weeks after engraftment, clinical (erythema, scaling) and histological (mononuclear cell infiltration, acanthosis) features of psoriasis appeared, and enhanced expression of Ki-67, major histocompability complex (MHC) class II antigen, TNF, IL-12, keratin 16, ICAM-1, and platelet/endothelial cell adhesion molecule (PECAM)-1 was detected. Neutralization of TNF significantly reduced papillomatosis and acanthosis indices, and was associated with a decrease in the number of T cells in the graft. These results suggest that development of psoriatic lesions and proliferation of resident T cells is dependent upon TNF.

5 Development of TNF-Specific Antibodies for Clinical Use

Convergence of a deeper understanding of the role of TNF as the primary mediator of pathogen-induced inflammation, along with advances in techniques to generate

hybridoma-derived monoclonal antibodies and manipulate genes, spurred the rapid development of monoclonal antibodies for clinical testing and commercial use. In 1986, a murine monoclonal antibody (muromonab-CD3) that recognized the CD3 antigen found on human T cells was approved by the Food and Drug Administration for the treatment of kidney transplant rejection. However, patients were limited to a single 10- to 14-day dose regimen with this product since the majority of patients developed a significant immune response that prevented further treatment (Goldstein et al. 1986). The development of methods to chimerize or humanize antibody sequences significantly reduced murine antibody immunogenicity (Knight et al. 1995), and has allowed maintenance dosing with these types of antibody constructs in humans.

Three anti-TNF monoclonal antibodies have been developed to block the effects of TNF in patients with immune-mediated inflammatory diseases (Table 1). These include two approved mAbs (infliximab and adalimumab) and a pegylated Fab′ (certolizumab pegol) that is in late-stage clinical development.

5.1 Infliximab

A straightforward and simple improvement applied to monoclonal antibody technology was the generation of a chimeric molecule, in which the murine constant domains were replaced with corresponding human constant domains. This strategy was used to produce infliximab (previously called cA2), thus the binding characteristics of the fully murine antibody were identical to those of the chimeric antibody (Knight et al. 1993) and were combined with the functional properties of the human IgG1 Fc region (Scallon et al. 1995). Plasmids encoding the heavy and light chain genes for infliximab were used to transfect a myeloma cell line, and a high producing cell clone was selected. This mammalian cell line maintained proper posttranslational processing and glycosylation of the antibody.

Infliximab binds human TNF with high affinity and specificity and inhibits the bioactivity of both soluble and transmembrane TNF (Scallon et al. 2002). These stable, high avidity complexes (two or three infliximab molecules bound to each TNF trimer) prevent TNF binding to cellular receptors. No TNF-mediated bioactivity was observed when these complexes were incubated with target cells that typically respond to TNF. Furthermore, the expression of adhesion molecules by human endothelial cells was significantly reduced by the addition of infliximab, as much as 10 h after addition of TNF (Nakada et al. 1998).

5.2 Adalimumab

Human monoclonal antibodies such as adalimumab (also known as D2E7) have also been derived from a murine monoclonal antibody template using a guided phage

display technique (Osbourn et al. 2005). The heavy chain of a mouse antibody directed against human TNF (MAK195) was cloned, paired with a repertoire of human light chains for display as Fab′ fragments, and screened for binding to human TNF. In parallel, a second library was prepared using the light chain from MAK195 combined with a repertoire of human heavy chains and screened in the same manner. The combined human light and heavy chains from the phage selected from these two libraries yielded an antibody that bound well to human TNF, and was affinity matured by site-specific mutagenesis in the complementarity determining regions (CDR) (Salfeld et al. 2000). Genes encoding the phage-derived human variable sequences were fused with human IgG1κ constant domain sequences and transfected into Chinese hamster ovary cells for selection of a production cell line. Despite its human origin, adalimumab is still reported to elicit an immune response in about 5% of RA patients (Humira® prescription label). Unique CDR sequences, as well as glycosylation, aggregation, route of administration, dose regimen, and formulation all influence the observed immune response (Clark 2000). Additionally, other clinical variables such as the timing of sample collection relative to dose administration can also significantly affect the reported antibody incidence.

Adalimumab has a reported affinity for human TNF of 100 pM (Santora et al. 2001). Mixtures of adalimumab and human TNF formed high molecular weight complexes that ranged in size from 600 to 5 000 kDa. The complex with the greatest thermal stability was 598 kDa and was postulated to comprise three molecules of adalimumab and three TNF trimers.

5.3 Certolizumab Pegol

Certolizumab pegol (formerly known as CDP-870) is a humanized anti-TNF monoclonal antibody derived from a murine monoclonal antibody by replacing the murine constant domains and framework sequences around each CDR with the corresponding human sequences (Kaushik and Moots 2005; Rose-John and Schooltink 2003). A Fab′ fragment of the humanized antibody is produced in an *Escherichia coli* expression system, and subsequently coupled with polyethylene glycol (PEG). Two 20 kDa PEG chains are attached via a maleimide linkage to the Fab′ fragment, extending the observed serum half-life in humans to approximately 14 days (Schreiber et al. 2005). The affinity and biologic potency of the pegylated Fab′ fragment for human TNF was similar to the intact antibody.

6 Pharmacokinetics of Anti-TNF Antibody Therapeutics

6.1 Infliximab

Infliximab is administered as an intravenous (IV) infusion for $\leq$2h, under various dosing regimens specific for each patient population. Analysis of single IV infusions

ranging from 3 to 20 mg kg^{-1} demonstrated a linear relationship between the dose administered and the maximum serum concentration (Remicade® prescription label). The volume of distribution at steady state is independent of dose, indicating that infliximab is distributed primarily within the vascular compartment. High serum levels of infliximab are achieved within 1 h after infusion, with a median concentration of 68.6 µg mL^{-1} after a 3-mg kg^{-1} dose, and 219.1 µg mL^{-1} after a 10-mg kg^{-1} dose (St Clair et al. 2002). These high serum levels of infliximab effectively neutralize the local levels of TNF in the synovium, gut mucosa, and skin; an immediate response to therapy is observed in most responding patients.

Pharmacokinetic studies of patients with infliximab at doses ranging from 3 to 10 mg kg^{-1} in RA (Lipsky et al. 2000; Nestorov 2005; St Clair et al. 2004), 5 mg kg^{-1} in ankylosing spondylitis (AS) (Brandt et al. 2000; van der Heijde et al. 2005), psoriatic arthritis (Antoni et al. 2002), and CD (Baert et al. 1999; Farrell et al. 2000), 5 or 10 mg kg^{-1} in ulcerative colitis (Rutgeerts et al. 2005), and doses ranging from 3 to 5 mg kg^{-1} in plaque psoriasis showed that the median terminal half-life of infliximab ranged from 7.7 to 9.5 days (Scheinfeld 2004). The pharmacokinetics in pediatric patients with CD (6–17 years of age) were similar to those of adult CD patients, with a median terminal half-life of 10.9 days for a 5-mg kg^{-1} dose (Remicade® prescription label). Following an initial administration of infliximab, infusions at weeks 2 and 6 resulted in predictable concentration–time profiles following each treatment. Further, there was no systemic accumulation of infliximab upon repeated treatment with a dose of either 3 or 10 mg kg^{-1}, administered at 4- or 8-week intervals. The clearance of infliximab was nonlinear and was reduced in the presence of methotrexate (Markham and Lamb 2000; Schwab and Klotz 2001). The presence of antibodies to infliximab increased the clearance of infliximab. There were no major differences in clearance or volume of distribution in patient subgroups defined by age or weight. It remains unknown if there are differences in clearance or volume of distribution in patients with hepatic or renal impairment. There have been two population pharmacokinetic studies with infliximab to date, a single-dose study in patients with CD (Fasanmade et al. 2002) and a multiple-dose study in patients with AS (Xu et al. 2006). Overall, the pharmacokinetics of infliximab appear to be consistent across patient populations. In general, higher serum concentrations of infliximab are associated with greater clinical benefits (St Clair et al. 2004; Zhu et al. 2006).

6.2 Adalimumab

Adalimumab is administered every 2 weeks as a 40 mg subcutaneous (SC) injection for patients with psoriatic arthritis or AS, and patients with RA receive 40 mg every 1 or 2 weeks depending on disease severity (Humira® prescription label). Treatment with adalimumab was safe and well tolerated when administered as a single IV administration at doses up to 10 mg kg^{-1} in patients with active RA (den Broeder et al. 2002a). In addition to rheumatic diseases, adalimumab is currently

being evaluated in clinical trials for the treatment of psoriasis, starting with an initial dose of 80 mg, followed by a maintenance dose of 40 mg administered either weekly or every 2 weeks (Chen et al. 2004).

The pharmacokinetics of adalimumab were observed to be linear over a dose range of 0.5 to 10 mg kg^{-1} following a single IV administration (Humira® prescription label). The volume of distribution at steady state (Vss) ranged from 4.7 to 6 L, and the systemic clearance was approximately 12 mL h^{-1}. The mean serum half-life ranged from 10 to 20 days. The steady-state concentration was approximately 5 µg mL^{-1} in the absence of methotrexate, and ranged from 8 to 9 µg mL^{-1} with concomitant methotrexate. A reduction in the clearance was observed in the presence of methotrexate (29% and 44% after single and multiple dosing, respectively) (Velagapudi et al. 2003). In long-term studies (longer than 2 years), there was no evidence of increased clearance over time. Serum adalimumab trough levels at steady state increased proportionally with dose following SC administration of 20, 40, and 80 mg either every week or every other week. The average absolute bioavailability was estimated to be 64% from three studies of single SC administrations of 40 mg (Humira® prescription label). Population pharmacokinetic analysis showed a trend toward a higher clearance of adalimumab in the presence of anti-adalimumab antibodies.

6.3 Certolizumab Pegol

Although not yet approved, certolizumab pegol has been studied for the treatment of both RA and CD. The optimal IV dose of certolizumab pegol in patients with RA was 5 mg kg^{-1} (Kaushik and Moots 2005). In a Phase II study of patients with RA, the peak plasma concentration occurred at the end of the infusion, and was proportional to the dose; the plasma concentration declined gradually thereafter (Choy et al. 2002). A similar pharmacokinetic profile was observed on redosing of certolizumab pegol as that of a single dose infusion. The plasma concentration profile was similar to that observed in healthy volunteers, with a half-life of 14 days. Antibodies to certolizumab pegol were low or undetectable after a single IV administration, but were detected in all treatment groups following a second cycle of treatment. A dose of 400 mg every 4 weeks has been proposed in patients with RA to achieve a clinical response (Kaushik and Moots 2005). In patients with moderate to severe CD, results from a dose ranging (100, 200, and 400 mg) study of SC administration of certolizumab pegol showed that this therapy was well tolerated when administered four times weekly (Schreiber et al. 2005).

7 Pharmacodynamics of Anti-TNF Antibody Therapeutics

Elevated levels of TNF have been found in the synovial fluid and tissues from patients with active RA, and in the intestinal biopsies and stool of patients with

active CD. High levels of TNF have also been observed in tissues and/or serum from patients with other immune-mediated inflammatory diseases including AS, psoriasis, and ulcerative colitis. Therapeutic approaches aimed at blocking the activity of TNF are effective for controlling disease signs and symptoms and improving quality of life in these patients, as well as inhibiting radiographic damage in patients with RA. These profound clinical effects of TNF inhibition are believed to be due to the blockade of several mechanisms involved in inflammation that are activated by TNF.

7.1 Infliximab

Currently, the majority of data in the literature describing the pharmacodynamic effects of anti-TNF antibody therapies have focused on infliximab. Pharmacodynamic investigations have demonstrated that infliximab binds to, and inhibits, the intended target, TNF (Charles et al. 1999). As a result of infliximab binding to membrane-bound and soluble TNF with high affinity and avidity, it effectively blocks the biological activities of TNF as a pivotal inflammatory mediator. Infliximab forms stable immune complexes with TNF trimers; the resulting complexes are biologically inactive, and it is postulated that these complexes are cleared by the reticuloendothelial system of the liver. Despite its potent effects on systemic markers of inflammation, infliximab does not appear to induce a generalized suppression of the immune system, specifically in CD (Cornillie et al. 2001).

7.1.1 Markers Associated with Inflammation and Angiogenesis

The elevated levels of C-reactive protein (CRP) commonly observed in many patients with immune-mediated inflammatory diseases underscore the role of CRP as a prognostic marker for active inflammation, resulting in joint structural damage in patients with RA, and its potential value as an indicator of response to TNF blockade. Particularly noteworthy is the number of diverse immune-mediated inflammatory diseases for which infliximab has been shown to be both clinically effective and effective in reducing CRP levels. Infliximab has been shown to normalize CRP levels in patients with early RA (St Clair et al. 2004), refractory psoriatic arthritis (Feletar et al. 2004), AS (Brandt et al. 2000; Braun et al. 2005; van der Heijde et al. 2005), spondyloarthropathy (Antoni et al. 2002; Mandl and Jacobsson 2002), and CD (Baldassano et al. 2003; van Dullemen et al. 1995), as well as in patients with RA who had no clinical improvement but did have radiographic benefit (Smolen et al. 2005). Further, CRP levels have been shown to be associated with greater joint damage progression in early RA patients treated with MTX, but not associated with progression in patients treated with infliximab plus MTX (Smolen et al. 2006). However, in a recent study, CRP polymorphisms were associated with a differential response to infliximab in patients with CD (Willot et al. 2006).

In patients with CD, treatment with infliximab has been shown to reduce the levels of several disease-specific markers, including a significant reduction in inflammatory markers, such as IL-6 (van Dullemen et al. 1995). The levels of these

markers often correlate with disease activity. When compared with patients who achieved remission after treatment with infliximab, patients with active CD had higher levels of IL-6, sIL-6R, sTNFR1, and sTNFR2 (Gustot et al. 2005). Detkova et al. observed a correlation between decreases in IL-10 levels and improvement in the CD activity index (CDAI) following infliximab treatment (Detkova et al. 2003). Also, reductions in serum levels of fibroblast growth factor (FGF) have been associated with decreases in perianal disease activity index and open fistula scores (Gao et al. 2004). Analyses of lamina propria mononuclear cells from the intestinal mucosa of patients with CD indicate that treatment with infliximab leads to a reduction in the number of cells that express TNF, IL-10, and IFN-γ (Plevy et al. 1997). Additional histological studies have provided evidence that infliximab treatment reduces the number of cells that stain positive for CD4, CD8, CD68, and MMP-9 (gelatinase B) in affected areas of the intestine, while also reducing the levels of detectable TNF, and other inflammatory markers, such as ICAM-1 (Baert et al. 1999; Geboes and Dalle 2002).

Infliximab has also been shown to induce caspase-3-dependent apoptosis of lamina propria T lymphocytes and peripheral monocytes in patients with steroid refractory CD (Lugering et al. 2001; ten Hove et al. 2002). Van den Brande et al. (2003) have shown that infliximab binds to transmembrane TNF on activated lamina propria T cells in patients with CD, resulting in apoptosis. Further, a significant increase in annexin V uptake has also been observed in active CD patients responding to infliximab therapy (Van den Brande et al. 2006). Additionally, the lamina propria T cells were identified in mucosal biopsies as target cells undergoing apoptosis in patients treated with infliximab. In T cell cultures from patients with CD, the production of IFN-γ was downregulated by infliximab (Agnholt and Kaltoft 2001). In contrast to the Van den Brande results, infliximab bound to the transmembrane form of TNF on activated T cells without inducing complement-mediated cytolysis or apoptosis, and without affecting proliferation. Infliximab has also been shown to reverse growth hormone resistance observed in patients with active inflammatory bowel disease through the suppression of systemic inflammation (Vespasiani Gentilucci et al. 2005).

In patients with RA, treatment with infliximab suppresses expression of markers related to inflammation (such as IL-6) and angiogenesis (such as vascular endothelial growth factor [VEGF]) (Charles et al. 1999; Strunk et al. 2006). Additionally, infliximab has been shown to reduce the synthesis of TNF, IL-1α, and IL-1β in the synovium within 2 weeks of treatment (Ulfgren et al. 2000). Also, patients with RA who were treated with infliximab had decreased serum levels of TNFR1, TNFR2, IL-1R antagonist, IL-6 and acute-phase proteins (serum amyloid A, haptoglobin, and fibrinogen) (Charles et al. 1999), IL-18 (Pittoni et al. 2002; van Oosterhout et al. 2005), as well as the chemokines GRO-α (Torikai et al. 2007), IL-8 (Visvanathan et al. 2007a), and CXCL16 (Kageyama et al. 2006). Low baseline serum levels of IL-2R have been associated with a clinical response to infliximab in patients with refractory RA (Kuuliala et al. 2006). Further, a recent study has shown that patients with RA who received infliximab therapy had decreased serum levels of sICAM-3 and sP-selectin (Gonzalez-Gay et al. 2006). Reduced levels of the cytokine

IL-18 have also been observed after infliximab treatment (Pittoni et al. 2002; van Oosterhout et al. 2005). The accumulation of CXCR3-positive T lymphocytes has been observed in the peripheral blood of RA patients treated with infliximab, suggesting altered lymphocyte trafficking (Aeberli et al. 2005). Treatment with infliximab has been shown to decrease activated p38 map kinase levels in CD4+ T cells in patients with RA (Garfield et al. 2005), and increase FOXP3 mRNA and protein expression by CD4+CD25hi regulatory T cells (Valencia et al. 2006). Infliximab therapy restored the suppressive function of the regulatory T cells (Ehrenstein et al. 2004), and appeared to induce a newly differentiated population of regulatory T cells (Nadkarni et al. 2007).

Elevated levels of anticyclic citrullinated peptide (CCP) antibodies have been associated with progression of structural damage in patients with RA (Meyer et al. 2003). Further, serum titers of anti-CCP antibodies and rheumatoid factor (RF) have been shown to decrease significantly after 6 months of treatment with infliximab (Ahmed et al. 2006; Alessandri et al. 2004). Decreases in anti-CCP antibody levels after initiation of treatment with infliximab were associated with decreases in IL-6 (Braun-Moscovici et al. 2006). After grouping the patients on the basis of their clinical response to infliximab, a significant decrease in serum anti-CCP antibodies and RF was observed only in those patients who had clinical improvement (Alessandri et al. 2004).

Infliximab therapy has also been shown to modulate inflammatory markers in patients with spondyloarthropathies. Treatment with infliximab resulted in a significant increase in IFN-γ and IL-2, and a transient decrease in IL-10 and natural killer (NK) T cells in patients who had high baseline values (Baeten et al. 2001). This switch in cytokine profile was observed in both the CD3+/CD8− and CD3+/CD8+ subsets. In psoriatic arthritis patients, a significant decrease in IL-6, VEGF, FGF, and E-selectin was observed after treatment with infliximab and reductions in VEGF, FGF, and MMP-2 were significantly correlated with improvement in psoriasis area and severity index (PASI) scores (Mastroianni et al. 2005). More recently, we have shown that treatment of AS patients with infliximab resulted in decreased serum levels of IL-6, VEGF, and CRP, and that these reductions were associated with an improvement in disease activity and spinal disease measures (Visvanathan et al. 2007b). Further, early decreases in IL-6 were associated with improvement in clinical measures in AS and psoriatic arthritis patients treated with infliximab (Visvanathan et al. 2006). Treatment with infliximab also resulted in decreases in the percentage of circulating CD4+ and CD8+ T cells expressing TNF or IFNγ in AS patients (Zou et al. 2003).

In a study of the mechanism of action of infliximab in the treatment of psoriasis vulgaris (Gottlieb et al. 2003), infliximab monotherapy was shown to disrupt the inflammatory processes associated with this dermatological disease. A reduction in the number of CD3-positive T cells and keratinocyte-derived ICAM-1 expression was demonstrated in lesional skin biopsies at weeks 2 and 10 after treatment initiation. Following treatment with infliximab, there was also a decrease in epidermal thickness and K16 and Ki67 expression levels, suggesting a role for TNF in the keratinocyte hyperproliferation observed in psoriasis.

7.1.2 Markers Associated with Bone and Cartilage Turnover

Several matrix metalloproteinases, including MMP-1 (interstitial collagenase) MMP-3 (stromelysin-1), and MMP-9 (gelatinase B), are up-regulated in inflammatory tissue, and TNF blockade with infliximab resulted in inhibition of these MMPs in patients with CD (Tchetverikov et al. 2005). More recently, Gao et al. observed decreased levels of MMP-9 and increased levels of MMP-2 in the sera of CD patients who responded to infliximab treatment (2007). Treatment with infliximab has been shown to provide a normalization of bone markers in patients with CD, with an increase in markers of bone formation (type-I procollagen N-terminal propeptide, bone-specific alkaline phosphatase, osteocalcin) and a decrease in markers of bone resorption (C-telopeptide of type-I collagen) in 30%–61% of patients (Franchimont et al. 2004).

In patients with RA, treatment with infliximab resulted in decreased levels of MMP-1, MMP-3 (Brennan et al. 1997), and MMP-9 (Klimiuk et al. 2004). Similar trends were observed in patients with spondyloarthropathies; infliximab treatment down-regulated MMPs and tissue inhibitors of metalloproteinases (TIMPs) in the synovium, and also resulted in a pronounced, rapid decrease in serum MMP-3 levels (Vandooren et al. 2004). More recently, we have shown that treatment of early RA patients with infliximab plus MTX not only resulted in a rapid decrease in inflammatory markers including MMP-3, but baseline levels of MMP-3 correlated with measures of clinical improvement at 1 year (Visvanathan et al. 2007a). Treatment with infliximab has also been shown to increase bone mineral density and modulate markers of bone metabolism in RA patients (Lange et al. 2005; Vis et al. 2006). Significant decreases in levels of the N-telopeptide of type I collagen (NTX) were observed 6 weeks after the initial treatment, and were maintained through 6 months. This decrease in NTX levels corresponded with an improvement in the number of swollen joints and modified Stanford Health Assessment Questionnaire (mHAQ) scores (Torikai et al. 2006). A recent small study of nine patients showed that infliximab therapy reduces the levels of serum cartilage oligomeric matrix protein (COMP) in patients with psoriatic arthritis, paralleling clinical improvement as measured by the American College of Rheumatology (ACR) response criteria (Cauza et al. 2006). Treatment of spondyloarthropathy patients with infliximab resulted in an increase in levels of procollagen type I N-terminal peptide (PINP) and insulin-like growth factor (IGF)-1, and a decrease in CTX-1 paralleled by an increase in bone mineral density, body weight, and lean mass (Briot et al. 2005).

7.1.3 Genes and Polymorphisms

Physiological responses to infliximab have been associated with multiple polymorphisms (Krejsa et al. 2006; Ranganathan 2005). In patients with CD, response to infliximab therapy has been associated with a single nucleotide polymorphism in the *FCGR3A* gene, which codes for the FcγRIIIa receptor (binds to the Fc portion of IgG) found on NK cells and macrophages (Louis et al. 2004). In patients

with elevated CRP levels, the *FCGR3A* gene was an independent variable that influenced response to infliximab treatment. In two studies of patients with RA, the −308 polymorphism in the promoter for the *TNF* gene was associated with a good response to infliximab (Cuchacovich et al. 2004; Mugnier et al. 2003). Further, another study has shown that the 196 *TT* polymorphism in the *TNFRSF1B* gene was associated with a better response to infliximab therapy (Fabris et al. 2002). Due to the proximity of the *HLA DR* genes to the TNF locus, it is not surprising that HLA Class III microsatellites BAT2 and D6S273 have also been significantly associated with response to infliximab treatment in RA patients (Martinez et al. 2004). More recently, additional genes (cell adhesion, cell migration, cytochromes, proteasome-mediated proteolysis) have been associated with responses to infliximab therapy in RA patients (Lequerre et al. 2006); however, larger studies will be required to confirm these results.

7.2 Adalimumab

7.2.1 Markers Associated with Inflammation and Angiogenesis

Treatment with adalimumab resulted in a reduction in CRP, and this decrease correlated with improvement in radiological scores in RA patients after 2 years (den Broeder et al. 2002b). Decreases in serum levels of COMP, sICAM-1, MMPs, and HC gp-39, but not sE-selectin, were also observed in these patients. Further, baseline levels of COMP and sICAM-1 were predictive of radiological outcome in RA patients treated with adalimumab. In patients with RA, treatment with adalimumab therapy rapidly decreased the influx of leukocytes into inflamed joints but did not impair neutrophil chemotaxis or the production of reactive oxygen species (den Broeder et al. 2003). Circulating IL-7 levels were significantly reduced in RA patients that responded to adalimumab (van Roon et al. 2007).

A reduction in RF and anti-CCP antibody titers has been associated with clinical measures of response in patients treated with adalimumab (Atzeni et al. 2006). In a recent study, a reduction in serum concentrations of RF was observed after 12 weeks of treatment with adalimumab, whereas the anti-CCP antibody level remained constant (Semmler et al. 2007). Further, no significant changes in the activation levels of both NF-κB subunits were detected.

7.3 Certolizumab Pegol

7.3.1 Markers Associated with Inflammation and Angiogenesis

Minimal data have been reported on the pharmacodynamic effects of certolizumab pegol in patients with immune-mediated inflammatory diseases. Certolizumab pegol has been shown to have the unique property of not being capable of fixing

complement and lysing cells, and therefore not activating other components of the immune system because of the lack of the IgG Fc domain (Kaushik and Moots 2005). Similar to the commercially available anti-TNF therapies, treatment with certolizumab pegol results in significant reductions in serum CRP levels in patients with CD, and to a lesser extent in patients with RA (Choy et al. 2002; Schreiber et al. 2005).

8 The Road Ahead for Anti-TNF Antibody Therapeutics

The postulated role of TNF in immune-mediated inflammatory diseases such as RA inflammatory bowel disease, and psoriasis has been confirmed in well-controlled clinical trials and to date, hundreds of thousands of patients have been successfully treated with infliximab and adalimumab. The impact of these breakthrough biologics on the patients' quality of life can be attributed to many scientists and clinicians, who continue to identify critical pathways and new targets that may further improve the benefits of these types of therapies, while minimizing unwanted side effects (Korzenik and Podolsky 2006; Myers et al. 2006; Strand et al. 2007), and to the patients who participate in these definitive clinical trials. With regard to the biology of TNF, there is persuasive data suggesting that activation of the TNFR1 receptor generates the proinflammatory properties of TNF, while the TNFR2 signaling pathway initiates the immunoregulatory actions attributed to TNF (Kollias 2005). This hypothesis suggests that blocking only TNF-mediated TNFR1 activation might be desirable, but this has not been tested in the clinic.

Researchers continue to develop new technologies to improve the efficiency of antibody selection and to further refine the specific properties of new, developing antibody therapeutics. These improvements range from transgenic mice engineered to produce human antibodies (Lonberg 2005) or new methods to enhance the affinity of existing antibodies (Rajpal et al. 2005), to the development of single domain antibodies (Holliger and Hudson 2005) whose unique small size and exceptional stability may allow topical, intranasal, or oral delivery options. In addition, a better characterization of patients who will respond to anti-TNF antibody therapies is of great importance. This can be achieved through more extensive studies examining relationships between pharmacodynamic markers and various clinical measures of response to anti-TNF treatment. Identification of associations between specific markers and a clinical response will aid in gaining a better understanding of the heterogeneity of individual patient populations, and their responses to anti-TNF therapies. Results from these types of studies will provide better insight into the profile of patients who will be the most likely to benefit from anti-TNF antibody therapy.

Despite new targets and technologies on the horizon, there is little doubt that anti-TNF antibodies will continue to be the biologic therapy of choice for immune-mediated inflammatory diseases for the foreseeable future. In addition, it is possible that other conditions with proinflammatory characteristics, such as Alzheimer's disease (Janelsins et al. 2005) or preterm labor (Sadowsky et al. 2006), may also

be treatable with anti-TNF agents. The rigorous clinical testing and monitoring of commercially available anti-TNF therapies will enable a greater understanding of whether these agents can continue to expand their potential as effective therapies for other debilitating diseases and to improve patients' lives.

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