Available online at www.sciencedirect.com
ScienceDirect
Pharmacology & Therapeutics 117 (2008) 244–279
Pharmacology & Therapeutics
www.elsevier.com/locate/pharmthera

Tumor necrosis factor antagonist mechanisms of action: A comprehensive review

Daniel Tracey [a], Lars Klareskog [b], Eric H. Sasso [c], Jochen G. Salfeld [a], Paul P. Tak [d,*]

[a] *Abbott Bioresearch Center, Worcester, MA, USA*
[b] *Rheumatology Unit, Department of Medicine, Karolinska Institutet and Karolinska University Hospital, Stockholm, Sweden*
[c] *Abbott Laboratories, Abbott Park, IL, USA*
[d] *Division of Clinical Immunology and Rheumatology, FOCIS Center of Excellence, Academic Medical Center/University of Amsterdam, F4-218, P.O. Box 22700, 1100 DE Amsterdam, The Netherlands*

Abstract

During the past 30 years, elucidation of the pathogenesis of rheumatoid arthritis, Crohn's disease, psoriasis, psoriatic arthritis and ankylosing spondylitis at the cellular and molecular levels has revealed that these diseases share common mechanisms and are more closely related than was previously recognized. Research on the complex biology of tumor necrosis factor (TNF) has uncovered many mechanisms and pathways by which TNF may be involved in the pathogenesis of these diseases. There are 3 TNF antagonists currently available: adalimumab, a fully human monoclonal antibody; etanercept, a soluble receptor construct; and infliximab, a chimeric monoclonal antibody. Two other TNF antagonists, certolizumab and golimumab, are in clinical development.The remarkable efficacy of TNF antagonists in these diseases places TNF in the center of our understanding of the pathogenesis of many immune-mediated inflammatory diseases. The purpose of this review is to discuss the biology of TNF and related family members in the context of the potential mechanisms of action of TNF antagonists in a variety of immune-mediated inflammatory diseases. Possible mechanistic differences between TNF antagonists are addressed with regard to their efficacy and safety profiles.

Keywords: Tumor necrosis factor; TNF antagonists; Mechanism of action; Inflammation; Rheumatoid arthritis; Immune-mediated inflammatory diseases

Abbreviations: ACPA, anti-citrullinated peptide/protein antibody; ADCC, antibody-dependent cellular cytotoxicity; ARE, adenine-uracil–rich elements; CCL, chemokine (C-C motif) ligand; CRP, C-reactive protein; CXCL, chemokine (C-X-C motif) ligand; ICAM-1, intercellular adhesion molecule; IL, interleukin; LPS, lipopolysaccharide; LT, lymphotoxin; mAbs, monoclonal antibodies; MCP-1, macrophage chemoattractant protein-1; MMP, matrix metalloproteinase; NF-κB, nuclear factor kappa-B; NK, natural killer; RA, rheumatoid arthritis; PI, package insert; RANKL, receptor activator of nuclear factor kappa-B ligand; RANTES, regulated on activation, normal T cell expressed and secreted; SPPL, signal peptide peptidase-like proteases; sTNF, soluble tumor necrosis factor; TACE, tumor necrosis factor-alpha–converting enzyme; THP-1, human acute monocytic leukemia cell line; TLR, toll-like receptor; tmTNF, transmembrane tumor necrosis factor; TNF, tumor necrosis factor; TNFR, tumor necrosis factor receptor; TRAF, tumor necrosis factor receptor-associated factor; TUNEL, terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end labeling; VCAM-1, vascular cell adhesion molecule; VEGF, vascular endothelial growth factor.

Contents

* Corresponding author. Tel.: +31 20 566 2171, +31 20 566 8998; fax: +31 20 691 9658.
E-mail address: P.P.Tak@amc.uva.nl (P.P. Tak).

0163-7258/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.pharmthera.2007.10.001

1. Introduction

Rheumatoid arthritis (RA) has emerged as a prototypic immune-mediated inflammatory disease in our understanding of pathophysiologic mechanisms and is a common focus of clinical studies of tumor necrosis factor (TNF) antagonists. RA is a chronic disease in which inflammation of the synovial tissue results in articular cartilage and bone destruction. Parallel advances in research on the pathogenesis of RA and cytokine biology converged on TNF and interleukin-1 (IL-1) as key factors in inflammation and matrix destruction (Saxne et al., 1988; Arend & Dayer, 1990). The concept arose that elevated concentrations of TNF at the sites of inflammation were driving disease pathology, and the removal of excess TNF from sites of inflammation became a therapeutic goal (Brennan et al., 1989; Knight et al., 1993). In addition, transgenic mice expressing high concentrations of TNF spontaneously developed arthritis that was clinically and histopathologically similar to RA (Keffer et al., 1991). A collagen-induced arthritis model demonstrated that blockade of TNF was efficient in ameliorating the disease (Thorbecke et al., 1992; Williams et al., 1992). After the demonstration of the role of TNF and the efficacy of TNF blockade in experimental models, a pilot study was performed in patients with RA using a neutralizing, chimeric, monoclonal anti-TNF antibody, cA2, now called infliximab. The result of this pilot study was very positive (Elliott et al., 1993), and this study was followed by a larger multicenter study with the same antibody that unequivocally demonstrated the efficacy of the anti-TNF antibody in reducing disease activity and signs and symptoms of RA (Elliott et al., 1994). Today, there are 3 registered TNF antagonists in the United States and the European Union: infliximab, etanercept and adalimumab; each is indicated for several immune-mediated inflammatory diseases (Table 1). The current status of registered clinical trials for all 5 TNF antagonists can be accessed at http://clinicaltrials.gov/, http://www.who.int/, or http://www.actr.org.au/. Although different immune-mediated inflammatory diseases involve distinct target organs or tissues, they appear to share some common underlying mechanisms involving TNF. All 3 TNF antagonists are parenterally administered protein therapeutics (biologics); infliximab and adalimumab are monoclonal antibodies (mAbs) that specifically bind TNF; and etanercept is a TNF-receptor Fc-fusion protein that binds TNF and lymphotoxin (LT) family members. In addition, 2 other TNF antagonists in development — certolizumab pegol, refered to as certolizumab hereafter, and golimumab — also will be covered in this review, although relatively little information is publicly available on these agents.

The clinical efficacy profiles, dosage and routes of administration, pharmacokinetic parameters and immunogenicity profiles of the TNF antagonists are listed in Table 1. The clinical efficacy profiles of infliximab, etanercept and adalimumab have been reviewed in detail (Bang & Keating, 2004; Furst et al., 2007; Haraoui, 2005; Atzeni et al., 2005a). Infliximab and adalimumab have very similar efficacy profiles and are highly efficacious in RA, psoriasis, psoriatic arthritis, ankylosing spondylitis and Crohn's disease. The available clinical data suggest that LT blockade by etanercept offers no additional benefit over TNF blockade in the treatment of RA (Weinblatt et al., 1999, 2003). Etanercept differs from infliximab and adalimumab primarily in the lack of efficacy of etanercept in granulomatous diseases, such as Crohn's disease, Wegener's granulomatosis and sarcoidosis (Sandborn et al., 2001; Utz et al., 2003; Wegener's Granulomatosis Etanercept Trial Research Group, 2005). In addition, although etanercept has efficacy comparable to infliximab and adalimumab in RA, etanercept may be less efficacious than infliximab or adalimumab in psoriasis (Leonardi et al., 2003; Gottlieb et al., 2004; Gordon et al., 2006). In the treatment of RA, combinations of TNF antagonists with low-dosage methotrexate have generally been more efficacious than either drug alone (Maini et al., 1998; Klareskog et al., 2004; Breedveld et al., 2006). Treatment of patients with RA with methotrexate alone reduced the recruitment of synovial fluid neutrophils and synovial tissue

Table 1
Clinical profile of TNF antagonists

	Infliximab	Etanercept	Adalimumab	Certolizumab	Golimumab	References
Brand name	REMICADE	ENBREL	HUMIRA	NA	NA	Enbrel PI, Humira PI, Remicade PI
Synonyms/historical	cA2	p75TNFR-Fc	D2E7	CDP870	CNTO-148	
EU registration	RA, PsA, AS, CD, UC, Ps	RA, PsA, AS, JIA, Ps	RA, PsA, AS, CD, Ps	NA	NA	Enbrel PI, Humira PI, Remicade PI
US registration	RA, PsA, AS, CD, UC, Ps	RA, PsA, AS, JIA, Ps	RA, PsA, AS, CD	NA	NA	Enbrel PI, Humira PI, Remicade PI
Efficacy in RA	+++	+++	+++	+++	+++	Enbrel PI, Furst, 2005, Haraoui, 2005, Humira PI, Kay et al., 2006, Remicade PI, Weir, 2006,
Efficacy in PsA	+++	+++	+++	ND	ND	Enbrel PI, Humira PI, Kay et al., 2006, Remicade PI, Weir, 2006,
Efficacy in AS	+++	+++	+++	ND	ND	Enbrel PI, Humira PI, Kay et al., 2006, Remicade PI, Weir, 2006,
Efficacy in CD	+++	–	+++	++	ND	Haraoui, 2005, Sandborn, 2001, Schreiber, 2005
Efficacy in UC	+++	ND	ND	ND	ND	Rutgeerts et al., 2005
Efficacy in Ps	+++	++	+++	ND	ND	Gordon, 2006, Gottlieb, 2004, Leonardi, 2003
Efficacy in JIA	++	++	ND	ND	ND	Carrasco et al., 2004, Furst, 2005
Efficacy in Wegener's granulomatosis	++	–	ND	ND	ND	Lamprecht et al., 2002, WGET Research Group, 2005
Efficacy in sarcoidosis	++	–	ND	ND	ND	Baughman et al., 2006, Utz, 2003
Administration	IV	SC	SC	SC	SC	Enbrel PI, Furst, 2005, Haraoui, 2005, Humira PI, Kay et al., 2006, Remicade PI, Weir, 2006
Dosages	3–10 mg/kg q4–8w	25 mg biw; 50 mg qw	40 mg eow; 40 mg qw	100, 200 or 400 mg q4w	50 or 100 mg q2w or q4w	Enbrel PI, Furst, 2005, Haraoui, 2005, Humira PI, Kay et al., 2006, Remicade PI, Weir, 2006
Pharmacokinetics						
Half-life (t ½)	8–10 days	4 days	10–20 days	~14 days	7–20 days	Enbrel PI, Humira PI, Remicade PI, Weir, 2006, Zhou et al., 2007
Volume of distribution (Vss)	4.3 +/−2.5 L [a]	8.0 L [b]	4.7–6.0 L [c]	ND	6.9 L [d]	Enbrel PI, Furst, 2006, Humira PI, Nestorov, 2005a, Remicade PI, Weisman et al., 2003, Zhou et al., 2004, Zhou et al., 2007
Clearance (C_L)	11 mL/h [a]	72 +/−5 mL/h [e]	12 mL/h [c]	ND	16.7 mL/h [f]	Enbrel PI, Furst, 2006, Humira PI, Nestorov, 2005a, Remicade PI, Zhou et al., 2004, Zhou et al., 2007
Cmax	118 μg/mL [a]	1.1 +/−0.6 μg/mL [e]	4.7 +/−1.6 μg/mL [g]	ND	70.8 +/−18.9 μg/mL [h]	Enbrel PI, Furst, 2006, Humira PI, Nestorov, 2005a, Remicade PI, Zhou, 2005, Zhou et al., 2007
Immunogenicity						
RA monotherapy	+++	+	+	ND	ND	Anderson, 2005, Baert, 2003, Enbrel PI, Humira PI, Remicade PI
RA with MTX	+	+/–	+/–	ND	ND	Anderson, 2005, Baert, 2003, Enbrel PI, Humira PI, Remicade PI
CD monotherapy	+++	+	+	+	ND	Anderson, 2005, Baert, 2003, Enbrel PI, Humira PI, Remicade PI

AS is ankylosing spondylitis; biw, twice a week; CD, Crohn's disease; IV, intravenous; JIA, juvenile idiopathic arthritis; MTX, methotrexate; NA, not applicable; ND, no data available; Ps, psoriasis; PsA, psoriatic arthritis; qw, every week; eow, every other week; RA, rheumatoid arthritis; SC, subcutaneous; UC, ulcerative colitis; +/–, very weak; +, weak; ++, moderate; +++, strong.

[a] 5 mg/kg IV.

[b] Vss (volume of distribution at steady state) estimated as the sum of Vc+Vp for the volumes of distribution in the central and peripheral compartments, respectively, from a 2-compartment population pharmacokinetic model based on 10 studies with 2–25 mg IV or SC single dose or biw.

[c] 0.25–10 mg/kg IV.

[d] Vss (volume of distribution at steady state) estimated as the sum of Vc+Vp for the volumes of distribution in the central and peripheral compartments, respectively, from a 2-compartment population pharmacokinetic model based on data from 0.1–10 mg/kg IV.

[e] Based on data from 2–20 mg IV and 2–50 mg SC.

[f] 0.1–10 mg IV.

[g] 40 mg SC.

[h] 3 mg/kg IV.

macrophages, as well as the expression of adhesion molecules and metalloproteinases (Kraan et al., 2000a, 2000b). Methotrexate also has immunosuppressive properties, including inhibition of activated T cells (Genestier et al., 1998) and selective inhibition of T cell–dependent animal models of RA (Lange et al., 2005). The mechanism of action of methotrexate is still under investigation, but its anti-inflammatory effects may be mediated by adenosine, folate antagonism, inhibition of spermine/spermidine production and/or alteration of cellular redox state (Montesinos et al., 2000; Cronstein, 2005). Thus, the enhanced efficacy of the combination of methotrexate with TNF antagonists may reflect true mechanistic synergism. The pharmacokinetic and immunogenicity profiles for infliximab, etanercept and adalimumab are dissimilar and will be discussed in detail later. Antibody concentrations against TNF antagonists were reduced by concomittant treatment with methotrexate, probably as a result of the immunosuppressive activity of methotrexate (Maini et al., 1998; Weinblatt et al., 2003; Anderson, 2005). Another interesting observation in the treatment of rheumatic diseases and Crohn's disease is that many patients who are nonresponsive, who have lost response or who are intolerant of one TNF antagonist responded when switched to a different TNF antagonist (van Vollenhoven et al., 2003; Barthel et al., 2005; Sandborn, 2005; Gomez-Reino & Group, 2006; Nikas et al., 2006; Bombardieri et al., 2007).

The overall safety profiles of infliximab, etanercept and adalimumab have been subject to more extensive scrutiny than most other drugs. TNF antagonists interfere with a key molecule in the immune defense system and their introduction came at a time when awareness of drug safety for new drugs had increased. Thus, apart from regular spontaneous adverse-event reportings, several extensive safety registries have been established in several countries. These safety registries have contributed to a more complete understanding of the risks and benefits of these drugs as compared with most other newly introduced pharmaceuticals. Some analyses of combined data from randomized, controlled trials or from safety registries indicated that TNF antagonists increased the overall risk of infections (Listing et al., 2005; Bongartz et al., 2006; Askling et al., 2007), but some studies have found there is no overall increased risk for infections after TNF blockade as compared with the frequency in patients with RA not treated with TNF antagonists (Dixon et al., 2006; Schiff et al., 2006a). However, the combined data from randomized controlled trials and safety registries have indicated that there is an increased risk for certain infections, particularly tuberculosis (TB) and other infections caused by intracellular microbes, after treatment with TNF antagonists (Askling et al., 2005; Carmona et al., 2005; Listing et al., 2005; Askling et al., 2006; Bongartz et al., 2006; Carmona et al., 2006; Schiff et al., 2006b). Cases of TB have been documented in patients treated with all TNF antagonists and the incidence has so far been shown to be greater and to occur earlier with infliximab and adalimumab than with etanercept (Keystone, 2005). Most of these cases were a result of reactivation of latent TB and occurred within the first few months of therapy (Bieber & Kavanaugh, 2004; Schiff et al., 2006a). Screening for latent TB prior to commencing therapy is advocated by the Centers for Disease Control and Prevention, the American Thoracic Society and others for all TNF antagonists. Screening with tuberculin skin tests and/or chest radiographs has markedly reduced the incidence of TB (Carmona et al., 2005; Lee & Kavanaugh, 2005; Schiff et al., 2006a). Further studies evaluating extended populations of patients and screening practices are warranted as part of the continued risk-management plans for these drugs.

The second major concern has been malignancies. An increased rate of lymphomas was initially detected in patients treated with TNF antagonists when compared with the risk for lymphomas in matched healthy controls from the population. However, there is strong evidence that disease activity is a driving force behind the increased risk for lymphomas in patients with RA irrespective of the treatment (Baecklund et al., 1998, 2004; Wolfe & Michaud, 2004; Baecklund et al., 2006). Data from safety registries indicate that the disease activity, rather than TNF antagonism, is likely to be responsible for the increased lymphoma risk that was initially reported in TNF antagonist–treated patients with RA (Wolfe & Michaud, 2004; Askling et al., 2006). There are, however, also reports that indicate that an increased frequency of lymphomas indeed may be associated with TNF blockade (Bongartz et al., 2006), and this issue merits close continued scrutiny in population-based surveillance registries. Currently, the risk for lymphomas after TNF blockade is considered limited enough not to exert a major influence over decisions to initiate or continue TNF-antagonist therapy in patients with RA with high disease activity or rapidly progressing joint destruction, but continued close scrutiny in registers is recommended by the United States Food and Drug Administration as well as the European Agency for the Evaluation of Medicinal Products. For solid cancers, no overall increased risk has been reported in registry studies, either in patients with RA treated with TNF antagonists or in other patients with RA (Gridley et al., 1993; Askling et al., 2006). However, Bongartz et al. (2006) reported an increased frequency of certain solid tumors, mainly from the skin, after TNF-antagonist treatment.

Other adverse events that have been associated with TNF-antagonist therapy include systemic lupus erythematosus-like syndromes and demyelinating diseases. This occurrence of other immune-mediated inflammatory diseases has been linked to the observation that removal of TNF may result in an increased activity of T and B cells that react with autoantigens and foreign antigens (Cope et al., 1994; Pasparakis et al., 1996; Berg et al., 2001; McDevitt et al., 2002). Increased frequencies of autoantibodies, in particular antinuclear antibodies and anti-dsDNA antibodies, has been reported after treatment with TNF antagonists, although less so with etanercept (de Rycke et al., 2005; Atzeni et al., 2005b). In clinical practice, the risk for development of systemic autoimmune diseases is low, and at present there is no recommendation for the monitoring of autoantibody titers during TNF-antagonist treatment. Interestingly, in a small open-label study, patients with systemic lupus erythematosus showed improvement of their inflammatory nephritis, despite elevation in autoantibody concentrations, after treatment with a TNF antagonist (Aringer et al., 2004).

2. Biology of tumor necrosis factor and lymphotoxin in health and disease

2.1. Overview

The biology of TNF and LT in health and disease is complex and continues to be illuminated by ongoing preclinical and clinical studies. Several review articles have addressed the molecular, cellular and physiologic aspects of TNF and LT biology (Bazzoni & Beutler, 1996; Gommerman & Browning, 2003; Schottelius et al., 2004; Hehlgans & Pfeffer, 2005; Kollias, 2005; Ware, 2005; Aloisi & Pujol-Borrell, 2006). TNF and some forms of LT play a role in lymphoid tissue development and have a homeostatic role in host defense against some bacterial infections. TNF has been called a sentinel cytokine or "the body's fire alarm" (Feldmann & Steinman, 2005), as it initiates the defense response to local injury. At low concentrations in tissues, TNF is thought to have beneficial effects, such as the augmentation of host defense mechanisms against infections. At high concentrations, TNF can lead to excess inflammation and organ injury. Acute release of very large amounts of TNF during sepsis may result in septic shock. In disease states, TNF is generally considered to be a proinflammatory cytokine, along with IL-1, IL-17, and other cytokines. A simplified view of the role of TNF in inflammation and some immune-mediated inflammatory diseases is that expression of TNF is increased in the affected tissues as a result of innate and adaptive immune responses. TNF then mediates a variety of direct pathogenic effects and induces the production of other mediators of inflammation and tissue destruction, placing it at the head of an inflammatory cascade within an inflammatory network, but TNF may also be considered as one particularly important proinflammatory cytokine in an intricate network rather than in an inflammatory cascade. Much less is known about the roles of the LT family in diseases, but at least some of its members' functions are similar to those of TNF.

2.2. Tumor necrosis factor and lymphotoxin nomenclature

The nomenclature for TNF, LT and related molecules has changed over time and can be cause for confusion. Upon recommendation by the organizers of the TNF Congress in 1998, the names for TNFα and TNFβ were changed to TNF and LTα, respectively. However, the term TNFα is still widely used and is synonymous with the term TNF used in this review. The terms used in this review are defined as follows:

- TNF is a general term that includes soluble TNF (sTNF) and transmembrane TNF (tmTNF) in the context of tissues or *in vivo* situations, but it can be synonymous with sTNF in the context of fluids.
- LT is a general term for the family of lymphotoxins; they are trimeric molecules composed of various combinations of α and/or β monomers, including LTα3, LTα1β2 and LTα2β1.
- LTα is the official name for LTα3 but is sometimes used in the literature to connote any LT molecule containing an LTα chain. The term LTα3 will be used here when referring to soluble LTα.
- LTαβ is a general term for the heterotrimeric membrane forms of LT, namely, LTα1β2 and LTα2β1.

2.3. Tumor necrosis factor biology

A schematic view of the network of ligands, receptors and signaling pathways that encompass TNF biology is shown in Fig. 1, which illustrates several layers of complexity. First, TNF is released from cells as a soluble cytokine (sTNF, a homotrimer of 17-kDa monomers) after being enzymatically cleaved from its cell surface–bound precursor (tmTNF, a homotrimer of 26-kDa monomers) by TNF-alpha–converting enzyme (TACE). Many different immune and nonimmune cell types can produce TNF, including macrophages, T cells, mast cells, granulocytes, natural killer (NK) cells, fibroblasts, neurons, keratinocytes and smooth muscle cells. Both sTNF and tmTNF are biologically active, and the relative amounts of each are collectively determined by the inducing stimuli, the cell types involved, the activation status of the cells, the amounts of active TACE and the amounts of natural TACE inhibitors, such as tissue inhibitor of metalloproteinases-3 (Smookler et al., 2006). Both sTNF and tmTNF ligands interact with either of 2 distinct receptors — TNF receptor 1 (TNFR1) (p55, CD120a) and TNFR2 (p75, CD120b) — on a wide variety of cell types to mediate their biologic functions. Both TNFR1 and TNFR2 are membrane glycoproteins that specifically bind TNF and LTα3, but they differ in their cellular expression profiles, affinities for ligands, cytoplasmic tail structures and signaling mechanisms. Trimers of receptor chains preassemble on the cell surface prior to ligand binding, owing to associations between TNF-receptor, subtype-specific, pre-ligand–binding assembly domains (Chan et al., 2000).

TNF-mediated biology gains additional complexity from the distinct signaling pathways mediated through TNFR1, TNFR2 or tmTNF, the last because tmTNF can function as a ligand and as a receptor. Receptor-mediated effects of sTNF and tmTNF can lead alternatively to activation of nuclear factor kappa-B (NF-κB) or to apoptosis, depending on the metabolic state of the cell. Interestingly, binding to tmTNF by TNFRs, or even TNF antagonists, can induce reverse signaling through this membrane-anchored ligand and can trigger cell activation, cytokine suppression or apoptosis of the tmTNF-bearing cell (Eissner et al., 2000; Harashima et al., 2001; Eissner et al., 2004). Reverse signaling through mTNF on monocytes is mediated by phosphorylation of the cytoplasmic tail, binding of casein kinase 1 and possibly other kinases, intracellular calcium elevation and signaling through the p38 and mitogen-activated protein kinase extracellular signal–regulated kinase/extracellular signal–regulated kinase pathways (Eissner et al., 2004). Recent evidence suggests that regulated intramembrane proteolysis of tmTNF by signal peptide peptidase–like proteases (SPPLs) releases a TNF intracellular domain to mediate reverse signaling in dendritic cells (Friedmann et al., 2006). Reverse signaling has been described for other ligands in the TNF superfamily (Bazzoni and Beutler, 1996), but the *in vivo* occurrence and functional significance of tmTNF-mediated reverse signaling remains to be elucidated.

The biosynthesis of TNF is tightly regulated, and TNF is barely detectable in quiescent cells. The production of TNF in

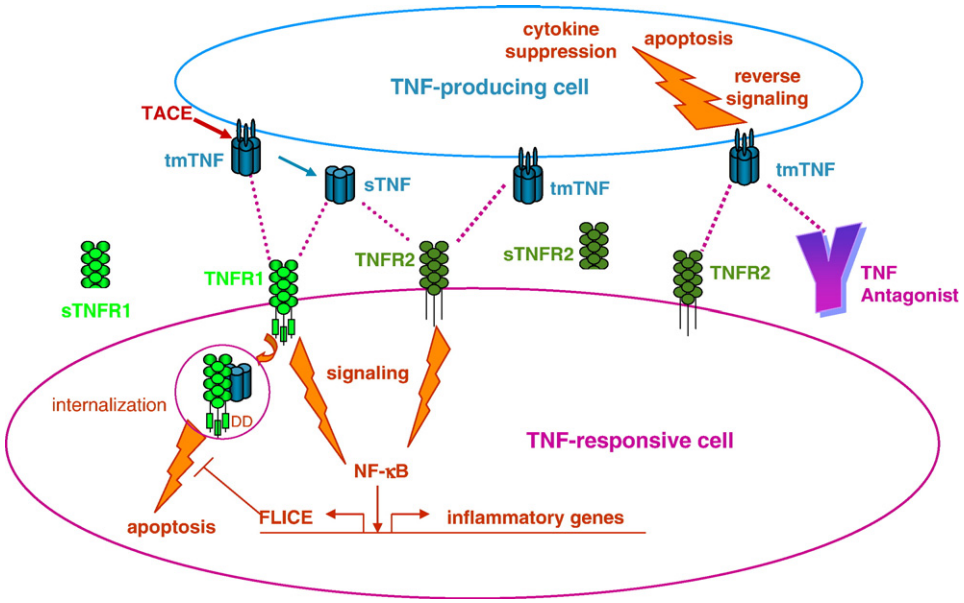

Fig. 1. Biology of TNF production, receptor interaction and signaling. Stimulation of a TNF-producing cell (top) results in cell surface expression of tmTNF trimers and enzymatic cleavage by TACE to release sTNF. Both tmTNF and sTNF can bind to cell surface TNFR1 or TNFR2 on a TNF-responsive cell (bottom), initiating signaling pathways that lead to apoptosis or NF-κB activation and inflammatory gene activation. The induction of apoptosis by sTNF via TNFR1 involves internalization of the ligand–receptor complex and association of death domains (DD) in the cytoplasmic tail of TNFR1 with adapter proteins and is normally blocked by FADD-like IL-1β–converting enzyme (FLICE). Reverse signaling can be initiated by TNFR2 or TNF antagonist binding to cell surface tmTNF, resulting in cytokine suppression or apoptosis. Soluble TNF receptors (sTNFR1 and sTNFR2) can be released from a TNF-responsive cell following enzymatic cleavage.

macrophages can be induced by a wide variety of stimuli, including bacteria, viruses, immune complexes, cytokines (e.g., IL-1, IL-17, granulocyte macrophage colony-stimulating factor, interferon-γ), complement factors, tumor cells, irradiation, ischemia/hypoxia and trauma. Many stimuli induce TNF mRNA within 30 min, but most regulation of TNF expression occurs post-transcriptionally. Adenine-uracil–rich elements (ARE) and flanking sequences in the 3-untranslated region regulate the translation and degradation of TNF mRNA (Han et al., 1990). Translation of TNF mRNA results in the intracellular production of trimeric pro-TNF protein, which lacks a signal peptide and is inserted into the plasma membrane as tmTNF. The production of TNF by cells is regulated by positive and negative feedback loops initiated by TNF-induced factors. For example, TNF induces the production of other cytokines, such as IL-1, interferon-γ and IL-2, which in turn can induce TNF production. TNF also induces some negative-feedback regulators, such as IL-10, prostaglandins and corticosteroids, that inhibit transcription of TNF mRNA.

TNFR1 is constitutively expressed on virtually all cell types except erythrocytes, whereas TNFR2 is generally inducible and is preferentially expressed on endothelial and hematopoietic cells. Both sTNF and tmTNF ligands can bind to both TNFR1 and TNFR2, but certain pairings are favored over others; namely, sTNF binding to TNFR1 and tmTNF binding to TNFR2. Although sTNF binds to both receptors on human cells with high affinity, it preferentially binds to TNFR1 (dissociation constant [K_d] ~ 20 pM) versus TNFR2 (K_d ~ 400 pM), owing to a 30-fold faster dissociation rate from TNFR2 than from TNFR1 (Grell et al., 1998). This observation has given rise to a ligand-passing hypothesis, whereby sTNF that binds to TNFR2 is quickly released and passed to TNFR1. Other data from *in vivo* studies corroborate the conclusion that most of the biologic activities of sTNF are mediated through TNFR1 (Ksontini et al., 1998). In contrast, tmTNF preferentially binds to TNFR2 (Grell et al., 1995) and is thought to exert most of its inflammatory and proapoptotic activities through TNFR2 or by reverse signaling through tmTNF. It should be noted, however, that studies on the relative roles of TNFR1 versus TNFR2 in sTNF-mediated and tmTNF-mediated biologic activities have not been entirely consistent and have been conducted primarily in mouse systems. The actual situation in humans *in vivo* is unclear.

Both the ligands and receptors in the TNF system are preformed, multivalent trimers. Structural and cellular studies have led to a model for the interaction of sTNF (and LTα3, described later) with cell-bound TNFR1 and TNFR2, whereby the receptor subunits bind to the grooves between sTNF subunits, resulting in cross-linking and clustering of receptors and initiation of signal transduction (Engelmann et al., 1990; Banner et al., 1993). Signaling initiated by sTNF (and LTα3) via TNFR1 and TNFR2 is mediated by adapter proteins that bind to the cytoplasmic domains of the receptors upon extracellular ligand binding. The cytoplasmic region of TNFR1 contains a death domain that couples TNFR1 to either of 2 distinct signaling pathways via binding of the adapter protein TNFR-associated death domain. The primary pathway leads to activation of nuclear factor kappa-B1 (NF-κB1), a family of transcription factors that control a large number of inflammatory genes, and a distinct signaling pathway leads to caspase-8– and caspase-3–dependent apoptosis. The apoptosis pathway is normally suppressed by FADD-like IL-1β–converting enzyme, a protein induced by the NF-κB1 activation pathway. However, if NF-κB1 activation is compromised, as often occurs in pathogen-infected cells, the apoptotic pathway

becomes the dominant TNFR1-mediated pathway (Hehlgans & Pfeffer, 2005; Ware, 2005). For the induction of apoptosis, the TNF/TNFR1 complex is internalized into endocytic vesicles in which various adapter proteins assemble and initiate the signaling cascades, leading to apoptosis (Higuchi & Aggarwal, 1994; Micheau & Tschopp, 2003; Schneider-Brachert et al., 2004). Association of the TNF/TNFR1 complex with lipid rafts, but not internalization, is required for the pathway leading to NF-κB1 activation (Legler et al., 2003; Schneider-Brachert et al., 2004; D'Alessio et al., 2005). TNF signaling is an active area of investigation, and the exact mechanisms involved in regulation of TNF-induced NF-κB1 activation and apoptosis are not fully understood.

TNFR1-mediated signaling can induce TACE-mediated enzymatic cleavage and shedding of the extracellular portion of TNFR2 in the form of sTNFR2 (Higuchi & Aggarwal, 1994). Shedding of sTNFR1 has also been reported, and both forms of sTNFRs are capable of binding and neutralizing sTNF, thus potentially serving as natural TNF antagonists. The concentrations of sTNFR1 and sTNFR2 are elevated in the serum of patients with RA and are good markers of disease activity (Roux-Lombard et al., 1993). However, the fine specificity of sTNFRs for various ligands in the TNF and LT families and their biologic activities *in vivo* are not well–understood. Signaling of TNF via TNFR2 has not been studied as intensively as signaling via TNFR1.

2.4. Lymphotoxin biology

As members of the TNF superfamily, LTs have many similarities to TNF, but there are some distinct molecular and biologic differences (Gommerman & Browning, 2003; Ware, 2005). A schematic view of the ligands, receptors and signaling pathways that encompass LT biology is shown in Fig. 2. First, there are several distinct ligands in the LT family. LTα3, formerly called TNFβ, is structurally similar to sTNF in that it is a soluble homotrimer composed of 17-kDa monomers and it binds specifically to TNFR1 and TNFR2 to exert its biologic activities. The affinities of LTα3 for TNFR1 and TNFR2 are comparable to those of TNF; but, unlike TNF, LTα3 does not rapidly dissociate from TNFR2 (Medvedev et al., 1996), suggesting that ligand passing of LTα3 from TNFR2 to TNFR1 is unlikely to occur. LTαβ is structurally distinct from LTα3 and comprises 2 membrane-anchored heterotrimers, the predominant LTα1β2 form and a minor LTα2β1 form. Both LTαβ forms interact with the LTβ receptor (LTβR), but the LTα2β1 form also binds less avidly to TNFR1 and TNFR2 than to the LTβR (Crowe et al., 1994; Williams-Abbott et al., 1997; Ware, 2005).

Unique among TNF superfamily members, the LTα monomer contains a traditional signal peptide; thus, LTα3 exists exclusively in a secreted, soluble form. The LTα monomer can only be membrane anchored when co-expressed and associated with LTβ monomers to form LTαβ heterotrimers. Follicular B cells and CD4 T cells in the spleen constitutively express LTαβ, but expression of LTαβ can be induced on splenic T cells by the cytokines IL-4 and IL-7 and the chemokine (C-C motif) ligands (CCL) 19 and 21 (Luther et al., 2002), and on a human T-cell line by TNF (Voon et al., 2004).

The LTβR that interacts with LTαβ-bearing lymphocytes is not expressed on T cells, B cells or NK cells but is constitutively expressed on stromal fibroblasts, epithelial cells and myeloid cells, such as monocytes/macrophages, dendritic cells and mast cells (Murphy et al., 1998; Ware, 2005). The cellular distribution of LTαβ ligands on lymphoid cells and LTβR on stromal and parenchymal cells, coupled with the requirement for cell–cell contact to initiate LTβR signaling, suggests a functional role of LTαβ in the interaction of lymphoid cells with surrounding stromal cells.

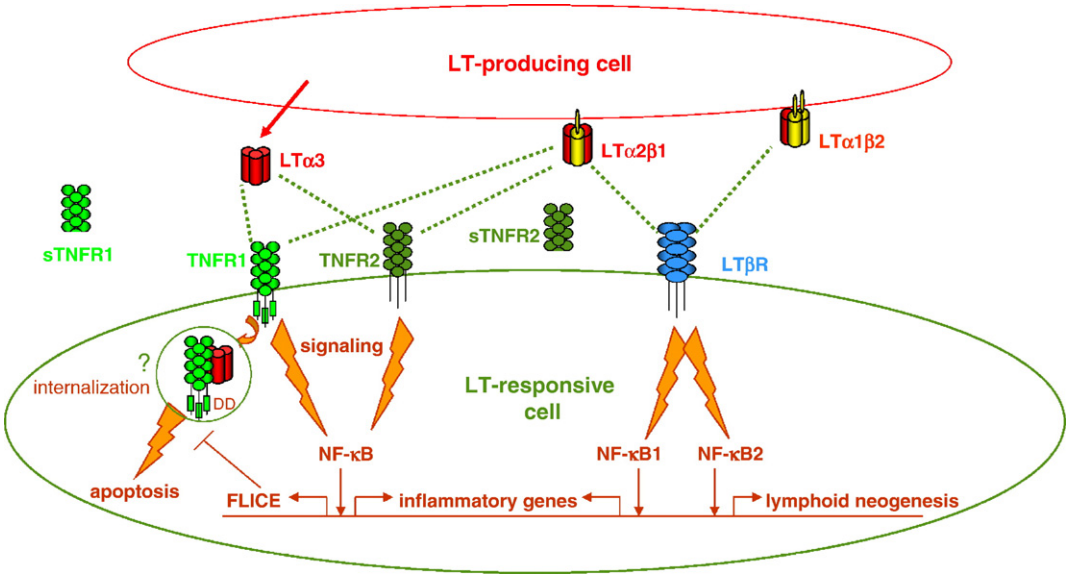

Fig. 2. Biology of LT production, receptor interaction and signaling. Stimulation of an LT-producing cell (top) results in the secretion of LTα3 or cell surface expression of LTα1β2 or LTα2β1. These ligands can interact with TNFR1, TNFR2 or LTbR as shown, resulting in apoptosis or NF-κB–dependent activation of inflammatory genes or events leading to lymphoid neogenesis. The TNFR1 or TNFR2 signaling pathways and internalization of TNFR1 with LTα3 are thought to be similar to those for TNF shown in Fig. 1.

Signaling via the LTβR is somewhat similar to that of TNFR1, using the adapter protein TNFR-associated factor 2 (TRAF2, or TRAF3) to couple the intracellular domains of the LTβR to both conventional and alternative NF-κB1 activation pathways, leading to the induction of inflammatory genes and genes involved in lymphoid tissue noegenesis, respectively (Ware, 2005). The LTβR does not contain a death domain; therefore, apoptosis pathways are not activated by LTαβ.

2.5. Tumor necrosis factor and lymphotoxin in the immune system

Ligands and receptors in the TNF and LT systems have a variety of roles in the development and function of the immune system (Gommerman & Browning, 2003; Hehlgans & Pfeffer, 2005; Ware, 2005). These include lymphoid organ development, as well as establishment and maintenance of lymphoid microenvironments, such as germinal centers. In established lymphoid tissues, certain TNF and LT family members are thought to play roles in innate immunity and in adaptive immunity, particularly in host defense against infections. However, much of the evidence for these roles is based on studies with genetically deficient mice; therefore, the relevance to human immune system development and function is less clear. Furthermore, some perplexing observations with "conventional" LTα-deficient mice, such as a reduction in LPS-induced TNF production (Alexopoulou et al., 1998), have been called into question by recent studies with a novel LTα-deficient mouse model, which showed unperturbed TNF expression (Liepinsh et al., 2006).

Mice genetically deficient in TNF or TNFR1 have a partially defective formation of B-cell follicles, follicular dendritic cell networks and germinal centers, but nearly normal humoral immune responses (Pasparakis et al., 1997). These observations suggest that TNF is not absolutely required but plays an ancillary role in these functions. A more dramatic phenotype is seen in mice genetically deficient for LTα, LTβ or LTβR, which are completely devoid of certain peripheral lymphoid tissues, such as lymph nodes and Peyer's patches (Fu & Chaplin, 1999). Secondary lymphoid organ formation involves a number of cytokines, chemokines and adhesion molecules and is thought to require at least 2 cell types: an LTαβ-expressing $CD4^+$ inducer cell and an LTβR-expressing embryonic stromal organizer cell (Nishikawa et al., 2003). Intravenous administration of an LTβR inhibitor (LTβR-immunoglobulin) to pregnant mice causes defects in lymph node and Peyer's patch development similar to those seen in LTα- or LTβ-deficient mice (Rennert et al., 1996). Although it is not entirely clear, it appears that LTαβ heterotrimers, and to some extent TNF, induce the expression of adhesion molecules, such as intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1) and mucosal addressin cell adhesion molecule-1 (MAdCAM-1), and C-C chemokine ligands (CCL), such as CCL19 and CCL21, as well as C-X-C chemokine ligands (CXCL) 12 and 13 that regulate lymphocyte homing and compartmentalization in lymphoid tissues (Gommerman & Browning, 2003; Aloisi & Pujol-Borrell, 2006).

Host defense against bacterial, fungal and parasitic infections initially entails innate immunity mediated by neutrophils, macrophages and other cells, followed by the engagement of antigen-specific adaptive immune responses involving T cells and/or B cells. The mechanisms of resistance to bacterial infections are complex and still under active investigation, but some implicate TNF and LT, particularly for intracellular bacteria such as *Mycobacterium* or *Listeria*. Control of these infections entails the formation of granulomas, bringing macrophages and T cells into proximity and walling off the bacteria. Killing of the intracellular bacteria within activated macrophages is primarily mediated by reactive oxygen species, including nitric oxide. Mice deficient in TNF, LTα, TNFR1 or LTβR are highly susceptible to experimental *Mycobacterium*, *Listeria* and *Staphylococcus* infections compared with normal mice (Rothe et al., 1993; Flynn et al., 1995; Pasparakis et al., 1996; Hultgren et al., 1998; Roach et al., 2001; Ehlers et al., 2003).

Dissecting the relative roles of the various TNF and LT ligands or their receptors in these studies is not possible. As discussed previously, mice deficient in TNF/LT ligands or receptors have marked defects in lymphoid organ development and organization, rendering their immune systems functionally impaired. Furthermore, the experimental models for measuring bacterial infections cannot be reliably compared between studies, owing to differences in bacterial strain virulence, as well as the genetic backgrounds and environmental conditions of the mice. Recent studies of *Listeria* infection in mice demonstrated that TNF from macrophages and neutrophils was more critical than TNF from T or B cells in resistance to infection (Grivennikov et al., 2005). Another study of resistance to *Listeria* infection in mice demonstrated the importance of a TNF/inducible nitric oxide synthase–producing dendritic cell subset in the spleen (Serbina et al., 2003). Several studies have demonstrated an LT requirement for granuloma formation and resistance to *Mycobacterium* infections, but the conclusions differ regarding whether these functions were mediated by LTα3 or LTαβ heterotrimers (Roach et al., 2001; Ehlers et al., 2003). Granuloma formation in response to *Leishmania* infection was shown to depend on both TNF and LTα (Engwerda et al., 2004). Mechanisms of host defense against intracellular pathogens, particularly *M. tuberculosis*, in humans have not been as well studied as those in mice, but they have been extensively discussed in the context of the clinical safety of TNF antagonists.

Host defense against malignancy has been another area of discussion regarding the use of TNF antagonists, but only a few studies shed light on potential roles of TNF or LT. NK cells are thought to be the primary component of innate immunity against tumors, as NK cells can recognize and kill tumor cells without prior sensitization or requirement for adaptive immunity. No requisite role for TNF in the development, activation or function of NK cells has been described. However, some studies in mice have shown a role for TNF and inflammation in the tumor promotion stages of skin tumor carcinogenesis (Moore et al., 1999). Several studies have demonstrated a requirement for LTα and stromal cell LTβR in the differentiation of active NK cells (Iizuka et al., 1999; Wu et al., 2001). LTα plays a role in the recruitment and antitumor activity of mature NK cells (Smyth et al., 1999), and LTα deficiency results in enhanced

tumor growth and metastasis (Ito et al., 1999). The relevance of these observations to the role of LT in host defense against tumors in humans has yet to be established.

Another area of interest is the role of TNF and LT in adaptive immunity. Adaptive immunity to foreign antigens involves antigen processing by dendritic cells, macrophages, or B cells and antigen presentation to various subsets of T cells and B cells, which result in cellular and/or humoral immunity to the inciting antigen. Beyond their roles in lymphoid organogenesis, TNF and LT are not required for adaptive immunity, but may modulate adaptive immune responses in several ways. TNF can skew monocyte differentiation to dendritic cells instead of macrophages (Chomarat et al., 2003) and can induce the production of a variety of chemokines (van Lieshout et al., 2005) that facilitate dendritic cell migration and initiation of immune responses during dendritic cell maturation. In T-cell responses to antigen, TNFR2 is functionally linked to CD28 and has a critical role in IL-2 induction and T-cell survival (Kim and Teh, 2004). TNF can stimulate T-cell proliferation but also can promote T-cell apoptosis and the termination of immune responses by activation-induced cell death (Kollias et al., 2002). TNF also promotes CXCL10-mediated T-cell chemotaxis by upregulating adhesion molecules on endothelial cells (Manes et al., 2006). Furthermore, TNF can cause a downregulation of T-cell reactivity by regulation of the expression of the CD3ζ chain of the T-cell–receptor complex (Isomaki et al., 2001). This downregulation can be reversed by TNF antagonism (Cope et al., 1994) and may result in increased T-cell reactivity (Cope et al., 1994; Berg et al., 2001).

Tolerance to self-antigens is maintained by several mechanisms, including deletion of autoreactive T-cell precursors in the thymus or periphery, by induction of anergy and by suppression of autoreactive T cells by regulatory T cells (Tregs). TNF may play multiple roles in tolerance and autoimmunity, but more studies are needed before definitive conclusions can be reached. When administered to type 1 diabetes–prone (nonobese diabetic [NOD]) mice during the first 3 weeks of age, TNF enhanced autoreactive T- and B-cell responses, whereas the opposite effect was seen if TNF was given after 4 weeks of age (Yang et al., 1994). Administration of anti-TNF antibodies to neonatal NOD mice prevented the onset of type 1 diabetes and elevated the numbers of Tregs (McDevitt et al., 2002). In certain strains of mice genetically prone to develop multiple sclerosis–like or lupus-like autoimmunity, TNF can exert an immunosuppressive role (Kollias et al., 2002). These findings suggest dual roles of TNF, either immunostimulatory or immunosuppressive, depending on the genetic background, timing and concentrations of TNF. A recent study demonstrating that TNF inhibits the suppressive function of human Tregs via signaling through constitutively expressed TNFR2 offers an intriguing explanation for the immunostimulatory activity of TNF (Valencia et al., 2006).

Autoimmunity arising from perturbed tolerance in the thymus has been observed in mice genetically deficient in LTα or LTβR (Chin et al., 2003). The transcription factor autoimmune regulator (Aire) has been described as a master regulator of tolerance by governing the expression of peripherally restricted antigens by medullary thymic epithelial cells (Anderson et al., 2002; Chin et al., 2003). The expression of Aire in thymic cells is regulated by LTαβ heterotrimers on activated thymocytes signaling through the LTβR on medullary thymic epithelial cells (Browning et al., 1997; Chin et al., 2003). Thus, there is evidence of a key role for LT in the maintenance of tolerance to autoantigens. Furthermore, transgenic expression of LTβR-Fc in NOD mice prevented the onset of type 1 diabetes (McDevitt et al., 2002).

2.6. Tumor necrosis factor and lymphotoxin in inflammation and disease

The biology of TNF has been extensively studied in experimental animals and in humans, particularly with regard to its many activities when expressed at high concentrations in the context of inflammation and disease. TNF is a pleiotropic cytokine, in that it mediates a wide variety of biologic activities. As shown in Fig. 3, some activities of TNF are common to a variety of diseases, such as those that modulate cell recruitment, cell proliferation, cell death and immune regulation (reviewed in Choy & Panayi, 2001; Feldmann, 2002; Schottelius et al., 2004). Other biologic activities of TNF may be restricted to certain diseases, such as matrix degradation and osteoclastogenesis in RA or granuloma formation in Crohn's disease (Fig. 3). Such disease-specific sequelae to the common inflammatory mechanisms may simply be related to the different cell types in the target tissues of various immune-mediated inflammatory diseases, or there may be some unique mechanisms underlying the pathogenesis of different diseases.

In recent years, the link between cytokine-mediated immunity, inflammation, and cancer has been the focus of considerable attention (reviewed in Lin & Karin, 2007). A central mediator in this relationship is TNF via its role in NF-κB regulation (Balkwill & Coussens, 2004). TNF-induced activation of NF-κB has been shown to induce the expression of genes that inhibit apoptosis, stimulate cell proliferation, and participate in tumor invasion and metastasis (reviewed in Mayo & Baldwin, 2000). In liver hepatocytes, Pikarsky et al. (2004) have shown that TNF produced by neighboring inflammatory stromal cells activates NF-κB in the hepatocytes, in this manner promoting malignant transformation. This suggests that TNF antagonists might be effective in the treatment of some types of cancer. On the basis of the success of TNF–antagonist therapies in treating RA and other inflammatory diseases, the potential role of TNF antagonists in the treatment of certain cancers is now being considered. However, important considerations for the use of TNF antagonists in cancer treatment include the tumor microenvironment, the type of cancer, and the possibility that the use of a TNF antagonist to treat one type of cancer may increase the risk of developing another type of malignancy.

As discussed previously, TNF has a particularly important role in the regulation of a cascade of pathogenic events in RA, Crohn's disease, psoriasis and other diseases, exemplified by the rapid induction of cytokines, such as IL-1β and IL-6, and the induction of acute-phase proteins, such as C-reactive protein (CRP) (Feldmann, 2002). However, many studies demonstrate that TNF acts within a complex network of cells and mediators

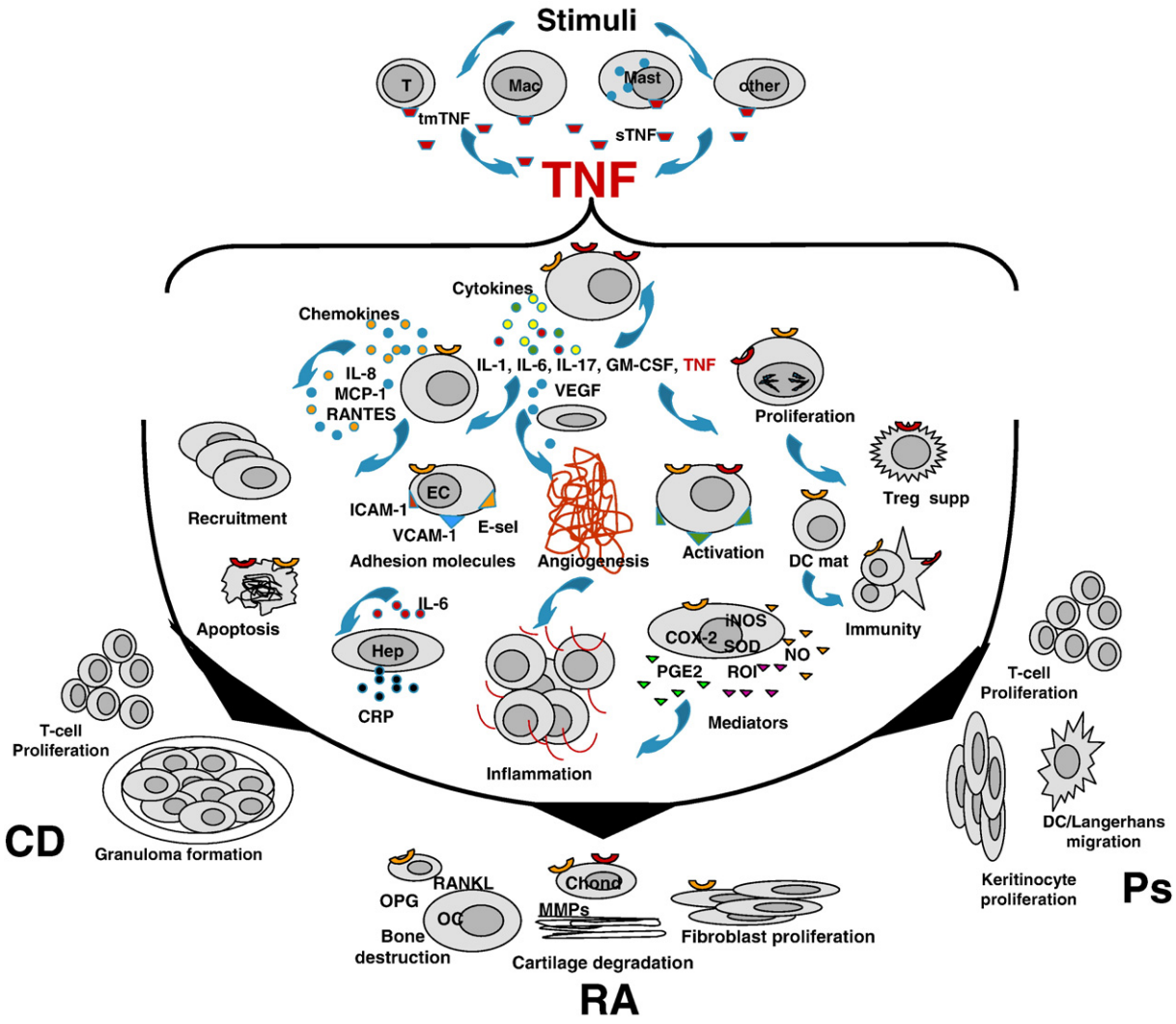

Fig. 3. In the pathophysiology of RA, Crohn's disease and psoriasis, TNF is produced at high concentrations by a variety of cell types, presumably induced by endogenous or microbial stimuli. A cascade and network of cellular responses mediated by TNF that are common to these 3 diseases are shown in the enclosed area in the center of the diagram. Mechanisms and cellular features restricted to a particular disease are shown outside of the enclosed area.

of inflammation, exemplified by the induction of TNF by IL-1β or IL-17. The network concept of the role of TNF in inflammation postulates that TNF is an early and important trigger for and mediator of downstream mechanisms and that a variety of positive and negative feedback loops govern the chronicity and pathogenic outcomes of inflammation. Many of these functions of TNF are thought to be inhibited by TNF antagonists and will be discussed in more detail in the following sections.

In patients with RA, the synovial membrane lining the joint space becomes inflamed as a consequence of increased vascularity and influx of inflammatory cells. Similar histopathology is seen in murine collagen-induced arthritis, for which TNF was observed within the intimal lining layer and synovial sublining at all stages of disease (Mussener et al., 1997). The staining of TNF was particularly intense at the invasive front, the cartilage–pannus junction. Synovial tissue from patients with RA contains TNF and other proinflammatory cytokines, such as IL-1, regardless of the duration of disease (Ulfgren et al., 1995; Tak et al., 1997; Ulfgren et al., 2000; Choy & Panayi, 2001). Activated macrophages are the primary source of TNF in inflamed synovial tissue, and both the number of macrophages and the degrees of TNF expression correlate with clinical scores for knee pain (Tak et al., 1997). Another study of patients with RA demonstrated by immunohistochemistry that TNF-producing cells were found predominantly in the sublining layer proximal to macrophage-like cells bearing TNFR1 and/or TNFR2 (Alsalameh et al., 1999). T cells in synovial lymphocyte aggregates exclusively expressed TNFR2. Both sTNFR1 and sTNFR2 have been detected in the synovial fluid and serum of patients with RA (Roux-Lombard et al., 1993). Neutralization of TNF in RA synovial tissue explant cultures blocked the production of IL-1 and other cytokines thought to be involved in the pathogenesis of RA (Brennan et al., 1989; Feldmann & Maini, 2002). These observations collectively support the hypothesis that TNF is instrumental in the pathogenesis of RA. The stimuli that induce and maintain TNF production in rheumatoid synovial macrophages are not known precisely, but

cell–cell interactions, cytokines, immune complexes, complement, microbial products, endogenous ligands for toll-like receptors (TLR) and hypoxia are all reasonable candidates. The cytokine IL-17 induces TNF from human macrophages (Jovanovic et al., 1998) and is thought to induce some of the TNF in an RA joint (Koenders et al., 2006). The RA joint also contains negative regulators of TNF production, such as IL-10, IFN-β and prostaglandins, but their effects appear to be overshadowed by positive regulators because TNF production is sustained in chronic inflammatory diseases such as RA.

The hypothesis that TNF drives much of the pathophysiology in a rheumatoid joint is supported by studies of TNF overexpression or TNF neutralization in animal models of RA (Keffer et al., 1991; Williams et al., 1992; Butler et al., 1997; Klareskog & McDevitt, 1999). Genetically engineered deletion of the TNF active-resistive–exercises (ARE) in mice led to overexpression of TNF, IL-1β and IL-6 in the synovial lining and resulted in the spontaneous development of chronic inflammatory polyarthritis (Butler et al., 1997; Kontoyiannis et al., 1999). Furthermore, transgenic mice expressing a TACE-resistant tmTNF mutant also developed spontaneous arthritis that was dependent on both TNFR1 and TNFR2 for maximum manifestation of disease (Alexopoulou et al., 1997; Edwards et al., 2006). Conversely, neutralization of TNF with monoclonal anti-TNF antibodies or sTNF-receptor fusion proteins ameliorated disease in rodent models of RA (summarized in Choy & Panayi, 2001; Feldmann & Maini, 2003; Schottelius et al., 2004; Edwards et al., 2006). Although many of the TNF neutralization studies in mouse models of RA demonstrated amelioration of clinical disease similar to that seen in studies of TNF antagonists in patients with RA, there have been apparent differences in the impact on bone erosion. TNF blockade in collagen-induced arthritis and streptococcal cell wall–induced arthritis in mice was most effective in early disease and primarily suppressed joint swelling and synovial inflammation, more so than cartilage or bone erosion (Joosten et al., 1999). However, TNF blockade in human TNF-transgenic mice suppressed joint destruction more than synovial inflammation (Zwerina et al., 2004). Other studies of TNF blockade in rat models of arthritis have also demonstrated significant suppression of cartilage and bone erosion (Bendele et al., 2000).

TNF ARE-deficient mice also develop Crohn's-like inflammatory bowel disease (Kontoyiannis et al., 1999), suggesting that TNF-driven pathways may also operate in Crohn's disease in humans. Interestingly, mature T and B cells played a major role in the development of TNF-driven inflammatory bowel disease, but not chronic inflammatory arthritis, in these mice (Kontoyiannis et al., 1999). TNF mRNA and protein have been demonstrated in the intestinal mucosa from patients with Crohn's disease and ulcerative colitis, predominantly in mast cells (Bischoff et al., 1999), monocytes, macrophages and T cells (Van Deventer, 1997). Furthermore, concentrations of TNF in the intestinal mucosa of patients with Crohn's disease and ulcerative colitis are elevated relative to healthy individuals (Van Deventer, 1997).

Likewise, elevated TNF expression has been seen in the skin lesions in patients with psoriasis (Schottelius et al., 2004). In one study, TNF in lesional skin was localized to papillary dermal macrophages, epidermal keratinocytes and intraepidermal Langerhans cells (Nickoloff et al., 1991). In another study, TNF was distributed throughout the epidermis and was localized to upper dermal blood vessels in lesional psoriasis skin and, to a lesser extent, in uninvolved psoriasis skin (Kristensen et al., 1993). In lesional skin, TNFR1 expression was associated with epidermal keratinocytes, a network of upper dermal dendritic cells and upper dermal blood vessels, whereas TNFR2 was expressed in association with upper dermal blood vessels and perivascular infiltrating cells. The hypothesis that TNF and TNFR expression are functionally linked to disease pathology has been definitively corroborated by the positive clinical trial results with TNF antagonists in RA, Crohn's disease, psoriasis and other diseases.

In recent years, emerging evidence has implicated TNF in the pathology of asthma, a chronic condition characterized by reversible airway obstruction, bronchial hyperresponsiveness, and chronic airway inflammation (Busse & Lemanske, 2001). Typical therapy for individuals with asthma involves the use of bronchodilators and anti-inflammatory agents; however, approximately 10% of people with asthma have severe/refractory asthma with symptoms unresponsive to inhaled corticosteroid treatment (Busse et al., 2000). In these cases, a marked increase in TNF production has been observed (Berry et al., 2006). Clinical trials using commercially available TNF antagonists have been initiated, and although preliminary results have been somewhat inconsistent, some reports have shown promising results. In particular, patients with chronic severe/refractory asthma treated with etanercept for 10 to 12 weeks demonstrated significant improvement in asthma symptoms (Howarth et al., 2005; Berry et al., 2006).

The role of the LT system in inflammation and disease has not been studied as intensively as that of TNF. Overexpression of LTα in the pancreas or kidneys of mice led to inflammation characterized by lymphoid neogenesis, a dynamic process during which sparse lymphocytic infiltrates evolve into lymphocyte aggregates that sometimes organize into B-cell follicles with germinal centers and T-cell–rich areas containing follicular dendritic cell networks and high endothelial venules (Kratz et al., 1996). These processes require LTαβ heterotrimers, and, to a lesser extent, TNF, to induce adhesion molecules and chemokines that mediate lymphocyte infiltration and organization into ectopic lymphoid structures (Gommerman & Browning, 2003; Browning et al., 2005; Weyand et al., 2005; Aloisi & Pujol-Borrell, 2006). In RA, Crohn's disease and other chronic immune-mediated inflammatory diseases, similar organized lymphoid structures, such as ectopic germinal centers and B-cell follicles, are seen within the inflamed tissue (Gommerman & Browning, 2003; Aloisi & Pujol-Borrell, 2006). Of interest, the presence of lymphoid follicles with germinal centers in RA synovial biopsies correlated with expression of LTβ on B and T cells and the chemokine CXCL13 in synovial fibroblasts and endothelial cells (Takemura et al., 2001).

Although the roles of LTα1β2 and LTα2β1 in immune-mediated inflammatory diseases remain to be defined, a critical role for signaling through the LTβR has been demonstrated by LTβR:Ig blockade in murine models of type 1 diabetes, multiple sclerosis and inflammatory bowel disease. However, some of

these effects may be mediated by LIGHT (lymphotoxin-like inducible protein that competes with glycoprotein D for herpes simplex virus entry mediator on T cells), an alternative ligand for LTβR (Gommerman & Browning, 2003; Hehlgans & Pfeffer, 2005). Studies in murine collagen-induced arthritis have yielded opposite results, depending on the timing of LTβR blockade with LTβR:Ig. On the one hand, the development of arthritis was suppressed if LTβR:Ig treatment was begun before collagen immunization and continued throughout the experiment (Fava et al., 2003). Conversely, treatment with LTβR:Ig after the onset of arthritis significantly exacerbated the disease and was associated with an enhanced type 1 T-helper cell response and elevated concentrations of anticollagen antibodies (Han et al., 2005). Furthermore, collagen-induced arthritis was more severe and prolonged in genetically LTα-deficient mice compared with wild-type mice (Han et al., 2005). These results are reminiscent of those with TNF in type 1 diabetes–prone mice (Yang et al., 1994), possibly suggesting a dual role for LTα/LTβR in this model. The relevance of these murine studies to the role of the LT system in human diseases remains to be elucidated.

3. Tumor necrosis factor antagonist structures and properties

3.1. Structures

The structures of the TNF antagonists infliximab, etanercept, adalimumab, certolizumab and golimumab are schematically represented in Fig. 4, which illustrates their similarities and differences. All agents except etanercept are anti-TNF mAbs or fragments thereof. Natural mAbs are derived from single B cells that clonally express copies of a unique heavy (H) chain and a unique light (L) chain that are covalently linked to form an antibody molecule of unique specificity. Engineered mAbs can be structurally identical to natural mAbs but are created by gene splicing and mutation procedures, mimicking natural gene rearrangement and somatic mutation events in B cells (Salfeld et al., 1998).

Infliximab, adalimumab and golimumab are full-length, bivalent IgG mAbs, whereas certolizumab is a monovalent Fab[1] antibody fragment covalently linked to polyethylene glycol. IgG antibody molecules are composed of 2 H and 2 L polypeptide chains, each of which contains 3 complementarity-determining regions in the N-terminal (V_H and V_L) domains. An IgG molecule is composed of 2 antigen-binding Fab arms, linked to a glycosylated Fc region via a flexible hinge region. The antigen-binding site on each Fab portion of a mAb is generally composed of amino acids from the 6 complementarity-determining regions in each H:L chain pair. Infliximab is a chimeric protein containing ~ 25% mouse-derived amino acids comprising the V_H and V_L domains and ~ 75% human-derived amino acids comprising the C_H1 and Fc constant regions. Certolizumab is a humanized protein containing amino acid sequences in the complementarity-determining regions derived from a mouse anti-TNF mAb and inserted into human V_H and

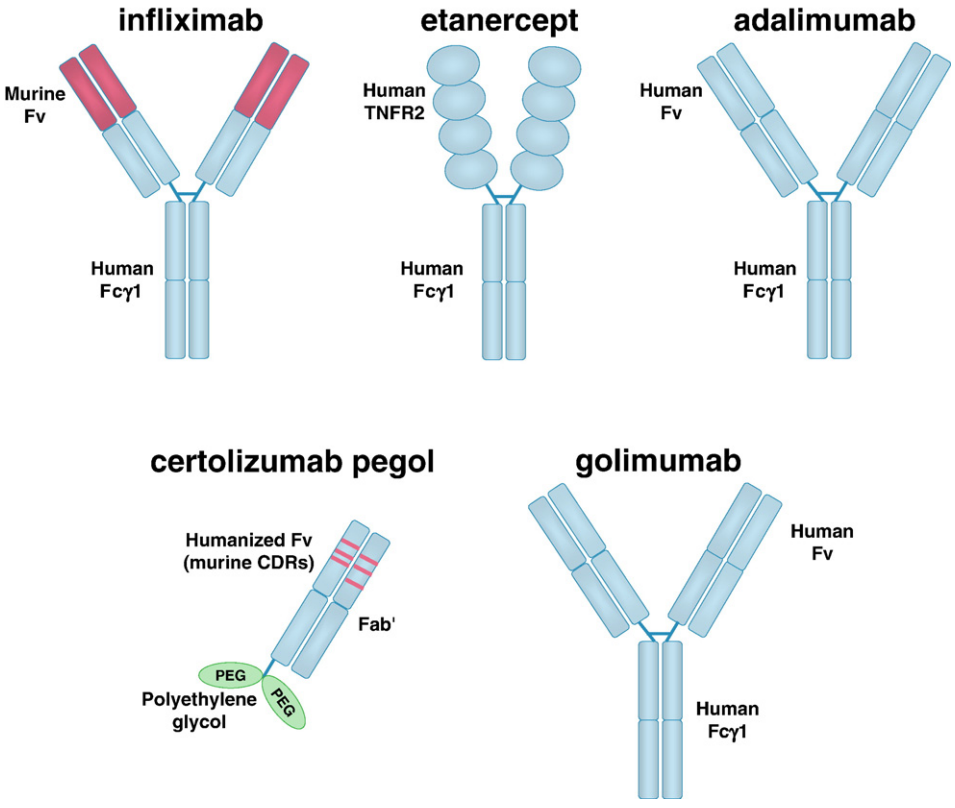

Fig. 4. Simplified diagrams of the molecular structures of 5 TNF antagonists. Infliximab is a mouse/human chimeric monoclonal anti-TNFantibody of IgG1 isotype. Adalimumab and golimumab are fully human IgG1 monoclonal anti-TNF antibodies. Etanercept is a fusion protein of TNFR2 (p75) and the Fc region of human IgG1. Certolizumab is a PEGylated Fab' fragment of a humanized IgG1 monoclonal anti-TNF antibody.

V_L domain frameworks. Adalimumab and golimumab are fully human mAbs. The TNF–antagonist mAbs also differ in their IgG isotypes, the Fc regions of which govern effector functions, like complement fixation and Fc receptor–mediated biologic activities. Infliximab, adalimumab and golimumab are IgG1 antibodies, which are capable of complement fixation and Fc-receptor binding. Certolizumab is an Fab[1] fragment of an IgG1 mAb and lacks effector functions because it has no Fc region. The hinge region of certolizumab is modified and covalently linked to 2 crosslinked chains of 20 kDa of polyethylene glycol to enhance solubility and half-life in vivo (Weir et al., 2006).

Etanercept is a genetically engineered fusion protein composed of a dimer of the extracellular portions of human TNFR2 fused to the Fc portion of human IgG1. The TNFR2 portion contains 4 domains, and the C-terminal domain includes a 57-residue region that contains 13 *O*-glycosylated residues and 11 proline residues (Kohno et al., 2005a). The plasma half-lives of antibodies appear to be largely governed by the binding of their Fc regions to the neonatal Fc receptor (FcRn) on endothelial cells (Lobo et al., 2004). Although the amino acid sequences of the Fc regions are identical, the markedly shorter plasma half-life of etanercept versus IgG1 mAbs or other Fc fusion proteins (Table 1; Lobo et al., 2004) suggests that the conformation or steric accessibility of the Fc region of etanercept may be different from those of the Fc regions of the IgG1 antibodies infliximab and adalimumab. The effect of the glycosylated C-terminal domain of TNFR2 on the structure and function of the adjacent Fc region of etanercept is unclear, as no data have been reported on the binding affinities of etanercept for FcRn or other Fc receptors. In comparison, the long plasma half-lives of infliximab, adalimumab and golimumab suggest that they bind to FcRn like natural IgG1 molecules.

3.2. Ligand binding

The key biochemical and mechanistic properties of infliximab, etanercept and adalimumab are shown in Table 2, including their ligand-binding properties. Published or publicly presented information on the properties of certolizumab and golimumab is also included in Table 2. Ligand-binding studies conducted with BIAcore surface plasmon resonance technology measure the on-rate and off-rate of an agent binding to a ligand. The ratio of these rates determines the binding affinity of the agent for the ligand, usually expressed as a dissociation constant, K_d. All of these agents bind sTNF with high affinity, with K_d values in the sub-nM range. However, there are some important differences between agents in their kinetic parameters of binding. Infliximab and adalimumab have been reported to have slower on-rates and off-rates than etanercept (Santora et al., 2001; Scallon et al., 2002). Recent studies with current BIAcore methodology found that the on-rate for etanercept was about twice that of infliximab or adalimumab; the off-rates of the 3 agents were comparable (Kaymakcalan et al., 2006a). Infliximab binds to both the 17-kDa monomer and the 51-kDa trimer forms of sTNF, whereas etanercept binds only to the trimer form, with each receptor arm contacting comparable epitopes on different faces of the trimer (Scallon et al., 2002). Thus, infliximab and etanercept probably bind to different epitopes on sTNF (Scallon et al., 2002). Similar studies comparing the binding of adalimumab to sTNF monomer and trimer have not been reported. Differences have been reported on the size, composition and stability of complexes formed between sTNF and the different agents. As bivalent mAbs, infliximab and adalimumab can bind 2 sTNF trimers simultaneously, which allows multimeric complexes to form under permissive stochiometric conditions (Santora et al., 2001; Scallon et al., 2002). In contrast, each molecule of etanercept appears to bind to sTNF by interacting with a single sTNF trimer, generally resulting in small 1:1 complexes (Scallon et al., 2002).

Following the discovery that tmTNF plays an important role in the proinflammatory functions of TNF, much attention has been paid to the binding of TNF antagonists to tmTNF. The concentrations of tmTNF on normal monocytes/macrophages and T cells are low, even after cell activation (Ware et al., 1992). In addition, tmTNF is subject to cleavage by TACE. Therefore, assessments of TNF–antagonist binding to tmTNF with immunofluorescence or radioligand binding techniques have obtained variable results, depending on the types of cells, reagents and protocols that were used (for cell culture and stimulation), as well as the sensitivity of the assays used to detect binding. To study cells with greater, more stable concentrations of tmTNF, investigators have used cell lines transfected with native or mutated (i.e., TACE-resistant) tmTNF. It is not known whether the tmTNF molecules derived from recombinant genes, particularly the mutated forms, are conformationally identical to natural tmTNF. SPPL2a and SPPL2b were recently identified as novel proteases that cleave tmTNF at intramembrane sites distinct from the extracellular TACE-cleavage site, possibly releasing extracellular portions of tmTNF from the cell (Friedmann et al., 2006). This finding raises the possibility that reported differences in the detection of binding to tmTNF by anti-TNF reagents may reflect differences in the activity of these proteases under the various experimental conditions used in these studies.

Several studies have demonstrated binding of infliximab, adalimumab, etanercept and/or certolizumab to cell lines expressing transfected tmTNF (Scallon et al., 2002; Mitoma et al., 2004; Mitoma et al., 2005; Fossati & Nesbitt, 2005a; Gramlick et al., 2006; Kaymakcalan et al., 2006a). The degree of cell binding was typically up to 3-fold greater with infliximab or adalimumab than with etanercept or certolizumab. These quantitative differences in binding may be a reflection of stoichiometric differences rather than differences in affinity, because up to 3 molecules of infliximab can bind to one tmTNF, whereas etanercept usually binds in a 1:1 ratio (Santora et al., 2001; Scallon et al., 2002). Furthermore, infliximab, adalimumab and etanercept have been reported to have similar, high binding affinities for transfected tmTNF ($4.5–4.8 \times 10^{-10}$ M), approximately 1 order of magnitude less than for sTNF (Kaymakcalan et al., 2006a). In a study of monocytes from normal human peripheral blood, adalimumab, etanercept and infliximab bound to cells in similar degrees, both with and without prestimulation with lipopolysaccharide (LPS) (Shen et al., 2005). In studies using T-cell stimulation, binding to normal human peripheral blood lymphocytes was observed for

Table 2
Biochemical and mechanistic profile of TNF antagonists

	Infliximab	Etanercept	Adalimumab	Certolizumab	Golimumab	References
Class	Monoclonal antibody	Fc-fusion protein	Monoclonal antibody	Monoclonal antibody fragment	Monoclonal antibody	Enbrel PI, Humira PI, Kay et al., 2006, Remicade PI, Weir, 2006
Structure	Mo/Hu chimeric IgG1κ	Hu sTNFR2-Fcγ1	Hu IgG1κ	PEG-Hu IgG1κ Fab[1]	Hu IgG1κ	Enbrel PI, Humira PI, Kay et al., 2006, Remicade PI, Weir, 2006
Molecular weight (kDa)	150	150	150	~95	150	Enbrel PI, Humira PI, Kay et al., 2006, Remicade PI, Weir, 2006
Specificity	TNF	TNF/LT	TNF	TNF	TNF	Enbrel PI, Humira PI, Kay et al., 2006, Remicade PI, Weir, 2006
TNF ligands	sTNF, tmTNF	sTNF, tmTNF,	sTNF, tmTNF	sTNF, tmTNF	sTNF, tmTNF	Enbrel PI, Humira PI, Kay et al., 2006, Remicade PI, Weir, 2006
LT ligands	–	LTα3, LTα2β1	–	–	–	Browning, 1995, Crowe, 1994, Scallon, 2002, Ware, 2005, Williams-Abbott, 1997
Neutralization potency						
sTNF (low conc)	++	+++	++	ND	ND	Kaymakcalan, 2006b
sTNF (high conc)	+++	+++	+++	+++	ND	Enbrel PI, Humira PI, Gramlick, 2006, Kay et al., 2006, Kaymakcalan, 2006b, Kohno, 2005a, Remicade PI, Van den Brande, 2003, Weir, 2006
tmTNF binding	+++	++	+++	+++	ND	Fossati, 2005a, Kaymakcalan, 2006a,b, Lugering, 2001, Mitoma, 2004, Scallon, 2002, Shen, 2005, van den Brande, 2003
tmTNF neutralization	+++	++	+++	+++	ND	Gramlick, 2006, Shen, 2005
Reverse signaling (apoptosis)	+++	++/–	+++	–	ND	Catrina, 2005, Chaudhary, 2006, Di Sabatino, 2004, Eissner, 2000, Fossati 2005b, Lugering, 2001, Shen, 2005, van den Brande, 2003
Reverse signaling (cytokine suppression)	+++	++/–	+++	+++	ND	Nesbitt, 2006, Kirchner, 2004, Mitoma, 2004, 2005, Scallon, 2002, Shen, 2005
FcγR binding						
Drug–TNF complexes 1:1 ratio	++	–	++	ND	ND	Kaymakcalan, 2006a, Kohno, 2005b
Drug–TNF complexes >10:1 ratio	–	–	–	ND	ND	Kaymakcalan, 2006a
CDC	+++	++/–	+++	–	ND	Fossati, 2005a, Gramlick, 2006, Kohno, 2005a, Scallon, 1995, van den Brande, 2003
ADCC	+++	++/–	+++	–	ND	Fossati, 2005a, Gramlick, 2006, Scallon, 1995, van den Brande, 2003

ADCC is antibody-dependent cellular cytotoxicity; CDC, complement–dependent cytotoxicity; Hu, human; IgG, immunoglobulin G; LT, lymphotoxin; Mo, mouse; ND, no data available; PEG, polyethylene glycol; sTNF, soluble TNF; tmTNF, transmembrane TNF; TNF, tumor necrosis factor; +/–, very weak; +, weak; ++, moderate; +++, strong.

infliximab, but not for etanercept following activation with anti-CD3/CD28 (Van den Brande et al., 2003); binding was observed for adalimumab, etanercept and infliximab following activation with PMA/ionomycin (Kaymakcalan et al., 2006a). Because the cell culture and stimulation protocols as well as the tmTNF detection methods of these tmTNF-binding experiments were quite varied, it is difficult to reconcile the divergent findings regarding etanercept. Nonetheless, the preponderence of evidence indicates that infliximab, adalimumab, etenercept and certolizumab can all bind strongly to tmTNF on human cells.

The cellular and biochemical consequences of binding to tmTNF by TNF antagonists may depend on tmTNF crosslinking and, thus, may be influenced by several factors. In contrast to the anti-TNF mAbs, which have the potential to crosslink 2 tmTNF trimers, it appears that etanercept preferentially binds with both receptor arms to a single tmTNF trimer, with little or no potential to crosslink 1 tmTNF trimer to another (Scallon et al., 2002). Variations in cell–surface density of tmTNF may underly some of the apparent discrepancies between cellular tmTNF-binding studies. Low-density expression of tmTNF might favor binding of infliximab, adalimumab and etanercept to a single tmTNF, without crosslinking, whereas high-density tmTNF expression might favor crosslinking and greater-avidity binding to tmTNF by infliximab or adalimumab, but probably not etanercept. Interestingly, certolizumab is a monovalent PEGylated Fab[1] molecule that should not be able to crosslink tmTNF, yet it has been found to induce reverse signaling in cells (Nesbitt et al., 2006). An additional consideration is that rheumatoid factors (RFs), which are IgM, IgG or IgA autoantibodies specific for the Fc region of IgG, are present in approximately 80% of patients with RA. Most IgM RF bind to IgG1 and are usually specific for Fc epitopes that are shared by adalimumab, etanercept and infliximab (Sasso et al.,

1988). It is thus conceivable that RF may crosslink cell-bound adalimumab, etanercept or infliximab molecules whether or not the drugs have crosslinked tmTNF. *In vitro*, the ability of cell-bound etanercept to inhibit cell proliferation was enhanced and became more similar to that of infliximab when anti-Fc antibodies were present (Mitoma et al., 2005). The relation between crosslinking of tmTNF and the clinical outcomes of anti-TNF therapy has not yet been established.

The only registered TNF antagonist that is known to bind and neutralize a ligand other than TNF is etanercept, which binds members of the LT family. There are no published values for binding affinities of etanercept to LT ligands, but reports indicate that etanercept binds to LTα3 with comparable or greater affinity than sTNF (Z. Kaymakcalan, personal communication 2006; Scallon et al., 2002). Serum from etanercept-treated patients was capable of binding more LTα3 than sTNF (Gudbrandsdottir et al., 2004). Both etanercept and native TNFR2 bind TNF and LTα3, suggesting that etanercept retains the ligand-binding specificity of its parent receptor. Etanercept also binds to a membrane-associated form of LT, LTα2β1 (Crowe et al., 1994; Browning et al., 1995; Williams-Abbott et al., 1997; Ware, 2005; Z. Kaymakcalan, unpublished data, 2006). The *in vivo* relevance and functional consequences of this binding remain to be elucidated.

3.3. Pharmacokinetics

The 3 licensed TNF antagonists, infliximab, etanercept and adalimumab, differ in their dosing regimens, pharmacokinetic properties and immunogenicity (Table 1), all of which may affect the efficacy and safety of these drugs. The pharmacokinetic profiles of these 3 drugs were reviewed recently (Nestorov, 2005a, 2005b; Furst et al., 2006). Three areas of note with regard to the pharmacokinetic differences are intravenous versus subcutaneous dosing regimens, drug half-lives in serum and the peak–trough ratios of the serum drug concentrations. According to the therapeutic window concept (Nestorov, 2005a), the steady-state range of serum or tissue drug concentrations should be adequate for the drug to neutralize surplus TNF, but not so high as to threaten safety because of neutralization of homeostatic concentrations of TNF required for host defense, or so low as to impair efficacy as a result of suboptimal neutralization of TNF. Thus, there is a logical preference for a low peak–trough ratio of serum drug concentration to minimize safety/efficacy risks outside the therapeutic window. A representation of the simulated steady-state serum concentrations of infliximab, etanercept and adalimumab based on a model developed using published pharmacokinetic parameters and dosing practice is shown in Fig. 5. The wide fluctuation in serum concentrations of infliximab reflects the fact that it is dosed in relatively large intravenous boluses and contrasts with the relatively constant concentrations of etanercept or adalimumab, which are administered in smaller, subcutaneous doses.

Infliximab achieves high initial concentrations in serum that are 13- to 40-fold greater than the peak concentrations of adalimumab or etanercept at steady state (Nestorov, 2005a). When infliximab was administered to patients with RA at 3 mg/kg every 8 weeks and had achieved steady state, the peak drug concentrations were at least 50-fold greater than the median trough concentrations, and trough concentrations were undetectable in 22% to 30% of patients (St. Clair et al., 2002). Trough concentrations of infliximab given at different doses and frequencies correlated with clinical and pharmacodynamic (CRP reduction) responses (St. Clair et al., 2002; Bendtzen et al., 2006), suggesting that drug exposure and efficacy are compromised at low infliximab trough concentrations.

Etanercept has a shorter serum half-life and greater clearance rate than infliximab or adalimumab (Table 1) and is administered by subcutaneous injection most commonly at either 25 mg twice weekly or 50 mg weekly. Peak plasma concentrations are achieved 48 to 60 h after the injection of etanercept, indicating that it is absorbed slowly (Zhou, 2005). Etanercept reaches steady-state plasma concentrations after 2 to 4 weeks, at which time peak, trough and mean concentrations are comparable for the two dosing regimens (Zhou, 2005; Nestorov, 2005a). The volume of distribution at steady state is at least as high as for infliximab or adalimumab (Table 1), which implies comparable or greater tissue penetration for etanercept, assuming similar

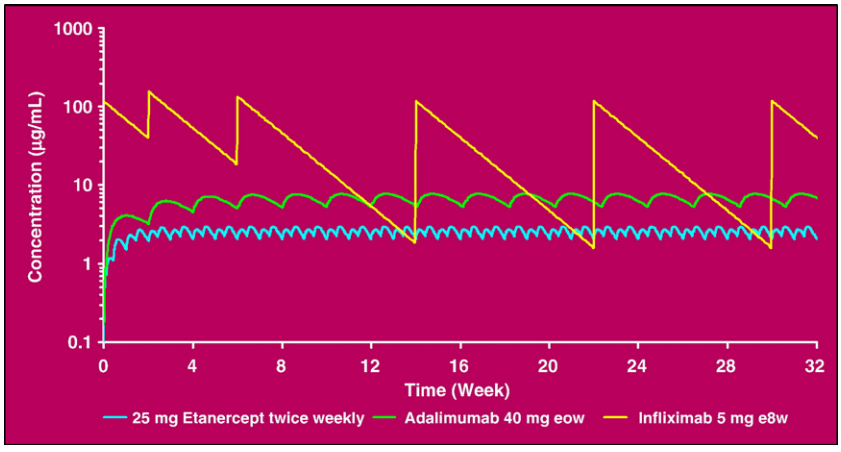

Fig. 5. A pharmacokinetics simulation of serum concentrations of infliximab, etanercept and adalimumab at steady state in patients with RA treated with each drug at the doses and schedules shown (Data on File, Abbott; St. Clair et al., 2002; Zhou et al., 2004).

distribution among tissues for each agent. In patients with RA treated with etanercept for 5 weeks, the concentration of etanercept in synovial fluid was comparable to that in the serum (Zhou, 2005). If the tissue penetration of etanercept in gut mucosa or skin is also high, this suggests that poor tissue penetration alone may not be the reason for the lack of efficacy of etanercept in Crohn's disease or its apparently lesser efficacy in psoriasis relative to infliximab or adalimumab. Rather, these differences in efficacy may be explained by other factors, including the peak and trough concentrations of drug in the target tissues, drug potencies and structure-based mechanisms of action.

Adalimumab given 40 mg subcutaneously every other week produces steady-state serum trough concentrations of 4 to 8 μg/mL, which are 3 to 7 times greater than the clinically effective serum concentrations (0.8–1.4 μg/mL) (Granneman et al., 2003). These data suggest that the mean steady-state concentrations of adalimumab are within the therapeutic window. As illustrated in Fig. 5, the steady-state serum concentrations of adalimumab are several-fold greater than for etanercept. Tissue penetration of adalimumab in patients with RA appears to be high, as concentrations of adalimumab in synovial fluid range from 31% to 96% of those in serum (Humira PI, 2007) and radioscintography demonstrated that adalimumab rapidly localized in joint tissues after intravenous administration (Barrera et al., 2003).

3.4. Tumor necrosis factor antagonist–tumor necrosis factor complexes

Complexes of TNF-antagonist drugs with sTNF (and LTα3 with etanercept) can vary widely in their composition and stability, depending on the drug and the relative concentrations of drug and TNF. The dynamics of drug distribution throughout the body and drug interaction with TNF or LT in various tissues are influenced by the nature of these complexes. Typically, antigen–antibody complexes are cleared by a combination of Fc receptor–dependent mechanisms in the reticuloendothelial system in spleen and liver, FcRn-dependent intracellular degradation and filtration through the kidney (Lobo et al., 2004). The amount of circulating TNF increases up to 7-fold in a dosage-related manner after administration of TNF antagonists, although most of the TNF is in the form of circulating complexes that lack TNF bioactivity (Suffredini et al., 1995; Charles et al., 1999; Barrera et al., 2001). For example, serum TNF concentrations of 15 pg/mL at baseline increased to 35 pg/mL and 105 pg/mL 7 days after administration of 1 mg/kg or 10 mg/kg of infliximab, respectively, to patients with RA (Charles et al., 1999). These observations have given rise to the concept of the TNF-carrier effect of these drugs. The rates of clearance of TNF antagonist–TNF complexes may differ for etanercept as compared with infliximab or adalimumab. One study comparing the clearance of etanercept, infliximab and adalimumab complexes in transgenic mice expressing human TNF showed that etanercept–TNF complexes circulated for weeks, whereas infliximab–TNF and adalimumab–TNF complexes were cleared quickly (Kaymakcalan et al., 2003). Similarly, etanercept–TNF complexes persisted for long periods in humans treated with etanercept (Suffredini et al., 1995; Fisher et al., 1996; Wee et al., 1997).

TNF antagonist–TNF complexes are not static but constantly bind and release bioactive TNF at a rate determined by the on-rate and off-rate of the drug for TNF, the relative concentrations of drug and TNF, and the stoichiometry of the complexes. Studies comparing the *in vitro* stability of infliximab–sTNF complexes with etanercept–sTNF complexes showed the latter to be relatively unstable, as they released bioactive sTNF more rapidly and in larger quantities (Scallon et al., 2002). Similar comparisons in mice have demonstrated the TNF-carrier effect of etanercept in vivo (Mohler et al., 1993) and the persistent release of bioactive TNF from complexes with a TNFR2-Fc protein in vivo (Evans et al., 1994). Patients with sepsis have very high concentrations of circulating sTNF, and a clinical trial of different doses of etanercept in patients with sepsis found a dose-related increase in mortality, possibly related to the TNF-carrier effect (Fisher et al., 1996). These studies raise the possibility that the TNF-carrier effect is more likely to occur with etanercept than with other TNF antagonists and may lead to redistribution of TNF from sites of inflammation to other tissues. The clinical consequences of this effect are currently unclear.

3.5. Immunogenicity

An important consideration for the use of any protein-based drug is its potential to induce antidrug antibodies, which may reduce efficacy of the drug or increase the potential for adverse effects. A recent review of the immunogenicity of a large number of therapeutic proteins, including infliximab, etanercept and adalimumab, confirmed that chimeric antibodies are generally more immunogenic than humanized or human antibodies (Hwang & Foote, 2005). Immunogenicity of human therapeutic antibodies resembles the formation of anti-idiotypic antibodies to unique determinants in endogenous antibodies, which is characteristic of the natural immune network (Jerne, 1974). The quantification and comparison of the immunogenicity of TNF antagonists are challenging, largely a result of variability in the design and sensitivities of the assays used to detect antibodies to TNF antagonists and interference in the assay by the drug itself (Bendtzen et al., 2006). Nevertheless, some general conclusions on the immunogenicity of infliximab, etanercept and adalimumab can be made, as reviewed recently (Anderson, 2005) and summarized in Table 1. As monotherapy, infliximab is the most immunogenic of the 3 drugs, and antidrug antibodies are reported in high percentages of patients with Crohn's disease and RA (Maini et al., 1998; Baert et al., 2003). In contrast, a low percentage of patients develop antidrug antibodies to etanercept or adalimumab (Anderson, 2005). High-dose tolerance is a well-known immunologic phenomenon and appears to explain the inverse dose-response in immunogenicity of TNF antagonists, as has been shown for infliximab, which was progressively less immunogenic at 1, 3 and 10 mg/kg (Maini et al., 1998). Concomitant use of any of the 3 drugs with methotrexate reduces their immunogenicities, probably owing to the immunosuppressive activity of methotrexate (Maini et al., 1998; Anderson, 2005; Bendtzen et al., 2006). Two recent studies of patients with RA treated with 3 mg/kg of infliximab found anti-infliximab antibodies in more than 40% of the patients, despite concomitant

methotrexate treatment (Wolbink et al., 2006). These findings differ from earlier studies using a different assay methodology that found that less than 10% of anti-infliximab–positive patients with RA were treated with 3 or 10 mg/kg of infliximab (Maini et al., 1998, 2004). The immunogenicity of certolizumab was studied in a Crohn's disease clinical trial (Schreiber et al., 2005). Twelve percent of patients developed antibodies to certolizumab, and there was evidence of increased clearance of certolizumab because of the antibody response (Schreiber et al., 2005).

Possible clinical consequences of the immunogenicity of TNF antagonists include acquired drug resistance and infusion- or injection-site reactions. A recent analysis of drug dosing and concomitant medication histories for more than 1,000 patients with RA concluded that use of infliximab required significant dosage increase and intensification of disease-modifying anti-rheumatic drug co-therapy over time compared with use of etanercept or adalimumab (Finckh et al., 2006). Several other studies in patients with RA treated with infliximab or adalimumab have clearly shown a correlation between the presence of antidrug antibodies and reductions in serum drug concentrations and clinical responses (Bendtzen et al., 2006; Wolbink et al., 2006; Bartelds et al., 2007). Antidrug antibodies can form multivalent immune complexes with the target drug, leading to rapid clearance and/or inactivation of the drug, thus requiring dosage escalation or concomitant therapy with another agent, such as methotrexate, to reduce the immunogenicity problem. Rapid clearance of immune complexes may occur regardless of whether the antidrug antibodies neutralize the TNF-binding activity of the drug or not. Clinical studies in patients with RA and Crohn's disease have confirmed the mechanistic correlation between presence of immune complexes of infliximab and antidrug antibodies and reduced clinical response to infliximab, accelerated clearance of infliximab, and development of infusion reactions to infliximab (Baert et al., 2003; van der Laken et al., 2006; Bendtzen et al., 2006; Wolbink et al., 2006). Some injection-site reactions may be caused by cytokine release or T cell–mediated hypersensitivity, so the contribution of immunogenicity-related mechanisms to these reactions needs to be clarified. In a study of injection-site reactions associated with etanercept therapy, immunohistochemical evidence was obtained for a delayed-type hypersensivity reaction mediated by $CD8^+$ T cells (Zeltser et al., 2001).

4. Mechanisms of action

The mechanisms of action of TNF antagonists have been intensively studied, particularly for infliximab and etanercept, but many questions remain unresolved. Some of the observed clinical differences between the TNF antagonists, such as the lack of efficacy of etanercept in Crohn's disease, sarcoidosis and Wegener's granulomatosis, could conceivably be because of differences in mechanism, pharmacokinetics, tissue distribution and/or potency. Possible mechanisms of TNF–antagonist action in patients are shown in Table 2 and Fig. 6 and are

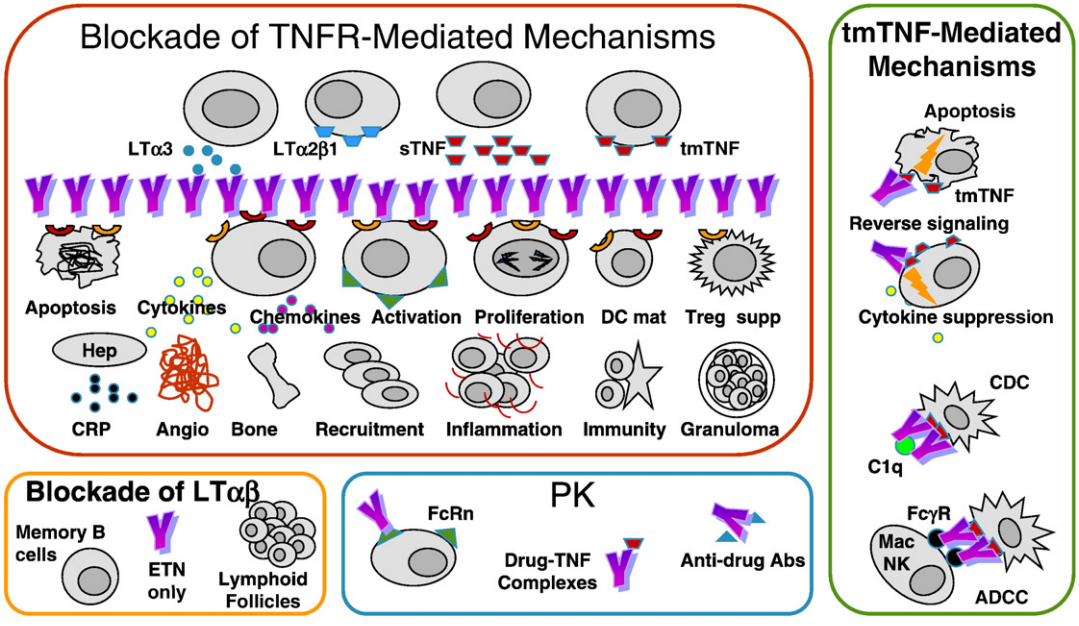

Fig. 6. Four categories of putative mechanisms of action of TNF antagonists are illustrated. The large panel illustrates the primary mechanisms of action, resulting from direct blocking of TNFR-mediated biologic activities. In these instances, the TNF antagonists bind to the cognate ligands (sTNF or tmTNF for all 5 TNF antagonists and additionally LTα3 and LTα2β1 for etanercept), thereby blocking their capacities to bind TNFR1 or TNFR2. The right panel illustrates several mechanisms induced by the binding of TNF antagonists to tmTNF, which can include reverse signaling via tmTNF or cytotoxicity of the tmTNF-bearing cell by CDC or ADCC. The small panel on the lower left illustrates 2 LTαβ-mediated mechanisms thought to be blocked by etanercept, the only TNF antagonist that binds LT family members. The lower center panel shows pharmacokinetic-related mechanisms that involve TNF antagonist binding to FcRn or forming complexes with sTNF or antidrug (TNF antagonist) antibodies. Denotes a TNF antagonist (infliximab, etanercept, adalimumab, certolizumab, golimumab). Denote TNFR1 and TNFR2. ETN = etanercept.

discussed in detail later. They generally fall into 2 categories: blockade of TNFR-mediated mechanisms and induction of tmTNF-mediated mechanisms (Fig. 6). It is likely that several of these mechanisms act in concert. The contribution of various mechanisms to drug efficacy remains an open question. For example, the relative roles of apoptosis and reversal of inflammation for reducing cellularity in rheumatoid synovial tissue during TNF-antagonist therapy are unclear.

4.1. Specificity and neutralization potency issues

Because both sTNF and tmTNF can be involved in inflammation and disease pathogenesis, the ideal TNF antagonist should block both ligands. All of the TNF antagonists listed in Table 1 neutralize sTNF with median inhibitory concentrations values in the nM range, as measured in various *in vitro* TNF bioassays. Because of the variability in cell lines, culture conditions and other factors in these bioassays, potency comparisons between TNF antagonists need to be determined in parallel. One study using both cytotoxicity and NF-κB reporter bioassays found etanercept to be slightly more potent than infliximab in neutralizing sTNF (Van den Brande et al., 2003). Another study found etanercept and certolizumab to have similar potencies that were greater than those for infliximab or adalimumab (Gramlick et al., 2006). Recent data suggest there may be differences between TNF antagonists in sTNF neutralization potency depending on the concentration of sTNF, which might have important implications *in vivo* (Kaymakcalan et al., 2006b). At high concentrations of sTNF (>2 ng/mL), as measured in inflamed tissues (Van Deventer, 1997; Choy & Panayi, 2001), infliximab, etanercept and adalimumab all neutralized sTNF with comparable potencies. However, at low concentrations of sTNF (~0.1 ng/mL), etanercept neutralized sTNF with more than 20-fold higher potency than did infliximab or adalimumab. Coupled with the pharmacokinetic data discussed previously (suggesting that the tissue penetration of etanercept is at least as great as that of infliximab or adalimumab), these results imply that when the TNF antagonist concentration is low, etanercept would more effectively neutralize TNF than would infliximab or adalimumab. The relevance of these findings to host defense and the safety of these drugs remains to be established.

Etanercept is unique among the 5 TNF antagonists in binding members of the LT family, namely soluble LTα3 and cell-surface LTα2β1. Because LTα3 exerts its biologic activities through TNFR1 and TNFR2, it is active in the same bioassays used to measure TNF activity. In these assays, etanercept and other sTNFR2:Fc constructs neutralize LTα3 and sTNF with similar potency (Z. Kaymakcalan, unpublished data, 2006; Scallon et al., 1995). Thus, it is possible that this polarized competition could leave some TNF un-neutralized if the LTα3 concentrations approximate or exceed the etanercept concentrations in a tissue. A second question raised by the LT specificity of etanercept is the functional impact of LTα2β1 binding. A recent study of the effect of TNF antagonists on B-cell dynamics in patients with RA found that etanercept, but not adalimumab, reduced the numbers of memory B cells in the peripheral blood of patients with RA (Anolik et al., 2005). Further, there was a paucity of follicular dendritic cell networks and germinal center structures in tonsil biopsies from the etanercept-treated patients. The authors concluded that these effects may be related to LTα inhibition by etanercept. These results are consistent with the known role of cell-associated LTαβ heterotrimers acting via the LTβR in lymphoid organ development as described previously, but it remains to be determined whether these effects actually resulted from inhibition of LTα2β1, LTα3, or even TNF by etanercept.

4.2. Reverse signaling

An emerging area of interest regarding the mechanisms of action of TNF antagonists centers on the functional outcomes of their interactions with tmTNF. Current evidence suggests that these drugs have dual functions and can act as antagonists by blocking tmTNF interactions with TNFR1/2, or as agonists by initiating reverse signaling, leading to apoptosis, cell activation or cytokine suppression. With regard to their tmTNF-antagonist activities, measured as inhibition of TNFR-mediated endothelial cell activation by tmTNF-transfected cells, infliximab, adalimumab and certolizumab had comparable activity when compared directly (Gramlick et al., 2006).

Reverse signaling through tmTNF has been shown *in vitro* to induce cytokine suppression and endotoxin resistance, suggesting that a similar mechanism may be operative in RA and other diseases during TNF antagonist therapy (Eissner et al., 2004). Endotoxin/LPS-activation of monocytes through TLR4 leads to the induction of cytokines, including TNF, IL-1β, IL-10 and IL-12. These cytokines are also produced by tmTNF-bearing macrophages in inflammatory sites. There is evidence of TLR pathway involvement in their induction (van Lent et al., 2006). Binding of TNF antagonists to tmTNF initiates reverse-signaling pathways that intersect with those induced by LPS, zymosan or other stimuli. Simultaneous engagement of these signaling pathways results in suppression of cytokine production, possibly by exhaustion of common signaling components (Eissner et al., 2004). The novel intramembrane proteases SPPL2a and SPPL2b were recently identified and were shown to be necessary for tmTNF-mediated reverse signaling in IL-12 production by human dendritic cells (Friedmann et al., 2006). It is possible that some reverse-signaling pathways initiated by TNF antagonists involve the activation of these proteases.

Although evidence from several studies establishes that all of the TNF antagonists bind to tmTNF, there is evidence for differential induction of cytokine suppression through reverse signaling (Scallon et al., 2002; Mitoma et al., 2004, 2005; Shen et al., 2005; Nesbitt et al., 2006). One study with a human monocytic cell line found that infliximab, but not etanercept, suppressed LPS-induced TNF and IL-1β production (Kirchner et al., 2004). Likewise, adalimumab and infliximab inhibited LPS-induced IL-10 and IL-12 production by human monocytes, whereas etanercept did not (Shen et al., 2005). Similar *in vitro* studies showed complete suppression of TNF and IL-1β production by infliximab, adalimumab and certolizumab, but only partial suppression by etanercept (Nesbitt et al., 2006).

However, both infliximab and etanercept suppressed the secretion of LPS-induced endothelial cell apoptotic factor (Death Factor X), suggesting that etanercept triggers at least 1 tmTNF reverse-signaling pathway (Kirchner et al., 2004). It is possible that tmTNF reverse-signaling mechanisms are operative in Crohn's disease and that bacterial antigens, such as LPS, may play a role in cytokine production and disease pathogenesis. Several analagous *in vivo* studies demonstrated an elevation in immunoreactive serum TNF concentrations in etanercept-treated humans (Suffredini et al., 1995) and mice (Mohler et al., 1993; Evans et al., 1994), possibly reflecting a lack of suppression of LPS-induced TNF production, coupled with the TNF-carrier effect of etanercept. Paradoxically, in LPS-treated humans, low-dosage etanercept suppressed IL-1β, IL-8, G-CSF and other cytokines, as well as cortisol and epinephrine, but high-dosage etanercept did not (Suffredini et al., 1995).

Reverse signaling through tmTNF in activated human T cells or tmTNF-transfected human T-cell lines can lead to the induction of the adhesion molecule E-selectin (Harashima et al., 2001; Mitoma et al., 2005). E-selectin expression on T cells is involved in the initial steps of cell adhesion to endothelium at sites of inflammation. The tmTNF-mediated expression of E-selectin in T cells was induced by both infliximab and etanercept (Mitoma et al., 2005). However, in the same studies, infliximab — but not etanercept — suppressed T-cell proliferation by inducing G0/G1 cell cycle arrest (Mitoma et al., 2005). Cross-linking of tmTNF-bound etanercept with anti-Fc antibodies increased the suppression of cell proliferation from a minimal degree to one that was approximately half that observed with infliximab (Mitoma et al., 2005). These *in vitro* results suggest that in patients, monovalent binding of etanercept to tmTNF might induce reverse signals that can induce E-selectin but not suppress cell proliferation. However, cross-linking of cell surface tmTNF by infliximab alone, or by etanercept plus rheumatoid factor (an endogenous anti-Fc antibody), may induce reverse signals of sufficient strength to suppress cell proliferation (see Section 3.2). Alternatively, there may be separate pathways of reverse signaling leading to E-selectin expression versus suppression of cell proliferation. Perhaps only the latter requires tmTNF cross-linking or aggregation. It is also possible that initiation of some reverse-signaling pathways, but not others, is mediated by regulated intramembrane proteolysis of tmTNF by SPPL2a/b. Nothing has been reported on the role of membrane forms of LTαβ in reverse signaling. In summary, reverse signaling initiated by TNF antagonists through tmTNF is emerging as a mechanism that may be important to apoptosis, cytokine suppression and/or other cellular events, but further investigation is needed to fully elucidate the molecular pathways and clinical significance of reverse signaling.

4.3. Apoptosis

Apoptosis, or programmed cell death, is a natural physiologic process in the regulation of cellular turnover, immune tolerance in normal states and the regulation of immune responses to pathogens. In chronic inflammatory diseases, such as Crohn's disease or RA, there is evidence that the frequency of apoptosis is subnormal in the inflamed tissues and that defective apoptosis may be a cause of inflammation (Sands, 2004; Tak, 2005). At a cellular level, apoptosis can be induced by metabolic perturbations, such as growth factor deprivation, or by ligand binding to receptors bearing cytoplasmic death domains, including TNFR1 (Monastra et al., 1996). Cellular perturbations from ultraviolet irradiation and chemotherapy lead to apoptosis in dermatologic diseases, such as psoriasis (Caffieri et al., 2007). Many different stimuli initiate apoptosis-signaling pathways that involve the activation of caspases 8, 9 and 3 and the release of cytochrome C from mitochondria. Both mitochondrial and extramitochondrial apoptosis-signalling pathways can induce DNA fragmentation and cell death. Readouts for apoptosis include staining for DNA fragmentation with annexin-V or terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end labeling (TUNEL) and measurement of activated caspase 3. It should be noted that TUNEL-positive cells exhibit DNA damage, but their detection in tissues is not necessarily evidence of apoptosis (Tak and Firestein, 1999; Smeets et al., 2003).

Whether stimulation of cells leads to activation or apoptosis depends on a complex interplay between the metabolic status and microenvironment of the cell. For example, sTNF generally stimulates cells through TNFR1 and the NF-κB pathway; however, if metabolism of the cell is altered by viral infection, the apoptosis pathway is favored (Ware, 2005). These pathways are influenced by the balance of intracellular proapoptotic factors, such as B-cell CLL/lymphoma 2-associated X protein (Bax), and antiapoptotic factors, such as B-cell CLL/lymphoma 2 (Bcl-2). Because tmTNF molecule has a cytoplasmic domain, it can induce apoptosis by acting either as a ligand for TNFRs on other cells (Monastra et al., 1996) or by acting as a receptor that transmits a reverse signal into the tmTNF-bearing cell (Eissner et al., 2000). Conceivably, both events may occur simultaneously. Thus, TNF antagonists might have a dual role in either blocking or inducing tmTNF-mediated apoptosis.

A common observation in the treatment of immune-mediated inflammatory diseases with TNF antagonists is the rapid reduction in cellularity at the site of inflammation, for which the relative roles of apoptosis, cytotoxicity, reduced cell influx and chemokine-mediated cell efflux are still being elucidated. Several studies have directly addressed the question of whether TNF antagonists induce apoptosis *in vivo* by measuring the frequencies of apoptotic cells in peripheral blood and biopsy samples from patients with Crohn's disease, RA or psoriasis following treatment with TNF antagonists. A study of 10 patients with Crohn's disease used TUNEL staining of lamina propria biopsy samples taken before and 24 h after treatment with infliximab at 5 mg/kg (ten Hove et al., 2002). The frequency of TUNEL-positive cells increased 4-fold and the majority of these cells were $CD3^+$ T cells. No changes were seen in the numbers of circulating apoptotic T cells, as measured by the Bax:Bcl-2 ratio. A similar study of 10 patients with Crohn's disease found a 5-fold increase in the percentages of TUNEL-positive lamina propria T cells 4 weeks after the last of 3 infusions of infliximab at 5 mg/kg (Di Sabatino et al., 2004).

Another study of 5 patients with Crohn's disease showed a 2-fold increase in the percentages of annexin-V–positive peripheral blood monocytes 4 h after an infusion of infliximab at 5 mg/kg (Lugering et al., 2001). Thus, 3 separate studies have demonstrated increased numbers of apoptotic lamina propria T cells or blood monocytes from patients with Crohn's disease 4 h to 4 weeks after infliximab treatment. Apoptosis observed 4 h or 24 h after infliximab treatment might reflect a direct drug effect, possibly via reverse signaling. Apoptosis observed 4 weeks after infliximab treatment is more likely a result of disease amelioration, although a direct drug effect cannot be excluded.

Two studies have measured apoptosis in patients with RA treated with infliximab or etanercept. One study examined TUNEL-positive cells in arthroscopic synovial biopsies taken before and 48 h after treatment of 12 patients with RA with infliximab at 3 mg/kg and 12 patients who received placebo (Smeets et al., 2003). A third arthroscopic synovial biopsy was obtained 28 days after the first infusion. Despite a significant reduction in the numbers of synovial macrophages at 48 h and 28 days post infliximab, there were no increases in TUNEL-positive cells at either time point. Furthermore, there was no increase in the number of apoptotic cells in the synovial tissue as determined by electron microscopy. In a study of RA synovial biopsies obtained from 9 patients treated with infliximab at 3 mg/kg at 0, 2 and 6 weeks and 12 patients treated with etanercept at 25 mg twice weekly, 2- to 5-fold increases in the percentages of TUNEL-positive or active caspase-3–positive cells were observed for both infliximab and etanercept after 8 weeks of therapy (Catrina et al., 2005). In addition, clinical responders to either infliximab or etanercept had a greater increase in synovial apoptosis than did clinical nonresponders. Furthermore, increased concentrations of apoptotic monocyte/macrophages were seen in RA synovium 8 weeks after administration of infliximab or etanercept (Catrina et al., 2005). However this effect could be secondary to the decrease in synovial inflammation rather than a direct pro-apoptotic effect of TNF blockade. The apparent differences between these 2 RA studies with infliximab may reflect differences between the groups of patients, the methodologies for measuring apoptosis or the time points of evaluation.

Apoptosis has also been studied in patients with psoriasis. One study, using TUNEL and caspase-3 staining in psoriatic lesional skin and synovial biopsies from patients with psoriatic arthritis, found no increases in the numbers of apoptopic cells at either site 48 h after the initiation of infliximab therapy (Goedkoop et al., 2004a). A recent report on etanercept in psoriasis has introduced the dendritic cell into the apoptosis arena (Malaviya et al., 2006a). In this study, psoriatic plaque biopsy specimens from 10 patients with psoriasis treated with etanercept, 25 mg twice weekly, were obtained at multiple time points over 24 weeks. Increases in the absolute number and percentages of activated caspase-3–positive myeloid dendritic cells were seen in the dermis of clinical responders to etanercept but not in clinical nonresponders. The largest apoptotic response was seen in the earliest biopsy, 1 month after the initiation of etanercept treatment, with lesser elevations also seen at 3 and 6 months. Other recent studies in patients with psoriasis have observed caspase-independent apoptosis of lesional plaque keratinocytes (Kruger-Krasagakis et al., 2006), lesional plaque T cells and dendritic cells, as well as peripheral blood T cells and monocytes (Malaviya et al., 2006b) following infliximab administration.

A number of *in vitro* studies have examined the mechanistic relationship between apoptosis and reverse signaling via tmTNF. Most used monocytes or T cells from healthy human donors or patients with Crohn's disease or RA. Typically, cells were isolated from blood or tissues, cultured for several days with various activating agents and then treated with TNF antagonists at various concentrations. Flow cytometry measurements were generally done in parallel to verify that the agents bound to tmTNF on the cells in question. Infliximab has been found to induce apoptosis *in vitro* in peripheral blood monocytes from healthy individuals and patients with Crohn's disease (Lugering et al., 2001). Similarly, infliximab induced apoptosis in lamina propria T cells from patients with Crohn's disease and, to a lesser extent, normal blood T cells (Di Sabatino et al., 2004), but another study found no apoptosis of RA synovial fluid T cells (Catrina et al., 2005). Studies comparing infliximab to adalimumab demonstrated that both agents induced apoptosis in normal blood monocytes (Shen et al., 2005), human acute monocytic leukemia cell line (THP-1) (Shen et al., 2006) and normal blood T cells (Fossati & Nesbitt, 2005a). Discordant results have been obtained in studies comparing etanercept with infliximab or adalimumab. In some studies, etanercept failed to induce apoptosis of normal blood monocytes (Shen et al., 2005), normal blood T cells (Van den Brande et al., 2003), a TNF-transfected Jurkat human T-cell line (Mitoma et al., 2005), lamina propria T cells from patients with Crohn's disease (Van den Brande et al., 2003) or synovial fluid T cells from patients with RA (Catrina et al., 2005), despite demonstrated binding of etanercept to tmTNF in most of these studies. However, other studies found that etanercept induced apoptosis of synovial fluid monocytes/macrophages from patients with RA and, to a lesser degree, normal blood monocytes (Catrina et al., 2005) and normal blood T cells (Fossati & Nesbitt, 2005a). The latter study also compared certolizumab to the other agents and found that certolizumab did not induce apoptosis in activated normal blood T cells, despite a substantial degree of binding to tmTNF, whereas infliximab, adalimumab and etanercept each induced apoptosis with similar potency. The failure of certolizumab to induce apoptosis via tmTNF is likely related to its inability to crosslink tmTNF as a monovalent Fab[1] fragment, but other factors, such as epitope specificity, may also be important. In this regard, the monovalent Fab fragment of infliximab has been reported to induce apoptosis *in vitro* with equal potency as the divalent parent molecule (Lugering et al., 2001). Cross-linking of cell-bound etanercept with anti-Fcγ antibodies suppressed proliferation and induced some apoptosis of tmTNF-transfected Jurkat T cells, whereas etanercept alone had little effect (Mitoma et al., 2005). These results with anti-Fcγ antibodies are clinically relevant because similar phenomena could occur in patients who are positive for rheumatoid factors, which are autoantibodies against the Fc region of autologous IgG. The collective apoptosis results are also particularly interesting in

light of the clinical efficacy of certolizumab, infliximab and adalimumab, but not etanercept, in Crohn's disease. The discordance between the efficacy of the TNF antagonists in Crohn's disease and their respective abilities to induce apoptosis of activated T cells *in vitro* suggests that apoptosis may not be important for efficacy in Crohn's disease.

The scientific basis for the disparities in these *in vitro* findings, especially with etanercept, is a matter of speculation. In addition to laboratory-to-laboratory and donor-to-donor variability, it is possible that the differences in the complex cell culture and assay protocols explain whether or not apoptosis is observed. These studies differ in the disease status of the donors, tissue sources of the cells, cell types being studied, cell preparation protocols, cell culture durations, cell stimuli, stimulation protocols, concentrations of TNF antagonists and apoptosis readout assays. These technical differences undoubtedly result in different degrees and kinetics of expression of tmTNF on the cells in question or in the activation of TACE, SPPL2 or other proteases that may modulate tmTNF expression (see Sections 2.3 and 3.2). Moreover, the metabolic states and microenvironments of the cells are likely to differ from one study to another and these factors are known to influence the sensitivity of the cells to the initiation of apoptosis pathways (Ware, 2005). In addition to the qualitative differences between studies outlined previously, the percentages of apoptotic cells vary widely, even for similar cell populations. For example, one *in vitro* study found that infliximab induced 10% apoptosis of normal blood monocytes (Lugering et al., 2001), whereas another study found that infliximab induced 70% apoptosis of normal blood monocytes (Shen et al., 2005). The times of assessment of apoptosis after the addition of TNF antagonists vary from 2 h to 72 h between the various in vitro studies. The concentrations of TNF antagonists also vary widely, and some studies used a single concentration, such as 10 μg/mL, that may exceed the pharmacologic concentrations of some of the agents in question (Fig. 5). Furthermore, several studies showed a clear concentration dependence for the induction of apoptosis by TNF antagonists. A recent study demonstrated concentration dependence of infliximab- and adalimumab-induced apoptosis of normal blood T cells (Chaudhary et al., 2006). Etanercept also induced apoptosis at 10 μg/mL, but not at 1.0 or 0.1 μg/mL. In another study, 1.0 μg/mL was the optimal concentration for etanercept to induce apoptosis of RA synovial fluid monocytes/macrophages (Catrina et al., 2005). Concentration dependence of apoptosis may be clinically relevant with regard to the therapeutic window concept, especially with the wide peak–trough fluctuations observed with administration of infliximab. Further studies are needed to investigate the incidence and role of apoptosis in vivo in TNF antagonist therapy and its relationship to *in vivo* drug concentrations and to the methodologic differences among the many *in vitro* studies. Most importantly, the relevance of apoptosis to the efficacy and safety of TNF antagonists in Crohn's disease, RA and psoriasis is still an open question.

4.4. Inflammation

The rationale for the first clinical study of a TNF antagonist in RA was based on the role of TNF in a pro-inflammatory cytokine cascade (Brennan et al., 1989). Indeed, subsequent analyses of the effects that TNF antagonists have on the cells and molecular mediators of inflammation have confirmed and extended this concept. Many of the hallmarks of chronic inflammation, such as leukocyte recruitment, activation and proliferation, and the production of inflammatory mediators, are reduced by TNF antagonist therapy and thus have had their mechanistic link to TNF empirically confirmed. As more than 100 cytokines and chemokines have been identified, many of them studied in TNF antagonist–treated patients, the concept has emerged that TNF is at the top of a proinflammatory cytokine cascade (Feldmann, 2002). However, as the complex interconnectivity and dynamics of cytokine biology have come to be better understood, the biologic relationships between cytokines might better be visualized as a network within a cascade. Cytokines can act independently, additively, or synergistically within this network, as exemplified by the various roles of TNF and IL-17 in synovial inflammation and joint destruction (Koenders et al., 2006). In any case, studies with TNF antagonists have conclusively shown that TNF plays a central role in the proinflammatory cytokine network (Fig. 3).

Many of the published studies on the effects of TNF antagonists on inflammation in humans have been conducted with infliximab-treated patients with RA (reviewed in Feldmann & Maini, 2001; Maini & Feldmann, 2002), and similar results have been observed with etanercept and adalimumab. Infliximab therapy has led to reductions in RA synovial tissue expression of IL-6, IL-8, granulocyte macrophage colony-stimulating factor, macrophage chemoattractant protein-1 (MCP-1), IL-1β, TNF and vascular endothelial growth factor (VEGF), as well as reductions in concentrations of these cytokines in synovial fluid or serum. Dramatic reduction in serum IL-6 concentrations occurred within 1 day of infliximab therapy, suggesting a direct effect of TNF neutralization (Charles et al., 1999). A similar reduction in the serum IL-6 concentration was seen within 24 h of adalimumab administration, as was a reduction in peripheral blood cell systemic IL-1β mRNA concentrations, but this was not accompanied by a decrease in synovial IL-1β expression (Ulfgren et al., 2000). Serum IL-18 concentrations were significantly reduced within 2 weeks of and up to 8 weeks after infliximab administration, but no changes in serum IL-12 or IL-13 concentrations were detected over this period (Pittoni et al., 2002). Downregulation of synovial TNF expression was seen 2 weeks after infliximab administration (Ulfgren et al., 2000). Not all of the cytokines or other factors suppressed by TNF antagonists are pro-inflammatory. Both infliximab and adalimumab rapidly reduced the serum concentrations of IL-1ra, a natural antagonist of IL-1 bioactivity, as well as sTNFR1 and sTNFR2, possibly as a consequence of reduced TNF or IL-1β concentrations (Charles et al., 1999; Barrera et al., 2001).

Acute-phase reactants, such as CRP, serum amyloid A and fibrinogen, are considered the hallmarks of systemic inflammation. CRP and serum amyloid A production by hepatocytes is predominantly regulated by IL-6. Simultaneous reductions in IL-6 and CRP have been seen in infliximab-treated (Elliott et al., 1993) and adalimumab-treated patients with RA (Barrera

et al., 2001; den Broeder et al., 2002). Strong correlations between the serum concentrations of IL-6, CRP and serum amyloid A were observed in infliximab-treated patients with RA (Charles et al., 1999). Significant reductions in CRP concentrations have also been seen with etanercept in RA (Moreland et al., 1997; Catrina et al., 2002), with etanercept in spondyloartropathies (Kruithof et al., 2005) and with infliximab (Van Deventer, 1997), adalimumab (Hanauer et al., 2006) or certolizumab (Schreiber et al., 2005) in Crohn's disease. Neuroendocrine axes involving the hypothalamus, pituitary gland, adrenal gland and liver, as well as metabolic responses resulting in dyslipidemia and metabolic syndrome, are all manifestations of a chronic-phase response and are normalized by TNF-antagonist therapy of immune-mediated inflammatory diseases (Bengmark, 2004; Straub et al., 2006).

VEGF is the predominant cytokine regulating angiogenesis, the formation of new blood vessels from endothelial cell precursor cells. Angiogenesis is a prominent feature in RA, psoriatic arthritis, psoriasis and other chronic inflammatory diseases in which increased blood vessel density facilitates cell trafficking in and out of the inflamed tissue. Elevated VEGF expression was seen in RA synovial tissue (Fraser et al., 2001) and psoriatic skin (Dvorak et al., 1995), and elevated serum VEGF concentrations were seen in patients with RA (Koch, 2003). Serum VEGF concentrations were reduced in patients with RA 1 to 4 weeks following infliximab and 3 to 12 weeks following etanercept administration (Paleolog et al., 1998; Agarwal et al., 2004). Markers of endothelium or neovasculature showed reduced vascularity in the synovial tissue of infliximab- or etanercept-treated patients with RA (Maini et al., 1999; Terslev et al., 2003). Vascularity, and expression of the neovascularization marker $\alpha_v\beta_3$ integrin were reduced in both psoriatic lesional skin and the synovium of patients with psoriatic arthritis 4 weeks after the initiation of infliximab in combination with stable methotrexate therapy (Goedkoop et al., 2004b). Likewise, other recent biopsy studies of patients with psoriasis demonstrated that angiogenic factors, such as angiopoietin-2, VEGF and metalloproteinase-9 (MMP-9) were reduced after infliximab treatment (Cordiali-Fei et al., 2006; Markham et al., 2006).

A common feature of the approved TNF antagonists is their ability to reduce the cellularity of inflamed tissue in a variety of diseases. In patients with RA, infliximab therapy was followed by a reduction in the cellularity of the inflamed synovial tissue that paralleled the rapid reduction in swollen joints. Significant reductions in the number of intimal and sublining macrophages and less pronounced reductions of plasma cells and T cells were seen 48 h after an infliximab infusion in patients with RA (Smeets et al., 2003). Likewise, 48 h after infusing infliximab in patients with psoriatic arthritis, reductions were seen in the frequency of synovial T cells and synovial sublining macrophages and of epidermal T cells in psoriatic lesions (Goedkoop, 2004a). In this study, no concomittant increases in the frequency of TUNEL-positive or caspase-3–positive cells were seen in the epidermis or synovium. In a more recent study of rheumatoid synovium, apoptosis induction was not observed during the first 24 h after infliximab infusion (Paul-Peter Tak, unpublished data). Thus, the rapid reduction in T cells and macrophages could not be explained by induction of apoptosis at the sites of inflammation. Etanercept has also been shown to induce a rapid and sustained reduction in global cellular infiltration, including macrophages and T cells, in peripheral joint synovitis in patients with spondyloarthropathies (Kruithof et al., 2005). Rapid reduction in cellularity might best be explained by a reduction in inflammatory cell recruitment, a process that involves adhesion molecule expression on endothelial cells and chemokine-mediated migration of leukocytes. Following infliximab treatment in RA, synovial-tissue expression of the adhesion molecules VCAM-1, ICAM-1 and E-selectin was reduced (Tak et al., 1996), as were serum concentrations of E-selectin and ICAM-1 (Paleolog et al., 1996). Likewise, in patients with psoriatic arthritis receiving stable methotrexate therapy, ICAM-1 and VCAM-1 expression in psoriatic lesional skin and ICAM-1 expression in the synovial sublining were reduced 4 weeks after an infliximab infusion (Goedkoop et al., 2004b). Direct evidence that infliximab reduced the migration and localization of cells in the joints of patients with RA was obtained using radiolabeled granulocytes. In parallel, the serum concentrations of the chemokines IL-8 and MCP-1 were also reduced (Taylor et al., 2000). Conceivably, TNF blockade also promotes cell egress from the synovial compartment through its effects on chemokines and adhesion molecules, as well as via possible effects on the lymphatic system, although this remains to be shown.

In Crohn's disease, reductions of the chemokines MCP-1, MIP-1α (macrophage inflammatory protein-1 alpha) and RANTES (regulated on activation, normal T-cell expressed and secreted) were detected by immunohistochemistry in the gut mucosa following infliximab therapy (Van Deventer, 1997). These chemokines are thought to play a critical role in recruiting macrophages and T cells to form granulomas, one of the histopathologic hallmarks of inflammation in Crohn's disease. In patients with psoriasis treated with etanercept, reduction in the chemokines IL-8, CXCL10 and CCL20 correlated with decreased infiltration of neutrophils, T cells and dendritic cells in plaques (Gottlieb et al., 2005). Likewise, reduced mononuclear cell infiltrates were seen in psoriatic lesions from adalimumab-treated patients with psoriasis, accompanied by a reversal of the reduced density of epidermal Langerhans cells (Gordon et al., 2005). Several biopsy studies of patients with psoriasis treated with infliximab also showed rapid reductions in the expression of cytokines, chemokines, adhesion molecules and activation markers (e.g. CD31) on endothelial cells, followed by reductions in cellularity (Goedkoop et al., 2004a; Markham et al., 2006). Thus, considerable evidence from treated patients suggests that TNF antagonists reduce inflammatory infiltrates by an effect on cell trafficking. Additional mechanisms that could contribute to the reduction of cellularity in inflamed tissues induced by TNF antagonists include cytotoxicity (discussed later), apoptosis and inhibition of cell proliferation.

4.5. Fc receptor interactions

The Fc region of IgG molecules binds to two distinct classes of Fc receptors: a family of Fcγ receptors found primarily on

leukocytes and FcRn, which is expressed primarily on endothelial cells and is involved in recycling of IgG. Binding of IgG Abs to FcRn is primarily mediated by residues 232–239 in the lower hinge region of the Fc portion of the IgG molecule (Radaev & Sun, 2002). Monomeric IgG binds to high-affinity FcγRI, whereas stable binding to the low-affinity receptors FcγRII or FcγRIII requires multimeric interactions, as can occur with IgG complexes. One form of FcγRII (FcγRIIb) mediates a suppressive signal, suggesting an immunoregulatory role. TNF antagonists infliximab, etanercept, adalimumab and golimumab all have an IgG1 Fc component via which they may bind to FcRn or other FcR, either as monomers or in complexes with TNF or antidrug antibodies. Engagement of Fc receptors by TNF antagonists or complexes could affect a number of cellular functions, including phagocytosis, ADCC, degranulation, cytokine release and regulation of antibody formation. FcγRIIIA is primarily expressed on monocytes, macrophages, NK cells and a subset of T cells. FcγRIIIA is polymorphic, with the F allele encoding receptors with lower affinity for Fcγ than the V allele. Recent studies found that the F/F genotype of FcγRIIIA correlated with positive clinical responses in patients with RA and psoriatic arthritis treated with either infliximab, etanercept or adalimumab (Tutuncu et al., 2005). As discussed previously, the relatively short half-life of etanercept suggests that etanercept may bind FcRn with lower affinity than infliximab, adalimumab or golimumab. This difference suggests that there may be conformational or steric differences in the Fc region of etanercept compared with IgG1 antibodies that result in rapid clearance due to poorer binding to FcRn. However, the relative affinities of etanercept, infliximab and adalimumab to FcRn or FcγRIIIA have not been reported. One study with the human monocytic cell line THP-1, bearing high-affinity FcγRI and low-affinity FcγRII receptors, found that etanercept bound to the cells with a 2- to 3-fold lower affinity than did infliximab or adalimumab in the absence of sTNF (Kohno et al., 2005a).

The avidity of binding of Fc-containing molecules to Fc receptors is a function of their intrinsic affinities and the degree of cross-linking by multimeric complexes. Complexes containing TNF and TNF antagonists can vary widely in composition and stoichiometry. In the previously mentioned THP-1 cell–binding study, complexes of infliximab–TNF and adalimumab–TNF, formed at approximately a 1:1 drug:TNF ratio, bound to cells to a much greater extent than did etanercept–TNF complexes and to a greater extent than the drugs alone, probably as a result of their larger size and ability to cross-link FcγR (Kohno et al., 2005b). However, in treated patients, the molar concentrations of TNF antagonists in plasma far exceed those of TNF, making it likely that small drug–TNF complexes predominate *in vivo*. In this regard, a recent study of the binding of adalimumab–TNF complexes prepared at 10:1 or 30:1 ratios found that these small complexes did not exhibit enhanced binding to Fc receptors on THP-1 cells (Kaymakcalan, 2006a). The results with THP-1 cells suggest that there may not be much difference in the ability of clinically relevant etanercept–TNF and infliximab/adalimumab–TNF complexes to bind to FcγRI/II receptors and that activation of myeloid cells by drug–TNF complexes through FcγR cross-linking would not be expected to occur at steady state in treated patients. In summary, infliximab, etanercept, adalimumab, golimumab and complexes of these agents with TNF are all likely to bind FcγR's and FcRn and to modulate a variety of cellular functions in vivo, but further research is needed to precisely define these interactions and possible differences among these agents.

4.6. Cytotoxicity

TNF antagonists may induce cytotoxicity of tmTNF-bearing cells by Fc-dependent mechanisms, including complement–dependent cytotoxicity (CDC) and ADCC. Complement activation by the classical pathway can be initiated by the binding of C1q to the C_H2 domain in the Fc region of cell-bound antibodies or Fc-fusion proteins. Cross-linking of cell-bound Fc-containing molecules by C1q can initiate the complement cascade, leading to formation of the membrane attack complex, pore formation and cell lysis. Macrophages and NK cells mediate ADCC by binding their FcγRs to the C_H2 domains of Fc-containing molecules bound to the target cell, thereby crosslinking the FcRs and inducing enzyme-mediated lysis of the target cell. Both CDC and ADCC require a threshold density level of target cell-bound Fc-containing molecules to trigger cell lysis.

One study compared complexes of TNF and infliximab, etanercept or adalimumab in their ability to bind the complement component C1q (Kohno et al., 2005a). It is possible that such complexes bound to a target cell via FcγRs could also bind C1q via free Fc components and trigger CDC of the target cell. None of the agents alone bound C1q, but infliximab–TNF and adalimumab–TNF complexes at an approximately 1:1 ratio bound C1q, whereas etanercept–TNF complexes did not. As discussed earlier, these types of large infliximab–TNF and adalimumab–TNF complexes are unlikely to occur or be clinically relevant. Studies of the potential of small, physiologically relevant drug–TNF complexes to fix complement or induce CDC have not been reported.

Only a few *in vitro* studies have been conducted to directly examine the CDC and ADCC potential of monomeric (uncomplexed) TNF antagonists. The Fc regions of infliximab, etanercept and adalimumab are all of the IgG1 isotype, which is capable of mediating CDC or ADCC. One study showed that infliximab bound to a human tmTNF-transfected murine cell line mediated CDC in the presence of rabbit complement and ADCC in the presence of human peripheral blood lymphocyte effector cells (Scallon et al., 1995). However, another study showed that infliximab, bound to activated human T cells, failed to mediate CDC in the presence of human complement or ADCC in the presence of human peripheral blood lymphocyte effector cells (Van den Brande et al., 2003). It is not clear whether these different results reflect the artificially high density of tmTNF on transfected cells, compared with normal cells, or some other methodologic differences between the studies. More recent studies have compared the CDC and ADCC activities of 4 TNF antagonists in parallel. Although each of the tested agents bound to a tmTNF-transfected human cell line, infliximab and adalimumab were somewhat more

active than etanercept in mediating CDC and ADCC, and certolizumab was inactive, as would be expected for an Fab' molecule (Fossati & Nesbitt, 2005a; Gramlick et al., 2006). No studies have examined the potential of etanercept to mediate CDC or ADCC of LTα2β1-bearing target cells. To date, there are no published reports describing the induction of CDC or ADCC by TNF antagonists in a patient. The widespread presence of multiple soluble and membrane-bound complement regulatory proteins, which are known to abrogate CDC of tumor-directed monoclonal antibodies in humans (Gelderman et al., 2005), raises the question as to whether CDC of tmTNF-bearing cells would be possible *in vivo*. Thus, while the limited *in vitro* data indicate that, under certain experimental conditions, infliximab, etanercept and adalimumab have the potential to mediate CDC and ADCC of tmTNF-bearing cells, there is no evidence that they induce CDC or ADCC in patients.

4.7. Immune regulation

Despite a vast amount of data supporting a role for TNF in lymphoid organization, innate immunity and adaptive immunity, there is relatively little direct evidence that TNF antagonists are immunosuppressive in clinical use. In fact, the overall effect of TNF antagonism on the immune system of patients appears to be one of normalizing immune homeostasis, with some evidence for immune enhancement (Maurice et al., 1999). Normalization of immune function by TNF antagonists involves down-regulation of the inflammation and immune reactions that drive RA, Crohn's disease, psoriasis and other diseases. Conversely, it appears that TNF antagonism can reverse some disease-related immune suppression and, in some cases, it enhances the immune response to foreign antigens. One exception to this generalization is the class effect of TNF antagonists whereby they appear to impair host defense against microbial infections, particularly reactivation of intracellular bacterial infections, which have been observed in a small percentage of treated patients.

A subset of T cells that is thought to play a central role in the suppression of autoreactivity and regulation of immune responses is the $CD4^+CD25^+$ Treg. The normal functions of Tregs, including the suppression of proinflammatory cytokine secretion by activated T cells and monocytes, are reduced in patients with RA compared with healthy individuals (Ehrenstein et al., 2004). Several recent clinical studies have provided evidence that TNF antagonists might normalize immune homeostasis by reversing compromised Treg function. The critical study that revealed this phenomenon demonstrated that infliximab treatment of patients with RA induced a significant increase in the number of circulating Tregs and a reversal of their anergic phenotype (Ehrenstein et al., 2004). Infliximab treatment was also found to restore the capacity of RA Tregs to inhibit cytokine production and convey a suppressive phenotype to conventional T cells (Ehrenstein et al., 2004). These findings were recently confirmed and extended in a study that directly demonstrated that TNF inhibits both naturally occurring and TGFβ1-induced Treg function and that infliximab treatment of patients with RA restored the ability of Tregs to suppress cytokine secretion and proliferation of $CD4^+$ T cells (Valencia et al., 2006). Likewise, the number and function of peripheral blood Tregs were increased from baseline in patients with RA following treatment with adalimumab (Vigna-Pérez et al., 2005). Because Tregs have been found to accumulate in the synovial fluid of patients with RA (Cao et al., 2003; Möttönen et al., 2005), they may play a role in regulating inflammatory effector cell function, as well as in the underlying mechanisms of tolerance and autoimmunity in RA. Reductions in anticitrullinated peptide/protein antibody (ACPA) and rheumatoid factor concentrations after infliximab (Alessandri et al., 2004), etanercept (Chen et al., 2006) and adalimumab (Atzeni et al., 2006) treatment of patients with RA is compatible with the TNF–Treg connection. It will be interesting to see whether TNF antagonists other than infliximab and adalimumab have effects on Treg numbers and function and whether these findings extend to other immune-mediated inflammatory diseases, such as Crohn's disease or psoriasis.

In light of the ability of TNF antagonists to suppress rheumatoid factor and anti-CCP autoantibody production, a somewhat paradoxical finding associated with the use of TNF antagonists has been their induction of antinuclear, anti-dsDNA and anticardiolipin antibodies in some patients with RA, ankylosing spondylitis and Crohn's disease (de Rycke et al., 2005; Atzeni et al., 2005b). These autoantibodies are generally of the IgM or IgA subclasses and are infrequently of the IgG subclass. Greater concentrations and frequencies of these autoantibodies, particularly the antinuclear antibodies, have been reported with infliximab than with etanercept or adalimumab (de Rycke et al., 2005; Atzeni et al., 2005b). Nevertheless, reversible lupus-like syndromes have been found infrequently in infliximab-treated patients (Charles et al., 2000). The possible mechanisms by which such autoantibodies may be induced by TNF antagonists include dysregulation of apoptosis and release of autoimmunogenic plasma nucleosomes from apoptotic cells or inhibition of a cytotoxic T-lymphocyte response that normally suppresses autoreactive B cells (Bendixen et al., 1984; D'Auria et al., 2004; Atzeni et al., 2006). The high antinuclear antibody response to infliximab may also relate to its unique pharmacokinetic profile (see Section 3.3), whereby high plasma concentrations shortly after infusion may trigger apoptosis of tmTNF-bearing cells and release of nucleosomes. In other studies, increased *ex vivo* peripheral blood T-cell reactivity to microbial antigens and autoantigens was seen after etanercept treatment of patients with RA (Berg et al., 2001). It is possible that these observations relate to reversal of TNF-mediated reduction in expression of the CD3ζ chain of the T cell–receptor complex (Isomaki et al., 2001), resulting in increased T-cell function (Cope et al., 1994), or to reversal of the T-cell suppressive function of activated monocytes (Berg et al., 2001). Differences between infliximab and etanercept in autoantibody suppression or induction may also relate to the dual role of TNF in immune tolerance, the role of LTαβ heterotrimers in the regulation of thymic tolerance, or differential effects on Treg subsets or T-cell–dependent antibody production (see Section 2.5).

There is a growing body of evidence to indicate the ways in which TNF antagonists modulate innate and adaptive immunity.

Each of the registered TNF antagonists has been assessed for immune-system effects in various ways, including *ex vivo* assays of cells from treated patients and assessments of the response of patients to vaccination with microbial antigens. The short-term effects of infliximab treatment of patients with RA included an increase in peripheral blood $CD4^+$ and $CD8^+$ T-cell frequencies on Day 1 and a decrease in monocyte frequencies on Day 7, with no significant change in B-cell or NK-cell frequencies (Lorenz et al., 1996). Another study demonstrated an increase in $CD4^+$ Th1 cells in the peripheral blood of patients with RA following infliximab treatment (Maurice et al., 1999). Functional changes included transient increases in proliferation and cytokine responsiveness of T cells to *ex vivo* CD28 costimulation, but not to CD3-mediated stimulation. These findings may relate to the separate observation that infliximab treatment rapidly reversed the deficient CD28 expression on $CD4^+$ T cells from patients with RA and restored responsiveness to CD28-mediated T-cell costimulation (Bryl et al., 2005). Similarly, deficient HLA-DR expression on antigen-presenting myeloid cells and the reduced capacity of these cells to stimulate T cells from patients with RA were reversed after infliximab treatment (Mueller et al., 2005). Chronic exposure of T cells to TNF can induce unresponsiveness to mitogen or antigen stimulation, and in patients with RA, this T-cell anergy was reversed after infliximab treatment (Cope et al., 1994). The humoral immune response to pneumococcal vaccination was generally preserved in infliximab-treated patients with RA, indicating no significant impact on T cell–dependent antibody production by B cells (Elkayam et al., 2004; Kapetanovic et al., 2006), which is consistent with previous studies showing preservation of some T cell–dependent humoral responses in TNF-deficient animals (Pasparakis et al., 1996).

A small clinical study was conducted to evaluate immune function in patients with RA treated with etanercept from 2 weeks to 6 months (Moreland et al., 2002). No significant or sustained differences between the etanercept and placebo groups were seen in the absolute numbers or percentages of peripheral blood leukocyte subsets as defined by phenotypic markers. No differences were seen in T-cell proliferative responses, DTH reactions, neutrophil function or serum Ig concentrations. In patients with psoriasis, etanercept reduced dendritic cell maturation and activation, leading to a reduction in T-cell activation (Gottlieb et al., 2005). Pneumococcal vaccination studies have shown diminished antibody responses in etanercept-treated patients with RA and ankylosing spondylitis (Elkayam et al., 2004), but not in those with psoriatic arthritis (Mease et al., 2004).

In a small substudy of a randomized trial of patients with RA, adalimumab treatment did not significantly alter the numbers of peripheral blood NK cells, monocytes/macrophages, B cells or major T-cell subsets (Kavanaugh et al., 2002). In addition, lymphocyte proliferation, DTH reactivity and antibody responses to pneumococcal antigen vaccination were not altered by adalimumab treatment. In a separate study, adalimumab did not diminish the capacity of patients with RA to develop protective antibody titers in response to influenza or pneumococcal vaccines (Kaine et al., 2007). Available data suggest that adalimumab-treated patients can be safely and effectively vaccinated against influenza and pneumococcal diseases. In summary, TNF antagonists can enhance or suppress immune function and autoantibody production to some extent, but on balance, they appear to normalize immune homeostasis by reversing the anergy in Treg cell function seen in RA patients. In general, administration of TNF antagonists has not led to major changes in immune cell subsets or in immune responses to vaccination, but further studies are needed to better assess their impact on immune function in patients.

4.8. Bone and cartilage destruction

Perhaps the most compelling clinical manifestation of the efficacy of TNF antagonists in patients with RA or psoriatic arthritis is the slowing, or even complete arrest, of bone destruction. Bone erosion is mediated by osteoclasts, which are multinucleated cells formed in the periphery from monocyte/macrophage-derived osteoclast precursors. The maturation and proliferation of osteoclast percursors, and the formation, activation and survival of osteoclasts, depend greatly on M-CSF and receptor activator of nuclear factor kappa-B ligand (RANKL), which exists both on the cell surface and as a soluble ligand (reviewed in Boyce et al., 2005; Schett et al., 2005; Walsh et al., 2005; Schett, 2006). The osteoclastogenic effects of RANKL are enhanced by TNF, IL-1, IL-6, IL-17 and other cytokines and are inhibited by IFNγ and IL-4 (O'Gradaigh et al., 2004; Mangashetti et al., 2005; Walsh et al., 2005; Palmqvist et al., 2006). RANKL is produced principally by osteoblasts in healthy individuals and is produced by activated T cells (Kotake et al., 2001) and fibroblast-like synoviocytes (Takayanagi et al., 2000) in patients with RA. The interaction of RANKL with its receptor RANK on osteoclasts is antagonized by osteoprotegerin (OPG), a soluble decoy/receptor specific for RANKL. In RA, an increase in the ratio of RANKL to OPG is thought to underly the increased osteoclast activity that causes erosions.

From the evidence of *in vivo* and *in vitro* experiments, it is now understood that TNF promotes osteoclastogenesis both directly, by acting on osteoclast precursors and osteoclasts, and indirectly, by promoting synovial inflammation and various associated osteoclastogenic factors (e.g.., RANKL, TNF, IL-1) (Boyce et al., 2005; Schett et al., 2005; Walsh et al., 2005). Transgenic mice that overexpress TNF develop a chronic inflammatory polyarthritis (Keffer et al., 1991) in which bone erosion, but not synovitis, is prevented by blockade of RANKL (Redlich et al., 2002a) or deletion of c-Fms, the receptor for M-CSF (Redlich et al., 2002b). TNF has been shown to upregulate expression of c-Fms in human bone marrow monocytes (Yao et al., 2006) and of M-CSF and RANK in mouse bone marrow stromal and mononuclear cells, respectively (Kitaura et al., 2005). These effects appear to precede RANKL-mediated steps in osteoclastogenesis.

Large clinical trials of patients with RA have clearly demonstrated that bone erosion and cartilage narrowing are prevented or greatly slowed in most patients treated with a TNF antagonist (Lipsky et al., 2000; Bathon et al., 2000; Keystone et al., 2004; Klareskog et al., 2004; St. Clair et al., 2004;

Breedveld et al., 2006). A study of patients with predominantly established RA (Klareskog et al., 2004) and a study of methotrexate-naïve patients with early RA (Breedveld et al., 2006) demonstrated a hierarchy of efficacy for preventing joint damage — the TNF antagonist plus methotrexate was more effective than the TNF antagonist alone, and methotrexate monotherapy was the least effective. In contrast, the ability of the 2 monotherapies to improve the signs and symptoms of arthritis were similar, both being less efficacious than combination therapy (Klareskog et al., 2004; Breedveld et al., 2006). Subanalyses indicated that treatment with a TNF antagonist plus methotrexate prevented radiographic progression with similar (excellent) efficacy in clinical responders and nonresponders, whereas, on average, methotrexate monotherapy led to markedly worse radiographic efficacy in clinical nonresponders (Lipsky et al., 2000; Genovese et al., 2005; Smolen et al., 2005; Landewé et al., 2006; Smolen et al., 2006). These observations are consistent with the scientific evidence for the direct roles of TNF in inflammatory joint destruction (described later) and support the concept that efficacy against clinical disease and joint damage can be dissociated from each other in patients treated with TNF antagonists. These findings also indicate that concomitantly used methotrexate has an important role in the optimal efficacy of TNF antagonists, the mechanism of which is not yet fully understood.

The mechanisms underlying the effects of TNF antagonists on bone destruction are beginning to be elucidated. Synovial macrophages in patients with RA have been shown to be osteoclast precursors (Danks et al., 2002). As a result, the rapid reduction in synovial macrophage numbers after TNF blockade (Smeets et al., 2003) may indirectly reduce the numbers of osteoclasts. In addition, osteoclast formation and activity *in vitro* were directly inhibited by infliximab and, to a lesser degree, by methotrexate, sulfasalazine and IL-4 in cocultures of human mononuclear cells and RA fibroblast-like synoviocytes (Lee et al., 2004). Furthermore, infliximab inhibited the expression of RANKL and RANK in these cultures, although the mechanism was not explored. Another *in vitro* study found that OPG mRNA expression was upregulated in cultured RA synovial fibroblasts and that OPG expression was increased by the addition of TNF to synovial fibroblasts from patients with RA or osteoarthritis (Kubota et al., 2004). Thus, there is *in vitro* evidence for several different mechanisms by which TNF can directly enhance osteoclast formation and activity, among which the regulation of RANKL activity is of key importance.

Studies of samples from patients with RA have revealed increased concentrations of RANKL in serum, synovial fluid or synovial tissue; however, reports differ as to whether OPG expression is increased or decreased in patients with RA (Feuerherm et al., 2001; Kotake et al., 2001; Ziolkowska et al., 2002; Catrina et al., 2006; Petit et al., 2006, Vis et al., 2006). These discrepancies may reflect the facts that 1) assays differ in their ability to accurately detect OPG bound to RANKL, 2) OPG concentrations vary between and within synovial tissues and 3) OPG activity and detection may be affected by its ability to bind TRAIL, a TNF-family member that shares homology with RANKL (Crotti et al., 2003; Haynes et al., 2003; Petit et al., 2006). An elevated ratio of RANKL to OPG in serum has been associated with greater radiographic progression in patients with recent-onset RA (Geusens et al., 2006). Moreover, TNF-antagonist therapy has been associated with reductions in 1) the ratio of RANKL to OPG in synovial tissue (Catrina et al., 2006), 2) the concentrations of RANKL and OPG in serum (Ziolkowska et al., 2002) and 3) the expression of DKK-1, a Wnt inhibitor that is induced by TNF and that suppresses osteoblast activity, promotes osteoclastogenesis and has increased serum concentrations in patients with RA (Diarra et al., 2007). Thus, it appears that TNF acts directly and indirectly in RA to shift the balance between RANKL and OPG in synovial/bone microenvironments to one that favors bone damage. TNF-antagonist therapy suppresses inflammatory damage to bone by reducing RANKL expression and restoring the balance with OPG.

Sites of bone formation are seen in ankylosing spondylitis and in psoriatic arthritis. However, in psoriatic arthritis, unlike ankylosing spondylitis, bone erosion is often a prominent feature of structural damage to joints. In psoriatic arthritis, osteoclast precursor numbers in blood were reduced by TNF-antagonist therapy (Ritchlin et al., 2003). In patients with ankylosing spondylitis, the serum concentrations of DKK-1 were lower than those seen in healthy individuals, whereas concentrations were above normal in patients with RA (Diarra et al., 2007). To date, it is not known whether the efficacy of infliximab, etanercept or adalimumab in these diseases is related to the balance between RANKL and OPG or to other mechanisms affecting osteoclasts and osteoblasts (Kavanaugh et al., 2006). However, the role of TNF may be different in diseases in which the arthritic bone disorder is predominantly proliferative, such as in ankylosing spondylitis compared with RA or some cases of psoriatic arthritis, where it is predominantly destructive.

Cartilage erosion is mediated by matrix metalloproteinases (MMPs) and other enzymes produced by synovial cells and chondrocytes when stimulated by cytokines, such as TNF, IL-1, IL-6, IL-17 and oncostatin M (Koshy et al., 2002). TNF and other cytokines synergize with IL-17 to induce MMP-1, MMP-3 and MMP-13 and the degradative release of proteoglycans and type II collagen (Koshy et al., 2002). Reductions in the concentrations of MMP-1, MMP-3 or their precursors following treatment with infliximab, etanercept or adalimumab indicate that TNF plays a critical role in the induction of these matrix-degrading enzymes in RA (Brennan et al., 1997; Catrina et al., 2002; Weinblatt et al., 2003). In a different study, adalimumab treatment of patients with RA led to reductions in the serum concentrations of MMP-1 and MMP-3, as well as markers of cartilage degradation, such as cartilage oligomeric matrix protein and gp-39 (den Broeder et al., 2002).

5. Other inhibitors of tumor necrosis factor action

In addition to the TNF antagonists described in this review, pharmacologic agents that either suppress TNF production or block its action have also been examined. For example, the phosphodiesterase inhibitor pentoxifylline inhibits TNF transcription (Doherty et al., 1991), whereas CNI-1493, a

tetravalent guanylhydrazone, inhibits TNF translation (Cohen et al., 1996). Thalidomide has been shown to inhibit TNF action by enhancing TNF mRNA degradation (Moreira et al., 1993). After promising results with thalidomide in a number of chronic diseases, including RA, thalidomide analogues were synthesized (e.g. lenalidomide). These analogues demonstrate both anti-inflammatory and antitumor effects and inhibit TNF production (Galustian et al., 2004).

A number of natural agents derived from fruits and vegetables have also been shown to function as TNF inhibitors. These inhibitors have been the subject of review by Paul et al. (2006). Based on their chemical structure, these natural products can be broadly categorized into polyphenolic and nonphenolic compounds. The polyphenolic compounds include the flavonoids, which are found in relatively high concentration in fruits, vegetables, nuts, and grains. Examples of flavonoids that modulate TNF signaling include naringenin (in grapefruit), resveratrol (in grapes), and quercetin (in garlic and onion). The nonphenolic compounds can be further categorized into alkaloids (e.g., lycorine found in several plant species including the bush lily *Clivia miniata*), terpenes (e.g., acanthotic acid isolated from *Acanthopanax koreanum*), fatty acids and their derivatives (e.g., 13-HOA isolated from linoleic acid by corn and rice lipoxygenase), sterols (e.g., guggulsterol isolated from *Commiphora mukul* gum), and retinoids (e.g., retinoic acid, a vitamin A metabolite). These natural compounds generally function by reducing TNF protein synthesis, reducing TNF release or inhibiting TNF mRNA expression. Additionally, some of these compounds have been found to interfere with various proinflammatory mediators, such as nitric oxide, cyclooxygenase-II, and prostaglandin E_2, and are thought to modulate TNF activity or production via these molecules.

Signal transduction pathways and transcription factors have also become targets in the management of chronic inflammatory diseases. Tas et al. (2005) published an extensive review of these pathways and described the progress to date in the development of small molecule inhibitors and gene therapy that target pathways involved in the pathogenesis of RA.

6. Summary and conclusions

The TNF antagonists infliximab, etanercept, adalimumab, certolizumab and golimumab are all effective therapeutic agents in RA that differ in their molecular structures and pharmacokinetic properties. Their strong clinical efficacy in RA and the potent neutralization of sTNF and tmTNF suggest that they achieve efficacy by preventing TNF from inducing TNFR-mediated cellular functions (Fig. 6). These functions include cell activation, cell proliferation, and cytokine and chemokine production, as well as the sequelae of these functions, such as cell recruitment, inflammation, immune regulation, angiogenesis, and extracellular matrix degradation. Supportive data for all of these mechanisms and for all of the TNF antagonists are incomplete, but the emerging picture is one in which TNF has a central role in a network of molecular and cellular events in the pathogenesis of RA. The rapid reduction in cellularity and inflammation in the rheumatoid synovium after TNF-antagonist therapy is likely the result of dampening of TNF-driven cytokine and chemokine cascades. Likewise, the longer-term reductions in cartilage and bone erosion are likely the result of dampening of TNF-driven production of matrix-degrading enzymes and osteoclastogenic factors, such as RANKL. When TNF antagonists bind to tmTNF, they inhibit its binding to TNFR on other cells and they may also induce direct effects upon the tmTNF-bearing cell. These effects include apoptosis, cytokine suppression, CDC and ADCC (Figs. 4 and 6). Ample evidence indicates that infliximab, etanercept, adalimumab and certolizumab can bind to tmTNF *in vitro*, but questions remain as to whether such binding has functional consequences in patients. Pharmacokinetic effects also may influence these functions, especially for infliximab, which achieves high concentrations in circulation following intravenous infusion. Infliximab may reach higher concentrations in tissue microenvironments than etanercept or adalimumab and, thereby, may have a greater opportunity to bind to tmTNF on cells and induce reverse signaling or FcR-mediated effects. It is particularly striking that certolizumab has been reported to not mediate apoptosis, CDC or ADCC *in vitro* but is clearly efficacious in patients with RA and Crohn's disease. However, all 4 agents have been found to directly suppress cytokine production, presumably by binding to tmTNF, regulating the proinflammatory cytokine cascade.

TNF plays a complex role in innate immunity and host defense, particularly against mycobacterial infections, and can both enhance or suppress adaptive immunity. In inflammatory diseases, chronic TNF exposure can suppress adaptive immunity and T-cell function. TNF antagonists have been associated with an increased risk of mycobacterial and other intracellular microbial infections, probably as a result of interference with innate immunity, but they have not been found to be broadly immunosuppressive. Less clear are the effects of TNF antagonists on host defense against malignancies, particularly lymphomas. Whereas recent data from large registries of patients with RA clearly indicate that disease activity, rather than TNF antagonism, is likely to be responsible for the observed increased risk for lymphoma, there is still some debate about this issue. TNF antagonists have mixed effects on autoantibody production, suppressing some responses and enhancing others. On balance, TNF antagonists restore some of the immune anergy associated with chronic inflammatory diseases. These observations may relate to recent exciting findings that TNF downregulates Tregs and that TNF antagonists restore Treg function. These Tregs may in turn suppress autoreactive T cells or other cells that drive inflammation in immune-mediated inflammatory diseases. Further investigations are needed to fully understand the effect of TNF antagonists on Treg function, immune tolerance and autoimmunity.

Evaluation of the many mechanisms by which TNF antagonists block TNF functions and ameliorate human inflammatory diseases has complemented and extended our understanding from animal studies of the roles of sTNF and mTNF in disease pathogenesis, but many questions about TNF antagonists remain unanswered. Relatively little attention has been paid to the contribution of LT to the pathogenesis of

immune-mediated inflammatory diseases or to whether the efficacy of etanercept depends at all on its ability to bind LT ligands, namely LTα3 and LTα2β1. These questions are still under investigation.

Only a few studies of mechanisms of TNF antagonists in other rheumatic diseases, such as ankylosing spondylitis, juvenile idiopathic arthritis or psoriatic arthritis, have been conducted. However, these initial data suggest that the effects of TNF blockade on synovial inflammation are comparable in different forms of arthritis. There has been great interest in understanding the mechanistic basis for the lack of efficacy of etanercept in Crohn's disease and other granulomatous diseases. Although much attention has been focused on tmTNF-mediated apoptosis as a differentiating mechanism, there is no convincing evidence for such a mechanistic explanation. Rather, the differential efficacy in Crohn's disease may relate to pharmacokinetic differences, or to differences in other consequences of reverse signaling, such as cytokine modulation.

Another intriguing mechanistic question is why most patients who fail to respond, have lost response or are intolerant of one TNF antagonist respond well when switched to another TNF antagonist. Pharmacokinetic analyses of drug concentrations in some patients who are nonresponders or who lost response to a particular TNF antagonist have revealed the presence of antidrug antibodies, which form complexes and promote the rapid clearance of the drug. Immunogenicity is most prevalent with infliximab and has been linked to the relatively high rate of acquired resistance to infliximab relative to etanercept or adalimumab, which are less immunogenic. In addition, immune-mediated inflammatory disease patient populations are heterogeneous and, even within a single disease, TNF may play a greater pathogenic role in some patients than in others. There is a need for biomarkers that can reliably identify different pathogenic subsets associated with response or lack of response to TNF antagonist therapy.

New insights into the mechanisms of action of TNF antagonists — and related distinctions between the agents — will undoubtedly emerge as greater numbers of diseases are treated by TNF blockade. As has been learned in biopsy studies from patients with RA, Crohn's disease and psoriasis, TNF antagonists share many common mechanisms of action across these diseases, but disease-specific mechanisms have also been observed. These mechanistic insights, coupled with improved management of the pharmacokinetics and immunogenicity of the TNF antagonists, should lead to further advances in realizing the full potential of this highly effective class of drugs.

Acknowledgments

The authors are grateful for the helpful discussions with Zehra Kaymakcalan, Brad McRae, Philip Sugerman, Steven Rozzo, Oscar Segurado and Taro Fujimori; for critical review of the manuscript by Philip Sugerman and Susan Paulson; for preparation of Fig. 5 by Susan Paulson; and for the excellent editorial assistance by Lori Lush and colleagues at JK Associates, Inc.

References

Agarwal, A., Panda, S., & Misra, R. (2004). Effect of etanercept on matrix metalloproteinases and angiogenic vascular endothelial growth factor: a time kinetic study. *Ann Rheum Dis 63*, 891−892.

Alessandri, C., Bombardieri, M., Papa, N., Cinquini, M., Magrini, L., Tincani, A., et al. (2004). Decrease of anti-cyclic citrullinated peptide antibodies and rheumatoid factor following anti-TNFalpha therapy infliximab in rheumatoid arthritis is associated with clinical improvement. *Ann Rheum Dis 63*, 1218−1221.

Alexopoulou, L., Pasparakis, M., & Kollias, G. (1997). A murine transmembrane tumor necrosis factor (TNF) transgene induces arthritis by cooperative p55/p75 TNF receptor signaling. *Eur J Immunol 27*, 2588−2592.

Alexopoulou, L., Pasparakis, M., & Kollias, G. (1998). Complementation of lymphotoxin alpha knockout mice with tumor necrosis factor-expressing transgenes rectifies defective splenic structure and function. *J Exp Med 188*, 745−754.

Aloisi, F., & Pujol-Borrell, R. (2006). Lymphoid neogenesis in chronic inflammatory diseases. *Nat Rev Immunol 6*, 205−217.

Alsalameh, S., Winter, K., Al-Ward, R., Wendler, J., Kalden, J. R., & Kinne, R. W. (1999). Distribution of TNF-alpha, TNF-R55 and TNF-R75 in the rheumatoid synovial membrane: TNF receptors are localized preferentially in the lining layer; TNF-alpha is distributed mainly in the vicinity of TNF receptors in the deeper layers. *Scand J Immunol 49*, 278−285.

Anderson, P. J. (2005). Tumor necrosis factor inhibitors: clinical implications of their different immunogenicity profiles. *Semin Arthritis Rheum 34*, 19−22.

Anderson, M. S., Venanzi, E. S., Klein, L., Chen, Z., Berzins, S. P., Turley, S. J., et al. (2002). Projection of an immunological self shadow within the thymus by the Aire protein. *Science 298*, 1395−1401.

Anolik, J. H., Owen, T., Barnard, J., & Sanz, I. (2005). Anti-tumor necrosis factor therapy in rheumatoid arthritis alters B lymphocyte dynamics. *Arthritis Rheum 52*(9), S677.

Arend, W., & Dayer, J. (1990). Cytokines and cytokine inhibitors or antagonists in rheumatoid arthritis. *Arthritis Rheum 33*, 305−315.

Aringer, M., Graninger, W. B., Steiner, G., & Smolen, J. S. (2004). Safety and efficacy of tumor necrosis factor alpha blockade in systemic lupus erythematosus: an open-label study. *Arthritis Rheum 50*, 3161−3169.

Askling, J., Fored, C. M., Brandt, L., Baecklund, E., Bertilsson, L., Cöster, L., et al. (2005). Risk and case characteristics of tuberculosis in rheumatoid arthritis associated with tumor necrosis factor antagonists in Sweden. *Arthritis Rheum 52*, 1986−1992.

Askling, J., Fored, C. M., Geborek, P., Jacobsson, L. T. H., van Vollenhoven, R., Feltelius, N., et al. (2006). Swedish registers to examine drug safety and clinical issues in RA. *Ann Rheum Dis 65*, 707−712.

Askling, J., Fored, C. M., Brandt, L., Baecklund, E., Bertilsson, L., Feltelius, N., et al. (2007). Time-dependent increase in risk of hospitalisation with infection among Swedish RA patients treated with TNF antagonists. *Ann Rheum Dis 66*, 1339−1344.

Atzeni, F., Sarzi-Puttini, P., Dell' Acqua, D., de Portu, S., Cecchini, G., Cruini, C., et al. (2006). Adalimumab clinical efficacy is associated with rheumatoid factor and anti-cyclic citrullinated peptide antibody titer reduction: a one-year prospective study. *Arthritis Res Ther 8*, R3.

Atzeni, F., Sarzi-Puttini, P., Doria, A., Iaccarino, L., & Capsoni, F. (2005a). Potential off-label use of infliximab in autoimmune and non-autoimmune diseases: a review. *Autoimmun Rev 4*, 144−152.

Atzeni, F., Turiel, M., Capsoni, F., Doria, A., Meroni, P., & Sarzi-Puttini, P. (2005b). Autoimmunity and anti-TNF-α agents. *Ann N Y Acad Sci 1051*, 559−569.

Baecklund, E., Askling, J., Rosenquist, R., Ekbom, A., & Klareskog, L. (2004). Rheumatoid arthritis and malignant lymphomas. *Curr Opin Rheumatol 16*, 254−261.

Baecklund, E., Ekbom, A., Sparén, P., Feltelius, N., & Klareskog, L. (1998). Disease activity and risk of lymphoma in patients with rheumatoid arthritis: nested case-control study. *BMJ 317*, 180−181.

Baecklund, E., Iliadou, A., Askling, J., Ekbom, A., Backlin, C., Granath, F., et al. (2006). Association of chronic inflammation, not its treatment, with increased lymphoma risk in rheumatoid arthritis. *Arthritis Rheum 54*, 692−701.

Baert, F., Noman, M., Vermeire, S., Van Assche, G., D' Haens, G., Carbonez, A., et al. (2003). Influence of immunogenicity on the long-term efficacy of infliximab in Crohn's disease. *N Engl J Med 348*, 601−608.

Balkwill, F., & Coussens, L. M. (2004). Cancer: an inflammatory link. *Nature 431*, 405−406.

Bang, L. M., & Keating, G. M. (2004). Adalimumab: a review of its use in rheumatoid arthritis. *Biodrugs 18*, 121−139.

Banner, D. W., D'Arcy, A., Janes, W., Gentz, R., Schoenfeld, H. J., Broger, C., et al. (1993). Crystal structure of the soluble human 55 kd TNF receptor-human TNF beta complex: implications for TNF receptor activation. *Cell 73*, 431−445.

Barrera, P., Joosten, L. A., den Broeder, A. A., van de Putte, L. B., van Riel, P. L., & van den Berg, W. B. (2001). Effects of treatment with a fully human anti-tumour necrosis factor alpha monoclonal antibody on the local and systemic homeostasis of interleukin 1 and TNF-alpha in patients with rheumatoid arthritis. *Ann Rheum Dis 60*, 660−669.

Barrera, P., Oyen, W. J. G., Boerman, O. C., & van Riel, P. L. C. M. (2003). Scintigraphic detection of tumour necrosis factor in patients with rheumatoid arthritis. *Ann Rheum Dis 62*, 825−828.

Bartelds, G. M., Wijbrandts, C. A., Nurmohamed, M. T., Stapel, S., Lems, W. F., Aarden, L., et al. (2007). Clinical response to adalimumab: the relationship with anti-adalimumab antibodies and serum adalimumab concentrations in rheumatoid arthritis. *Ann Rheum Dis 66*, 921−926.

Barthel, H. R., Gille, T., Halbsguth, A., & Kramer, M. (2005). Successful treatment with adalimumab in infliximab-resistant Crohn's disease. *J Gastroenterol Hepatol 20*, 1464−1465.

Bathon, J. M., Martin, R. W., Fleischmann, R., Tesser, J. R., Schiff, M., Keystone, E., et al. (2000). A comparison of etanercept and methotrexate in patients with early rheumatoid arthritis. *N Engl J Med 343*, 1586−1593.

Baughman, B. P., Drent, M., Kavuru, M., Judson, M. A., Costabel, U., du Bois, R., et al. (2006). Infliximab therapy in patients with chronic sarcoidosis and pulmonary involvement. *Am J Respir Crit Care Med 174*, 795−802.

Bazzoni, F., & Beutler, B. (1996). The tumor necrosis factor ligand and receptor families. *N Engl J Med 334*, 1717−1725.

Bendele, A. M., Chlipala, E. S., Scherrer, J., Frazier, J., Sennello, G., Rich, W. J., et al. (2000). Combination benefit of treatment with the cytokine inhibitors interleukin-1 receptor antagonist and PEGylated soluble tumor necrosis factor receptor type I in animal models of rheumatoid arthritis. *Arthritis Rheum 43*, 2648−2659.

Bendixen, G., Hadidi, T., Manthorpe, R., Permin, H., Struckmann, J., Wilk, A., et al. (1984). Antibodies against nuclear components in schistosomiasis. Results compared to values in patients with rheumatoid arthritis, systemic lupus erythematosus, and osteoarthrosis. *Allergy 39*, 107−113.

Bendtzen, K., Geborek, P., Svenson, M., Larsson, L., Kapetanovic, M. C., & Saxne, T. (2006). Individualized monitoring of drug bioavailability and immunogenicity in rheumatoid arthritis patients treated with the tumor necrosis factor α inhibitor infliximab. *Arthritis Rheum 54*, 3782−3789.

Bengmark, S. (2004). Acute and "chronic" phase reaction: a mother of disease. *Clin Nutr 23*, 1256−1266.

Berg, L., Lampa, J., Rogberg, S., van Vollenhoven, R., & Klareskog, L. (2001). Increased peripheral T cell reactivity to microbial antigens and collagen type II in rheumatoid arthritis after treatment with soluble TNFalpha receptors. *Ann Rheum Dis 60*, 133−139.

Berry, M. A., Hargadon, B., Shelley, M., Parker, D., Shaw, D. E., Green, R. H., et al. (2006). Evidence of a role of tumor necrosis factor alpha in refractory asthma. *N Engl J Med 354*, 697−708.

Bieber, J., & Kavanaugh, A. (2004). Consideration of the risk and treatment of tuberculosis in patients who have rheumatoid arthritis and receive biologic treatments. *Rheum Dis Clin North Am 30*, 257−270.

Bischoff, S. C., Lorentz, A., Schwengberg, S., Weier, G., Raab, R., & Manns, M. P. (1999). Mast cells are an important cellular source of tumour necrosis factor alpha in human intestinal tissue. *Gut 44*, 643−652.

Bombardieri, S., Ruiz, A., Fardellone, P., McKenna, P., Unnebrink, F., Oezer, K., et al. (2007). Effectiveness of adalimumab in rheumatoid arthritis in patients with a history of TNF antagonists in clinical practice. *Rheumatology 46*, 1191−1199.

Bongartz, T., Sutton, A. J., Sweeting, M. J., Buchan, I., Matteson, E. L., & Montori, V. (2006). Anti-TNF antibody therapy in rheumatoid arthritis and the risk of serious infections and malignancies: systematic review and meta-analysis of rare harmful effects in randomized controlled trials. *JAMA 295*, 2275−2285.

Boyce, B. F., Li, P., Yao, Z., Zhang, Q., Badell, I. R., Schwarz, E. M., et al. (2005). TNFα and pathologic bone resorption. *Keio J Med 54*, 127−131.

Breedveld, F. C., Weisman, M. H., Kavanaugh, A. F., Cohen, S. B., Pavelka, K., van Vollenhoven, R., et al. (2006). The PREMIER study: a multicenter, randomized, double-blind clinical trial of combination therapy with adalimumab plus methotrexate versus methotrexate alone or adalimumab alone in patients with early, aggressive rheumatoid arthritis who had not had previous methotrexate treatment. *Arthritis Rheum 54*, 26−37.

Brennan, F. M., Browne, K. A., Green, P. A., Jaspar, J. M., Maini, R. N., & Feldmann, M. (1997). 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 36*, 643−650.

Brennan, F. M., Chantry, D., Jackson, A., Maini, R., & Feldmann, M. (1989). Inhibitory effect of TNF alpha antibodies on synovial cell interleukin-1 production in rheumatoid arthritis. *Lancet 2*, 244−247.

Browning, J. L., Allaire, N., Ngam-ek, A., Notidis, E., Hunt, J., Perrin, S., et al. (2005). Lymphotoxin-beta receptor signaling is required for the homeostatic control of HEV differentiation and function. *Immunity 23*, 539−550.

Browning, J. L., Dougas, I., Ngam-ed, A., Bourdon, P. R., Ehrenfels, B. N., Miatkowski, K., et al. (1995). Characterization of surface lymphotoxin forms. Use of specific monoclonal antibodies and soluble receptors. *J Immunol 154*, 33−46.

Browning, J., Sizing, I., Lawton, P., Bourdon, P., Rennert, P., Majeau, G., et al. (1997). Characterization of lymphotoxin-alpha beta complexes on the surface of mouse lymphocytes. *J Immunol 159*, 3288−3298.

Bryl, E., Vallejo, A. N., Matteson, E. L., Witkowski, J. M., Weyand, C. M., & Goronzy, J. J. (2005). Modulation of CD28 expression with anti-tumor necrosis factor alpha therapy in rheumatoid arthritis. *Arthritis Rheum 52*, 2996−3003.

Busse, W. W., Banks-Schlegel, A., & Wenzel, S. E. (2000). Pathophysiology of severe asthma. *J Allergy Clin Immunol 106*, 1033−1042.

Busse, W. W., & Lemanske, R. F., Jr. (2001). Asthma. *N Engl J Med 344*, 350−362.

Butler, D., Malfait, A., Mason, L., Warden, P., Kollias, G., Maini, R., et al. (1997). DBA/1 mice expressing the human TNF-alpha transgene develop a severe, erosive arthritis: characterization of the cytokine cascade and cellular composition. *J Immunol 159*, 2867−2876.

Caffieri, S., Di Lisa, F., Bolesani, F., Facco, M., Semenzato, G., Dall'Acqua, F., et al. (2007). The mitochondrial effects of novel apoptogenic molecules generated by psoralen photolysis as a crucial mechanism in PUVA therapy. *Blood 109*, 4988−4994.

Cao, D., Malmström, V., Baecher-Allan, C., Hafler, D., Klareskog, L., & Trollmo, C. (2003). Isolation and functional characterization of regulatory T cells from the target organ of patients with rheumatoid arthritis. *Eur J Immunol 33*, 215−223.

Carmona, L., Gómez-Reino, J. J., Rodríguez-Valverde, V., Montero, D., Pascual-Gómez, E., Mola, E. M., et al. (2005). Effectiveness of recommendations to prevent reactivation of latent tuberculosis infection in patients treated with tumor necrosis factor antagonists. *Arthritis Rheum 52*, 1766−1772.

Carmona, L., & Gomez-Reino, J. J., & BIOBADASER Group. (2006). Survival of TNF antagonists in spondylarthritis is better than in rheumatoid arthritis. Data from the Spanish registry BIOBADASER. *Arthritis Res Ther 8*, R72.

Carrasco, R., Smith, J. A., & Lovell, D. (2004). Biologic agents for the treatment of juvenile rheumatoid arthritis: current status. *Paediatr Drugs 6*, 137−146.

Catrina, A. I., af Klint, E., Ernestam, S., Catrina, S. B., Makrygiannakis, D., Botusan, I., et al. (2006). Anti-tumor necrosis factor therapy increases synovial osteoprotegerin expression in rheumatoid arthritis. *Arthritis Rheum 54*, 76−81.

Catrina, A. I., Lampa, J., Ernestam, S., af Klint, E., Bratt, J., Klareskog, L., et al. (2002). Anti-tumour necrosis factor (TNF)-alpha therapy (etanercept) down-regulates serum matrix metalloproteinase (MMP)-3 and MMP-1 in rheumatoid arthritis. *Rheumatology 41*, 484−489.

Catrina, A. I., Trollmo, C., af Klint, E., Engstrom, M., Lampa, J., Hermansson, Y., et al. (2005). Evidence that anti-tumor necrosis factor therapy with both etanercept and infliximab induces apoptosis in macrophages, but not lymphocytes, in rheumatoid arthritis joints: extended report. *Arthritis Rheum 52*(1), 61−72.

Chan, F. K. -M., Chun, H. J., Zheng, L., Siegel, R. M., Bui, K. L., & Lenardo, M. J. (2000). A domain in TNF receptors that mediates ligand-independent receptor assembly and signaling. *Science 288*, 2351−2354.

Charles, P., Elliott, M. J., Davis, D., Potter, A., Kalden, J. R., Antoni, C., et al. (1999). Regulation of cytokines, cytokine inhibitors, and acute-phase proteins following anti-TNF-alpha therapy in rheumatoid arthritis. *J Immunol 163*, 1521−1528.

Charles, P. J., Smeenk, R. J., De Jong, J., Feldmann, M., & Maini, R. N. (2000). Assessment of antibodies to double-stranded DNA induced in rheumatoid arthritis patients following treatment with infliximab, a monoclonal antibody to tumor necrosis factor alpha: findings in open-label and randomized placebo-controlled trials. *Arthritis Rheum 43*, 2382−2390.

Chaudhary, R., Butler, M., Playford, R. J., & Ghosh, S. (2006). Anti-TNF antibody induced stimulated T lymphocyte apoptosis depends on the concentration of the antibody and etanercept induces apoptosis at rates equivalent to infliximab and adalimumab at 10 micrograms per ml concentration. *Gastroenterology 130*(4), A696.

Chen, H. A., Lin, K. C., Chen, C. H., Liao, H. T., Wang, H. P., Chang, H. N., et al. (2006). The effect of etanercept on anti-cyclic citrullinated peptide antibodies and rheumatoid factor in patients with rheumatoid arthritis. *Ann Rheum Dis 65*, 35−39.

Chin, R. K., Lo, J. C., Kim, O., Blink, S. E., Christiansen, P. A., Peterson, P., et al. (2003). Lymphotoxin pathway directs thymic Aire expression. *Nat Immunol 4*, 1121−1127.

Chomarat, P., Dantin, C., Bennett, L., Banchereau, J., & Palucka, A. K. (2003). TNF skews monocyte differentiation from macrophages to dendritic cells. *J Immunol 171*, 2262−2269.

Choy, E. H. S., & Panayi, G. S. (2001). Cytokine pathways and joint inflammation in rheumatoid arthritis. *N Engl J Med 344*, 907−916.

Cohen, P. S., Nakshatri, H., Dennis, J., Caragine, T., Bianchi, M., Cerami, A., et al. (1996). CNI-1493 inhibits monocyte/macrophage tumor necrosis factor by suppression of translation efficiency. *Proc Natl Acad Sci U S A 93*, 3967−3971.

Cope, A. P., Londei, M., Chu, N. R., Cohen, S. B., Elliott, M. J., Brennan, F. M., et al. (1994). Chronic exposure to tumor necrosis factor (TNF) in vitro impairs the activation of T cells through the T cell receptor/CD3 complex; reversal in vivo by anti-TNF antibodies in patients with rheumatoid arthritis. *J Clin Invest 94*, 749−760.

Cordiali-Fei, P., Trento, E., D'Agosto, G., Bordignon, V., Mussi, A., Ardigo, M., et al. (2006). Decreased levels of metalloproteinase-9 and angiogenic factors in skin lesions of patients with psoriatic arthritis after therapy with anti-TNF-alpha. *J Autoimmune Dis 3*, 5.

Cronstein, B. N. (2005). Low-dose methotrexate: a mainstay in the treatment of rheumatoid arthritis. *Pharmacol Rev 57*, 163−172.

Crotti, T. N., Ahern, M. J., Lange, K., Weedon, H., Coleman, M., Roberts, P. J., et al. (2003). Variability of RANKL and osteoprotegerin staining in synovial tissue from patients with active rheumatoid arthritis: quantification using color video image analysis. *J Rheumatol 30*, 2319−2324.

Crowe, P., VanArsdale, T., Walter, B., Ware, C., Hession, C., Ehrenfels, B., et al. (1994). A lymphotoxin-beta-specific receptor. *Science 264*, 707−710.

D'Alessio, A., Al-Lamki, R. S., Bradley, J. R., & Pober, J. S. (2005). Caveolae participate in tumor necrosis factor receptor 1 signaling and internalization in a human endothelial cell line. *Am J Pathol 166*, 1273−1282.

Danks, L., Sabokbar, A., Gundle, R., & Athanasou, N. A. (2002). Synovial macrophage-osteoclast differentiation in inflammatory arthritis. *Ann Rheum Dis 61*, 916−921.

D'Auria, F., Rovere-Querini, P., Giazzon, M., Ajello, P., Baldissera, E., Manfredi, A. A., et al. (2004). Accumulation of plasma nucleosomes upon treatment with anti-tumour necrosis factor-alpha antibodies. *J Intern Med 255*, 409−418.

de Rycke, L., Baeten, D., Kruithof, E., Van den Bosch, F., Veys, E. M., & de Keyser, F. (2005). Infliximab, but not etanercept, induces IgM anti-double-stranded DNA autoantibodies as main antinuclear reactivity: biologic and clinical implications in autoimmune arthritis. *Arthritis Rheum 52*, 2192−2201.

den Broeder, A. A., Joosten, L. A. B., Saxne, T., Heinegard, D., Fenner, H., Miltenburg, A. M. M., et al. (2002). Long term anti-tumour necrosis factor alpha monotherapy in rheumatoid arthritis: effect on radiological course and prognostic value of markers of cartilage turnover and endothelial activation. *Ann Rheum Dis 61*, 311−318.

Di Sabatino, A., Ciccocioppo, R., Cinque, B., Millimaggi, D., Morera, R., Ricevuti, L., et al. (2004). Defective mucosal T cell death is sustainably reverted by infliximab in a caspase dependent pathway in Crohn's disease. *Gut 53*, 70−77.

Diarra, D., Stolina, M., Polzer, K., Zwerina, J., Ominsky, M. S., Dwyer, D., et al. (2007). Dickkopf-1 is a master regulator of joint remodeling. *Nat Med 13*, 156−163.

Dixon, W. G., Watson, K., Lunt, M., Hyrich, K. L., Silman, A. J., & Symmons, D. P., British Society for Rheumatology Biologics Register. (2006). Rates of serious infection, including site-specific and bacterial intracellular infection, in rheumatoid arthritis patients receiving anti-tumor necrosis factor therapy: results from the British Society for Rheumatology Biologics Register. *Arthritis Rheum 54*, 2368−2376.

Doherty, G. M., Jensen, J. C., Alexander, H. R., Buresh, C. M., & Norton, J. A. (1991). Pentoxifylline suppression of tumor necrosis factor gene transcription. *Surgery 110*, 192−198.

Dvorak, H., Brown, L., Detmar, M., & Dvorak, A. (1995). Vascular permeability factor/vascular endothelial growth factor, microvascular hyperpermeability, and angiogenesis. *Am J Pathol 146*, 1029−1039.

Edwards, C. K., III, Bendele, A. M., Reznikov, L. I., Fantuzzi, G., Chlipala, E. S., Li, L., et al. (2006). Soluble human p55 and p75 tumor necrosis factor receptors reverse spontaneous arthritis in transgenic mice expressing transmembrane tumor necrosis factor? *Arthritis Rheum 54*, 2872−2885.

Ehlers, S., Hölscher, C., Scheu, S., Tertilt, C., Hehlgans, T., Suwinski, J., et al. (2003). The lymphotoxin beta receptor is critically involved in controlling infections with the intracellular pathogens *Mycobacterium tuberculosis* and *Listeria monocytogenes*. *J Immunol 170*, 5210−5218.

Ehrenstein, M. R., Evans, J. G., Singh, A., Moore, S., Warnes, G., Isenberg, D. A., et al. (2004). Compromised function of regulatory T cells in rheumatoid arthritis and reversal by anti-TNFalpha therapy. *J Exp Med 200*, 277−285.

Eissner, G., Kirchner, S., Lindner, H., Kolch, W., Janosch, P., Grell, M., et al. (2000). Reverse signaling through transmembrane TNF confers resistance to lipopolysaccharide in human monocytes and macrophages. *J Immunol 164*, 6193−6198.

Eissner, G., Kolch, W., & Scheurich, P. (2004). Ligands working as receptors: reverse signaling by members of the TNF superfamily enhance the plasticity of the immune system. *Cytokine Growth Factor Rev 15*, 353−366.

Elkayam, O., Caspi, D., Reitblatt, T., Charboneau, D., & Rubins, J. B. (2004). The effect of tumor necrosis factor blockade on the response to pneumococcal vaccination in patients with rheumatoid arthritis and ankylosing spondylitis. *Semin Arthritis Rheum 33*, 283−288.

Elliott, M. J., Maini, R. N., Feldmann, M., Long, F. A., Charles, P., Katsikis, P., et al. (1993). Treatment of rheumatoid arthritis with chimeric monoclonal antibodies to tumor necrosis factor alpha. *Arthritis Rheum 36*, 1681−1690.

Elliott, M., Maini, R., Feldmann, M., Kalden, J., Antoni, C., Smolen, J., et al. (1994). Randomised double-blind comparison of chimeric monoclonal antibody to tumour necrosis factor alpha (cA2) versus placebo in rheumatoid arthritis. *Lancet 344*, 1105−1110.

Enbrel (etanercept) prescribing information. (2007). *Immunex Corporation, Thousand Oaks, CA.*

Engelmann, H., Holtmann, H., Brakebusch, C., Avni, Y., Sarov, I., Nophar, Y., et al. (1990). Antibodies to a soluble form of a tumor necrosis factor (TNF) receptor have TNF-like activity. *J Biol Chem 265*, 14497−14504.

Engwerda, C. R., Ato, M., Stager, S., Alexander, C. E., Stanley, A. C., & Kaye, P. M. (2004). Distinct roles for lymphotoxin-alpha and tumor necrosis factor in the control of *Leishmania donovani* infection. *Am J Pathol 165*, 2123−2133.

Evans, T. J., Moyes, D., Carpenter, A., Martin, R., Loetscher, H., Lesslauer, W., et al. (1994). Protective effect of 55- but not 75-kD soluble tumor necrosis factor receptor-immunoglobulin G fusion proteins in an animal model of gram-negative sepsis. *J Exp Med 180*, 2173−2179.

Fava, R. A., Notidis, E., Hunt, J., Szanya, V., Ratcliffe, N., Ngam-ek, A., et al. (2003). A role for the lymphotoxin/LIGHT axis in the pathogenesis of murine collagen-induced arthritis. *J Immunol 171*, 115−126.

Feldmann, M. (2002). Development of anti-TNF therapy for rheumatoid arthritis. *Nat Rev Immunol 2*, 364−371.

Feldmann, M., & Maini, R. N. (2001). Anti-TNF alpha therapy of rheumatoid arthritis: what have we learned? *Ann Rev Immunol 19*, 163−196.

Feldmann, M., & Maini, R. N. (2002). Discovery of TNF-alpha as a therapeutic target in rheumatoid arthritis: preclinical and clinical studies. *Joint Bone Spine 69*, 12−18.

Feldmann, M., & Maini, R. N. (2003). Lasker Clinical Medical Research Award: TNF defined as a therapeutic target for rheumatoid arthritis and other autoimmune diseases. *Nat Med 9*, 1245−1250.

Feldmann, M., & Steinman, L. (2005). Design of effective immunotherapy for human autoimmunity. *Nature 435*, 612−619.

Feuerherm, A. J., Borset, M., Seidel, C., Sundan, A., Leistad, L., Ostensen, M., et al. (2001). Elevated levels of osteoprotegerin (OPG) and hepatocyte growth factor (HGF) in rheumatoid arthritis. *Scand J Rheumatol 30*, 229−234.

Finckh, A., Simard, J. F., Gabay, C., & Guerne, P. -A. (2006). Evidence for differential acquired drug resistance to anti-tumour necrosis factor agents in rheumatoid arthritis. *Ann Rheum Dis 65*, 746−752.

Fisher, C. J., Agosti, J. M., Opal, S. M., Lowry, S. F., Balk, R. A., Sadoff, J. C., et al. (1996). Treatment of septic shock with the tumor necrosis factor receptor:Fc fusion protein. *N Engl J Med 334*, 1697−1702.

Flynn, J. L., Goldstein, M. M., Chan, J., Triebold, K. J., Pfeffer, K., Lowenstein, C. J., et al. (1995). Tumor necrosis factor-[alpha] is required in the protective immune response against mycobacterium tuberculosis in mice. *Immunity 2*, 561−572.

Fossati, G., & Nesbitt, A. M. (2005a). *In vitro* complement-dependent cytotoxicity and antibody-dependent cellular cytotoxicity by the anti-TNF agents adalimumab, etanercept, infliximab, and certolizumab pegol (CDP870). *Am J Gastroenterol 100*, S299 (Suppl S).

Fossati, G., & Nesbitt, A. M. (2005b). Effect of the anti-TNF agents, adalimumab, etanercept, infliximab, and certolizumab pegol (CDP870) on the induction of apoptosis in activated peripheral blood lymphocytes and monocytes. *Am J Gastroenterol 100*, S298−S299 (Suppl S).

Fraser, A., Fearon, U., Reece, R., Emery, P., & Veale, D. J. (2001). Matrix metalloproteinase 9, apoptosis, and vascular morphology in early arthritis. *Arthritis Rheum 44*, 2024−2028.

Friedmann, E., Hauben, E., Maylandt, K., Schleeger, S., Vreugde, S., Lichtenthaler, S. F., et al. (2006). SPPL2a and SPPL2b promote intramembrane proteolysis of TNF[alpha] in activated dendritic cells to trigger IL-12 production. *Nat Cell Biol 8*, 843−848.

Fu, Y. -X., & Chaplin, D. D. (1999). Development and maturation of secondary lymphoid tissues. *Ann Rev Immunol 17*, 399−433.

Furst, D. E., Breedveld, F. C., Kalden, J. R., Smolen, J. S., Burmester, G. R., Sieper, J., et al. (2007). Updated consensus statement on biological agents for the treatment of rheumatic diseases, 2007. *Ann Rheum Dis 66*, iii2−iii22.

Furst, D. E., Wallis, R., Broder, M., & Beenhouwer, D. O. (2006). Tumor necrosis factor antagonists: different kinetics and/or mechanisms of action may explain differences in the risk for developing granulomatous infection. *Semin Arthritis Rheum 36*, 159−167.

Galustian, C., Labarthe, M. C., Bartlett, J. B., & Dalgleish, A. G. (2004). Thalidomide-derived immunomodulatory drugs as therapeutic agents. *Expert Opin Biol Ther 4*, 1963−1970.

Gelderman, K. A., Lam, S., & Gorter, A. (2005). Inhibiting complement regulators in cancer immunotherapy with bispecific mAbs. *Expert Opin Biol Ther 5*, 1593−1601.

Genestier, L., Paillot, R., Fournel, S., Ferraro, C., Miossec, P., & Revillard, J. -P. (1998). Immunosuppressive properties of methotrexate: apoptosis and clonal deletion of activated peripheral T cells. *J Clin Invest 102*, 322−328.

Genovese, M. C., Kavanaugh, A. F., Cohen, S. B., Emery, P., Sasso, E. H., & Spencer-Green, G. T. (2005). The relationship of radiographic progression to clinical response in patients with early RA treated with adalimumab (Humira®) plus MTX or MTX alone. *Arthritis Rheum 52*, S451 (Suppl).

Geusens, P. P., Landewé, R. B. M., Garnero, P., Chen, D., Dunstan, C. R., Lems, W. F., et al. (2006). The ratio of circulating osteoprotegerin to RANKL in early rheumatoid arthritis predicts later joint destruction. *Arthritis Rheum 54*, 1772−1777.

Goedkoop, A. Y., Kraan, M. C., Picavet, D. I., de Rie, M. A., Teunissen, M. B., Bos, J. D., et al. (2004b). Early effects of tumour necrosis factor {alpha} blockade on skin and synovial tissue in patients with active psoriasis and psoriatic arthritis. *Ann Rheum Dis 63*, 769−773.

Goedkoop, A. Y., Kraan, M. C., Teunissen, M. B., Picavet, D. I., de Rie, M. A., Bos, J. D., et al. (2004a). Deactivation of endothelium and reduction in angiogenesis in psoriatic skin and synovium by low dose infliximab therapy in combination with stable methotrexate therapy: a prospective single-centre study. *Arthritis Res Ther 6*, R326−R334.

Gomez-Reino, J., & Group, C. L. B. (2006). Switching TNF antagonists in patients with chronic arthritis: an observational study of 488 patients over a four-year period. *Arthritis Res Ther 8*, R29.

Gommerman, J. L., & Browning, J. L. (2003). Lymphotoxin/light, lymphoid microenvironments and autoimmune disease. *Nat Rev Immunol 3*, 642−655.

Gordon, K. B., Bonish, B. K., Patel, T., Leonardi, C. L., & Nickoloff, B. J. (2005). The tumour necrosis factor-alpha inhibitor adalimumab rapidly reverses the decrease in epidermal Langerhans cell density in psoriatic plaques. *Br J Dermatol 153*, 945−953.

Gordon, K. B., Langley, R. G., Leonardi, C., Toth, D., Menter, M. A., Kang, S., et al. (2006). Clinical response to adalimumab treatment in patients with moderate to severe psoriasis: double-blind, randomized controlled trial and open-label extension study. *J Am Acad Dermatol 55*, 598−606.

Gottlieb, A. B., Chamian, F., Masud, S., Cardinale, I., Abello, M. V., Lowes, M. A., et al. (2005). TNF inhibition rapidly down-regulates multiple proinflammatory pathways in psoriasis plaques. *J Immunol 175*, 2721−2729.

Gottlieb, A. B., Evans, R., Li, S., Dooley, L. T., Guzzo, C. A., Baker, D., et al. (2004). Infliximab induction therapy for patients with severe plaque-type psoriasis: a randomized, double-blind, placebo-controlled trial. *J Am Acad Dermatol 51*, 534−542.

Gramlick, A., Fossati, G., & Nesbitt, A. M. (2006). Neutralization of soluble and membrane tumor necrosis factor-alpha (TNF- alpha) by infliximab, adalimumab, or certolizumab pegol using P55 or P75 TNF-alpha receptor-specific bioassays. *Gastroenterology 130*(4), A697.

Granneman, R. G., Zhang, Y., Noertersheuser, P. A., Velagapudi, R. B., Awni, W. M., Locke, C. S., et al. (2003). Pharmacokinetic/pharmacodynamic (PK/PD) relationships of adalimumab (HUMIRA, Abbott) in rheumatoid arthritis (RA) patients during phase II/III clinical trials. *Arthritis Rheum 48*, S140−S141.

Grell, M., Douni, E., Wajant, H., Lohden, M., Clauss, M., Maxeiner, B., et al. (1995). The transmembrane form of tumor necrosis factor is the prime activating ligand of the 80 kDa tumor necrosis factor receptor. *Cell 83*, 793−802.

Grell, M., Wajant, H., Zimmermann, G., & Scheurich, P. (1998). The type 1 receptor (CD120a) is the high-affinity receptor for soluble tumor necrosis factor. *PNAS 95*, 570−575.

Gridley, G., McLaughlin, J. K., Ekbom, A., Klareskog, L., Adami, H. -O., Hacker, D. G., et al. (1993). Incidence of cancer among patients with rheumatoid arthritis. *J Natl Cancer Inst 85*, 307−311.

Grivennikov, S. I., Tumanov, A. V., Liepinsh, D. J., Kruglov, A. A., Marakusha, B. I., Shakhov, A. N., et al. (2005). Distinct and nonredundant in vivo functions of TNF produced by T cells and macrophages/neutrophils: protective and deleterious effects. *Immunity 22*, 93−104.

Gudbrandsdottir, S., Larsen, R., Sorensen, L. K., Nielsen, S., Hansen, M. B., Svenson, M., et al. (2004). TNF and LT binding capacities in the plasma of arthritis patients: effect of etanercept treatment in juvenile idiopathic arthritis. *Clin Exp Rheumatol 22*, 118−124.

Han, J., Brown, T., & Beutler, B. (1990). Endotoxin-responsive sequences control cachectin/tumor necrosis factor biosynthesis at the translational level [published erratum appears in J Exp Med 171(3), 971−972]. *J Exp Med 171*, 465−475.

Han, S., Zhang, X., Marinova, E., Ozen, Z., Bheekha-Escura, R., Guo, L., et al. (2005). Blockade of lymphotoxin pathway exacerbates autoimmune arthritis by enhancing the Th1 response. *Arthritis Rheum 52*, 3202−3209.

Hanauer, S. B., Sandborn, W. J., Rutgeerts, P., Fedorak, R. N., Lukas, M., MacIntosh, D., et al. (2006). Human anti-tumor necrosis factor monoclonal antibody (adalimumab) in Crohn's disease: the CLASSIC-I trial. *Gastroenterology 130*, 323−333.

Haraoui, B. (2005). Differentiating the efficacy of the tumor necrosis factor inhibitors. *Semin Arthritis Rheum 34*, 7−11.

Harashima, S. -I., Horiuchi, T., Hatta, N., Morita, C., Higuchi, M., Sawabe, T., et al. (2001). Outside-to-inside signal through the membraneTNF-alpha induces E-selectin (CD62E) expression on activated human CD4+ T cells. *J Immunol 166*, 130−136.

Haynes, D. R., Barg, E., Crott, T. N., Holding, C., Weedon, H., Atkins, G. J., et al. (2003). Osteoprotegerin expression in synovial tissue from patients with rheumatoid arthritis, spondyloarthropathies and osteoarthritis and normal controls. *Rheumatology 42*, 123−134.

Hehlgans, T., & Pfeffer, K. (2005). The intriguing biology of the tumour necrosis factor/tumour necrosis factor receptor superfamily: players, rules and the games. *Immunology 115*, 1−20.

Higuchi, M., & Aggarwal, B. (1994). TNF induces internalization of the p60 receptor and shedding of the p80 receptor. *J Immunol 152*, 3550−3558.

Howarth, P. H., Babu, K. S., Arshad, H. S., Lau, L., Buckley, M., McConnell, W., et al. (2005). Tumour necrosis factor (TNFalpha) as a novel therapeutic target in symptomatic corticosteroid dependent asthma. *Thorax 60*, 1012−1018.

Hultgren, O., Eugster, H. -P., Sedgwick, J. D., Korner, H., & Tarkowski, A. (1998). TNF/lymphotoxin-alpha double-mutant mice resist septic arthritis but display increased mortality in response to *Staphylococcus aureus*. *J Immunol 161*, 5937−5942.

Humira (adalimumab) prescribing information. (2007). Abbott Laboratories, North Chicago, IL.

Hwang, W. Y. K., & Foote, J. (2005). Immunogenicity of engineered antibodies. *Methods Humanized Antibodies Applications vol. 36*. (pp. 3−10).

Iizuka, K., Chaplin, D. D., Wang, Y., Wu, Q., Pegg, L. E., Yokoyama, W. M., et al. (1999). Requirement for membrane lymphotoxin in natural killer cell development. *PNAS 96*, 6336−6340.

Isomaki, P., Panesar, M., Annenkov, A., Clark, J. M., Foxwell, B. M. J., Chernajovsky, Y., et al. (2001). Prolonged exposure of T cells to TNF down-regulates TCR zeta and expression of the TCR/CD3 complex at the cell surface. *J Immunol 166*, 5495−5507.

Ito, D., Back, T. C., Shakhov, A. N., Wiltrout, R. H., & Nedospasov, S. A. (1999). Mice with a targeted mutation in lymphotoxin-alpha exhibit enhanced tumor growth and metastasis: impaired NK cell development and recruitment. *J Immunol 163*, 2809−2815.

Jerne, N. K. (1974). Towards a network theory of the immune system. *Ann Immunol (Paris) 125C*, 373−389.

Joosten, L. A. B., Helsen, M. M. A., Saxne, T., van de Loo, F. A. J., & van den Berg, W. B. (1999). IL-1aβ blockade prevents cartilage and bone destruction in murine type II collagen-induced arthritis, whereas TNF-á blockade only ameliorates joint inflammation. *J Immunol 163*, 5049−5055.

Jovanovic, D. V., Di Battista, J. A., Martel-Pelletier, J., Jolicoeur, F. C., He, Y., Zhang, M., et al. (1998). IL-17 stimulates the production and expression of proinflammatory cytokines, IL-1β and TNF-a, by human macrophages. *J Immunol 160*, 3513−3521.

Kaine, J. L., Kivitz, A. J., Birbara, C., & Luo, A. Y. (2007). Immune responses following administration of influenza and pneumococcal vaccines to patients with rheumatoid arthritis receiving adalimumab. *J Rheumatol 34*, 272−279.

Kapetanovic, M. C., Saxne, T., Sjoholm, A., Truedsson, L., Jonsson, G., & Geborek, P. (2006). Influence of methotrexate, TNF blockers and prednisolone on antibody responses to pneumococcal polysaccharide vaccine in patients with rheumatoid arthritis. *Rheumatology 45*, 106−111.

Kavanaugh, A. F., Greenwald, M., Zizic, T., Rao, V., Fischkoff, F., Hoffman, R., et al. (2002). Treatment with adalimumab (D2E7) does not affect normal immune responsiveness. *Arthritis Rheum 46*, S132 (Suppl).

Kavanaugh, A., Tutuncu, Z., & Catalan-Sanchez, T. (2006). Update on anti-tumor necrosis factor therapy in the spondyloarthropathies including psoriatic arthritis. *Curr Opin Rheumatol 18*, 347−353.

Kay, J., Matteson, E. L., Dasgupta, B., Nash, P., Durez, P., Hall, S., et al. (2006). One-year results of golimumab compared with placebo in patients with active RA despite treatment with methotrexate: a phase II, randomized, double-blind, placebo-controlled, dose-ranging trial. *Arthritis Rheum 54* (Suppl 2123), S833.

Kaymakcalan, Z., Beam, C., & Salfeld, J. (2003). Murine model for assessing adalimumab, infliximab, and etanercept to prevent polyarthritis. *Ann Rheum Dis 62*(Suppl 1), 136−137.

Kaymakcalan, Z., Sakorafas, P., Bose, S., & Scesney, S. (2006a). Adalimumab, etanercept, and infliximab bind to soluble and transmembrane TNF with similar affinities. *Ann Rheum Dis 65*(Suppl II), 458.

Kaymakcalan, Z., Kalghatgi, L., & Xiong, L. (2006b). Differential TNF-neutralizing potencies of adalimumab, etanercept, and infliximab. *Ann Rheum Dis 65*(Suppl II), 458.

Keffer, J., Probert, L., Cazlaris, H., Georgopoulos, S., Kaslaris, E., Kioussis, D., et al. (1991). Transgenic mice expressing human tumour necrosis factor: a predictive genetic model of arthritis. *EMBO J 10*, 4025−4031.

Keystone, E. C. (2005). Safety of biologic therapies: an update. *J Rheumatol Suppl 32*, 8−12.

Keystone, E., Kavanaugh, A., Sharp, J., Tannenbaum, H., Hua, Y., Teoh, L., et al. (2004). Radiographic, clinical, and functional outcomes of treatment with adalimumab a human anti-tumor necrosis factor monoclonal antibody in patients with active rheumatoid arthritis receiving concomitant methotrexate therapy: a randomized, placebo-controlled, 52-week trial. *Arthritis Rheum 50*, 1400−1411.

Kim, E. Y., & Teh, H. -S. (2004). Critical role of TNF receptor type-2 (p75) as a costimulator for IL-2 induction and T cell survival: a functional link to CD28. *J Immunol 173*, 4500−4509.

Kirchner, S., Holler, E., Haffner, S., Andreesen, R., & Eissner, G. (2004). Effect of different tumor necrosis factor TNF reactive agents on reverse signaling of membrane integrated TNF in monocytes. *Cytokine 28*, 67−74.

Kitaura, H., Zhou, P., Kim, H-J., Novack, D. V., Ross, F. P., & Teitelbaum, S. L. (2005). M-CSF mediates TNF-induced inflammatory osteolysis. *J Clin Invest 115*, 3418−3427.

Klareskog, L., & McDevitt, H. (1999). Rheumatoid arthritis and its animal models: the role of TNF-[alpha] and the possible absence of specific immune reactions. *Curr Opin Immunol 11*, 657−662.

Klareskog, L., van der Heijde, D., de Jager, J. P., Gough, A., Kalden, J., Malaise, M., et al. (2004). Therapeutic effect of the combination of etanercept and methotrexate compared with each treatment alone in patients with rheumatoid arthritis: double-blind randomised controlled trial. *Lancet 363*, 675−681.

Knight, D., Trinh, H., Le, J., Siegel, S., Shealy, D., McDonough, M., et al. (1993). Construction and initial characterization of a mouse-human chimeric anti-TNF antibody. *Mol Immunol 30*, 1443−1453.

Koch, A. E. (2003). Angiogenesis as a target in rheumatoid arthritis. *Ann Rheum Dis 62*, 60−67.

Koenders, M. I., Joosten, L. A. B., & van den Berg, W. B. (2006). Potential new targets in arthritis therapy: interleukin (IL)-17 and its relation to tumour necrosis factor and IL-1 in experimental arthritis. *Ann Rheum Dis 65*, iii29−iii33.

Kohno, T., Louie, J. S., & Stevens, S. R. (2005a). Differences in Fc receptor and C1q binding in tumor necrosis factor (TNF) antagonists may contribute to differences in mechanisms of action. *J Invest Dermatol 124*(4), A111.

Kohno, T., Tam, L., Ting, T., Bass, R. B., & Stevens, S. R. (2005b). Adalimumab and infliximab bind to Fc-receptor and C1q and generate immunoprecipitation: a different mechanism from etanercept. *Arthritis Rheum 52*(9), S562−S563.

Kollias, G. (2005). TNF pathophysiology in murine models of chronic inflammation and autoimmunity. *Semin Arthritis Rheum 34*, 3−6.

Kollias, G., Kontoyiannis, D., Douni, E., & Kassiotis, G. (2002). The role of TNF/TNFR in organ-specific and systemic autoimmunity: implications for the design of optimized 'anti-TNF' therapies. *Curr Dir Autoimmun 5*, 30−50.

Kontoyiannis, D., Pasparakis, M., Pizarro, T. T., Cominelli, F., & Kollias, G. (1999). Impaired on/off regulation of TNF biosynthesis in mice lacking TNF AU-rich elements: implications for joint and gut-associated immunopathologies. *Immunity 10*, 387−398.

Koshy, P. J., Henderson, N., Logan, C., Life, P. F., Cawston, T. E., & Rowan, A. D. (2002). Interleukin 17 induces cartilage collagen breakdown: novel synergistic effects in combination with proinflammatory cytokines. *Ann Rheum Dis 61*, 704−713.

Kotake, S. l., Udagawa, N., Hakoda, M., Mogi, M., Yano, K., Tsuda, E., et al. (2001). Activated human T cells directly induce osteoclastogenesis from human monocytes. *Arthritis Rheum 44*, 1003−1012.

Kraan, M. C., De Koster, B. M., Elferink, J. G. R., Post, W. J., Breedveld, F. C., & Tak, P. P. (2000a). Inhibition of neutrophil migration soon after initiation of treatment with leflunomide or methotrexate in patients with rheumatoid arthritis: findings in a prospective, randomized, double-blind clinical trial in fifteen patients. *Arthritis Rheum 43*, 1488−1495.

Kraan, M. C., Reece, R. J., Barg, E. C., Smeets, T. J. M., Farnell, J., Rosenburg, R., et al. (2000b). Modulation of inflammation and metalloproteinase expression in synovial tissue by leflunomide and methotrexate in patients with active rheumatoid arthritis: findings in a prospective, randomized, double-blind, parallel-design clinical trial in thirty-nine patients at two centers. *Arthritis Rheum 43*, 1820−1830.

Kratz, A., Campos-Neto, A., Hanson, M., & Ruddle, N. (1996). Chronic inflammation caused by lymphotoxin is lymphoid neogenesis. *J Exp Med 183*, 1461−1472.

Kristensen, M., Chu, C. Q., Eedy, D. J., Feldmann, M., Brennan, F. M., & Breathnach, S. M. (1993). Localization of tumour necrosis factor-alpha (TNF-alpha) and its receptors in normal and psoriatic skin: epidermal cells express the 55-kD but not the 75-kD TNF receptor. *Clin Exp Immunol 94*, 354−362.

Kruger-Krasagakis, S., Galanopoulos, V. K., Giannikaki, L., Stefanidou, M., & Tosca, A. D. (2006). Programmed cell death of keratinocytes in infliximab-treated plaque-type psoriasis. *Br J Dermatol 154*, 460−466.

Kruithof, E., De Rycke, L., Roth, J., Mielants, H., Van den Bosch, F., De Keyser, F., et al. (2005). Immunomodulatory effects of etanercept on peripheral joint synovitis in the spondylarthropathies. *Arthritis Rheum 52*, 3898−3909.

Ksontini, R. M. D., MacKay, S. L. D. P., & Moldawer, L. L. P. (1998). Revisiting the role of tumor necrosis factor alpha and the response to surgical injury and inflammation. *Arch Surg 133*, 558−567.

Kubota, A., Hasegawa, K., Suguro, T., & Koshihara, Y. (2004). Tumor necrosis factor-á promotes the expression of ostoprotegrin in rheumatoid synovial fibroblasts. *J Rheumatol 31*, 426−435.

Lamprecht, P., Voswinkel, J., Lilienthal, T., Nolle, B., Heller, M., Gross, W. L., et al. (2002). Effectiveness of TNF-á blockade with infliximab in refractory Wegener's granulomatosis. *Rheumatology 41*, 1303−1307.

Landewé, R., van der Heijde, D., Klareskog, L., van Vollenhoven, R., & Fatenejad, S. (2006). Disconnect between inflammation and joint destruction after treatment with etanercept plus methotrexate: results from the trial of etanercept and methotrexate with radiographic and patient outcomes. *Arthritis Rheum 54*, 3119−3125.

Lange, F., Bajtner, E., Rintisch, C., Nandakumar, K. S., Sack, U., & Holmdahl, R. (2005). Methotrexate ameliorates T cell dependent autoimmune arthritis and encephalomyelitis but not antibody induced or fibroblast induced arthritis. *Ann Rheum Dis 64*, 599−605.

Lee, C. K., Lee, E. Y., Chung, S. M., Mun, S. H., Yoo, B., Moon, H. B., et al. (2004). Effects of disease-modifying antirheumatic drugs and antiinflammatory cytokines on human osteoclastogenesis through interaction with receptor activator of nuclear factor kappa-B, osteoprotegerin, and receptor activator of nuclear factor kappa-B ligand. *Arthritis Rheum 50*, 3831−3843.

Lee, S. J., & Kavanaugh, A. (2005). Adalimumab for the treatment of rheumatoid arthritis. *Therapy 2*, 13−21.

Legler, D. F., Micheau, O., Doucey, M. -A., Tschopp, J., & Bron, C. (2003). Recruitment of TNF receptor 1 to lipid rafts is essential for TNF[alpha]-mediated NF-[kappa]B activation. *Immunity 18*, 655−664.

Leonardi, C. L., Powers, J. L., Matheson, R. T., Goffe, B. S., Zitnik, R., Wang, A., et al. (2003). Etanercept as monotherapy in patients with psoriasis. *N Engl J Med 349*, 2014−2022.

Liepinsh, D. J., Grivennikov, S. I., Klarmann, K. D., Lagarkova, M. A., Drutskaya, M. S., Lockett, S. J., et al. (2006). Novel lymphotoxin alpha (LTalpha) knockout mice with unperturbed tumor necrosis factor expression: reassessing LTalpha biological functions. *Mol Cell Biol 26*, 4214−4225.

Lin, W. W., & Karin, M. (2007). A cytokine-mediated link between innate immunity, inflammation, and cancer. *J Clin Invest 117*, 1175−1183.

Lipsky, P. E., van der Heijde, D. M. F. M., St. Clair, E. W., Furst, D. E., Breedveld, F. C., Kalden, J. R., et al. (2000). Infliximab and methotrexate in the treatment of rheumatoid arthritis. *New Engl J Med 343*, 1594−1602.

Listing, J., Strangfeld, A., Kary, A., Rau, R., von Hinueber, U., Stoyanova-Scholz, M., et al. (2005). Infections in patients with rheumatoid arthritis treated with biologic agents. *Arthritis Rheum 52*, 3403−3412.

Lobo, E. D., Hansen, R. J., & Balthasar, J. P. (2004). Antibody pharmacokinetics and pharmacodynamics. *J Pharm Sci 93*, 2645−2668.

Lorenz, H. M., Antoni, C., Valerius, T., Repp, R., Grünke, M., Schwerdtner, N., et al. (1996). 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 156*, 1646−1653.

Lugering, A., Schmidt, M., Lugering, N., Pauels, H. G., Domschke, W., & Kucharzik, T. (2001). Infliximab induces apoptosis in monocytes from patients with chronic active Crohn's disease by using a caspase-dependent pathway. *Gastroenterology 121*, 1145−1457.

Luther, S. A., Bidgol, A., Hargreaves, D. C., Schmidt, A., Xu, Y., Paniyadi, J., et al. (2002). Differing activities of homeostatic chemokines CCL19, CCL21, and CXCL12 in lymphocyte and dendritic cell recruitment and lymphoid neogenesis. *J Immunol 169*, 424−433.

Maini, R. N., & Feldmann, M. (2002). How does infliximab work in rheumatoid arthritis? *Arthritis Res 4*, S22−S28.

Maini, R. N., Breedveld, F. C., Kalden, J. R., Smolen, J. S., Davis, D., MacFarlane, J. D., et al. (1998). 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 41*, 1552−1563.

Maini, R. N., Breedveld, F. C., Kalden, J. R., Smolen, J. S., Furst, D., Weisman, M. H., et al. (2004). Sustained improvement over two years in physical function, structural damage, and signs and symptoms among patients with rheumatoid arthritis treated with infliximab and methotrexate. *Arthritis Rheum 50*, 1051−1065.

Maini, R. N., Taylor, P. C., Paleolog, E., Charles, P., Ballara, S., Brennan, F. M., et al. (1999). Anti-tumour necrosis factor specific antibody (infliximab) treatment provides insights into the pathophysiology of rheumatoid arthritis. *Ann Rheum Dis 58*(Suppl 1), I56−I60.

Malaviya, R., Sun, Y., Tan, J. K., Wang, A., Magliocco, M., Yao, M., et al. (2006a). Etanercept induces apoptosis of dermal dendritic cells in psoriatic plaques of responding patients. *J Am Acad Dermatol 55*, 590−597.

Malaviya, R., Sun, Y., Tan, J. K., Magliocco, M., & Gottlieb, A. B. (2006b). Induction of lesional and circulating leukocyte apoptosis by infliximab in a patient with moderate to severe psoriasis. *J Drugs Dermatol 5*, 890−893.

Manes, T. D., Pober, J. S., & Kluger, M. S. (2006). Endothelial cell-T lymphocyte interactions: iP-10 stimulates rapid transendothelial migration of human effector but not central memory CD4+T cells. Requirements for shear stress and adhesion molecules. *Transplantation 82*, S9−S14.

Mangashetti, L. S., Khapli, S. M., & Wani, M. R. (2005). IL-4 inhibits bone-resorbing activity of mature osteoclasts by affecting NF-kappa and calcium signaling. *J Immunol 175*, 917−925.

Markham, T., Mullan, R., Golden-Mason, L., Rogers, S., Bresnihan, B., FitzGerald, O., et al. (2006). Resolution of endothelial activation and down-regulation of Tie2 receptor in psoriatic skin after infliximab therapy. *J Am Acad Dermatol 54*, 1003−1012.

Maurice, M. M., Van Der Graaff, W. L., Leow, A., Breedveld, F. C., Van Lier, R. A. W., & Verweij, C. L. (1999). Treatment with monoclonal anti-tumor necrosis factor α antibody results in an accumulation of Th1 CD4+T cells in the peripheral blood of patients with rheumatoid arthritis. *Arthritis Rheum 42*, 2166−2173.

Mayo, M. W., & Baldwin, A. S. (2000). The transcription factor NF-kappaB: control of oncogenesis and cancer therapy resistance. *Biochim Biophys Acta 1470*, M55−M62.

McDevitt, H., Munson, S., Ettinger, R., & Wu, A. (2002). Multiple roles for tumor necrosis factor-alpha and lymphotoxin alpha/beta in immunity and autoimmunity. *Arthritis Res 4*(Suppl 3), S141−S152.

Mease, P. J., Ritchlin, C. T., Martin, R. W., Gottlieb, A. B., Baumgartner, S. W., Burge, D. J., et al. (2004). Pneumococcal vaccine response in psoriatic arthritis patients during treatment with etanercept. *J Rheumatol 31*, 1356−1361.

Medvedev, A. E., Espevik, T., Ranges, G., & Sundan, A. (1996). Distinct roles of the two tumor necrosis factor (TNF) receptors in modulating TNF and lymphotoxin alpha effects. *J Biol Chem 271*, 9778−9784.

Micheau, O., & Tschopp, J. (2003). Induction of TNF receptor I-mediated apoptosis via two sequential signaling complexes. *Cell 114*, 181−190.

Mitoma, H., Horiuchi, T., & Tsukamoto, H. (2004). Binding activities of infliximab and etanercept to transmembrane tumor necrosis factor-alpha. *Gastroenterology 26*, 934−935.

Mitoma, H., Horiuchi, T., Hatta, N., Tsukamoto, H., Harashima, S. -I., Kikuchi, Y., et al. (2005). Infliximab induces potent anti-inflammatory responses by outside-to-inside signals through transmembrane TNF-[945]. *Gastroenterology 128*, 376−392.

Mohler, K., Torrance, D., Smith, C., Goodwin, R., Stremler, K., Fung, V., et al. (1993). Soluble tumor necrosis factor (TNF) receptors are effective therapeutic agents in lethal endotoxemia and function simultaneously as both TNF carriers and TNF antagonists. *J Immunol 151*, 1548−1561.

Monastra, G., Cabrelle, A., Zambon, A., Rosato, A., Macino, B., Collavo, D., et al. (1996). Membrane form of TNFalpha induces both cell lysis and apoptosis in susceptible target cells. *Cell Immunol 171*, 102−110.

Montesinos, M. C., Yap, J. S., Desai, A., Posadas, I., McCrary, C. T., & Cronstein, B. N. (2000). Reversal of the antiinflammatory effects of methotrexate by the nonselective adenosine receptor antagonists theophylline and caffeine: evidence that the antiinflammatory effects of methotrexate are mediated via multiple adenosine receptors in rat adjuvant arthritis. *Arthritis Rheum 43*, 656−663.

Moore, R. J., Owens, D. M., Stamp, G., Arnott, C., Burke, F., East, N., et al. (1999). Mice deficient in tumor necrosis factor-alpha are resistant to skin carcinogenesis. *Nat Med 5*, 828−831.

Moreira, A. L., Sampaio, E. P., Zmuidzinas, A., Frindt, P., Smith, K. A., & Kaplan, G. (1993). Thalidomide exerts its inhibitory action on tumor necrosis factor alpha by enhancing mRNA degradation. *J Exp Med 177*, 1675−1680.

Moreland, L. W., Baumgartner, S. W., Schiff, M. H., Tindall, E. A., Fleischmann, R. M., Weaver, A. L., et al. (1997). Treatment of rheumatoid arthritis with a recombinant human tumor necrosis factor receptor (p75)-Fc fusion protein. *N Engl J Med 337*, 141−147.

Moreland, L. W., Bucy, R. P., Weinblatt, M. E., Mohler, K. M., Spencer, G. G. T., & Chatham, W. W. (2002). Immune function in patients with rheumatoid arthritis treated with etanercept. *Clin Immunol 103*, 13−21.

Möttönen, M., Heikkinen, J., Mustonen, L., Isomäki, P., Luukkainen, R., & Lassila, O. (2005). CD4+CD25+T cells with the phenotypic and functional characteristics of regulatory T cells are enriched in the synovial fluid of patients with rheumatoid arthritis. *Clin Exp Immunol 140*, 360−367.

Mueller, R. B., Skapenko, A., Grunke, M., Wendler, J., Stuhlmuller, B., Kalden, J. R., et al. (2005). Regulation of myeloid cell function and major histocompatibility complex class II expression by tumor necrosis factor. *Arthritis Rheum 52*, 451−460.

Murphy, M., Walter, B. N., Pike-Nobile, L., Fanger, N. A., Guyre, P. M., Browning, J. L., et al. (1998). Expression of the lymphotoxin beta receptor on follicular stromal cells in human lymphoid tissues. *Cell Death Differ 5*, 497−505.

Mussener, A., Litton, M. J., Lindroos, E., & Klareskog, L. (1997). Cytokine production in synovial tissue of mice with collagen-induced arthritis (CIA). *Clin Exp Immunol 107*, 485−493.

Nesbitt, A. M., Fossati, G., & Brown, D. T. (2006). Comparison of certolizumab pegol, etanercept, adalimumab, and infliximab: effect on lipopolysaccharide-induced cytokine production by human peripheral blood monocytes. *Am J Gastroenterol 101*(Suppl 2), S420−S470.

Nestorov, I. (2005a). Clinical pharmacokinetics of TNF antagonists: how do they differ? *Semin Arthritis Rheum 34*, 12−18.

Nestorov, I. (2005b). Clinical pharmacokinetics of tumor necrosis factor antagonists. *J Rheumatol Suppl 74*, 13−18.

Nickoloff, B., Karabin, G. D., Barker, J. N., Griffiths, C. E., Sarma, V., Mitra, R. S., et al. (1991). Cellular localization of interleukin-8 and its inducer, tumor necrosis factor-alpha in psoriasis. *Am J Pathol 138*, 129−140.

Nikas, S. N., Voulgari, P. V., Alamanos, Y., Papadopoulos, C. G., Venetsanopoulou, A. I., Georgiadis, A. N., et al. (2006). Efficacy and safety of switching from infliximab to adalimumab: a comparative controlled study. *Ann Rheum Dis 65*, 257−260.

Nishikawa, S. -I., Honda, K., Vieira, P., & Yoshida, H. (2003). Organogenesis of peripheral lymphoid organs. *Immunol Rev 195*, 72−80.

O'Gradaigh, D., Ireland, D., Bord, S., & Compston, J. E. (2004). Joint erosion in rheumatoid arthritis: interactions between tumour necrosis factor á, interleukin 1, and receptor activator of nuclear factor κB ligand (RANKL) regulate osteoclasts. *Ann Rheum Dis 63*, 354−359.

Paleolog, E. M., Hunt, M., Elliott, M. J., Feldmann, M., Maini, R. N., & Woody, J. N. (1996). Deactivation of vascular endothelium by monoclonal anti-tumor necrosis factor alpha antibody in rheumatoid arthritis. *Arthritis Rheum 39*, 1082−1091.

Paleolog, E. M., Young, S., Stark, A. C., McCloskey, R. V., Feldmann, M., & Maini, R. N. (1998). Modulation of angiogenic vascular endothelial growth factor by tumor necrosis factor alpha and interleukin-1 in rheumatoid arthritis. *Arthritis Rheum 41*, 1258−1265.

Palmqvist, P., Lundberg, P., Persson, E., Johansson, A., Lundgren, I., Lie, A., et al. (2006). Inhibition of hormone and cytokine-stimulated ostoclastogenesis and bone resorption by interleukin-4 and interleukin-13 is associated with increased osteoprotegerin and decreased RANKL and RANK in a STAT6-dependent pathway. *J Biol Chem 281*, 2414−2429.

Pasparakis, M., Alexopoulou, L., Episkopou, V., & Kollias, G. (1996). Immune and inflammatory responses in TNF alpha-deficient mice: a critical requirement for TNF alpha in the formation of primary B cell follicles, follicular dendritic cell networks and germinal centers, and in the maturation of the humoral immune response. *J Exp Med 184*, 1397−1411.

Pasparakis, M., Alexopoulou, L., Grell, M., Pfizenmaier, K., Bluethmann, H., & Kollias, G. (1997). Peyer's patch organogenesis is intact yet formation of B lymphocyte follicles is defective in peripheral lymphoid organs of mice deficient for tumor necrosis factor and its 55-kDa receptor. *PNAS 94*, 6319−6323.

Paul, A. T., Gohil, A. M., & Bhutani, K. K. (2006). Modulating TNF-alpha signaling with natural products. *Drug Discov Today 11*, 725−732.

Petit, A. R., Walsh, N. C., Manning, C., Goldring, S. R., & Gravallese, E. M. (2006). RANKL protein is expressed at the pannus–bone interface at sites of articular bone erosion in rheumatoid arthritis. *Rheumatology 45*, 1068−1076.

Pikarsky, E., Porat, R. M., Stein, I., Abramovitch, R., Amit, S., Kasem, S., et al. (2004). NF-kappaB functions as a tumour promoter in inflammation-associated cancer. *Nature 431*, 461−466.

Pittoni, V., Bombardieri, M., Spinelli, F. R., Scrivo, R., Alessandri, C., Conti, F., et al. (2002). Anti-tumour necrosis factor (TNF) {alpha} treatment of rheumatoid arthritis (infliximab) selectively down regulates the production of interleukin (IL) 18 but not of IL12 and IL13. *Ann Rheum Dis 61*, 723−725.

Radaev, S., & Sun, P. (2002). Recognition of immunoglobulins by Fcgamma receptors. *Mol Immunol 38*, 1073−1083.

Redlich, K., Hayer, S., Maier, A., Dunstan, C. R., Tohidast-Akrad, M., Lang, S., et al. (2002a). Tumor necrosis factor alpha-mediated joint destruction is inhibited by targeting osteoclasts with osteoprotegerin. *Arthritis Rheum 46*, 785−792.

Redlich, K., Hayer, S., Ricci, R., David, J. P., Tohidast-Akrad, M., Kollias, G., et al. (2002b). Osteoclasts are essential for TNF-alpha-mediated joint destruction. *J Clin Invest 110*, 1419−1427.

Remicade (infliximab) prescribing information. (2006). *Centocor, Inc. Malvern, PA.*

Rennert, P., Browning, J., Mebius, R., Mackay, F., & Hochman, P. (1996). Surface lymphotoxin alpha/beta complex is required for the development of peripheral lymphoid organs. *J Exp Med 184*, 1999−2006.

Ritchlin, C. T., Haas-Smith, S. A., Li, P., Hicks, D. G., & Schwarz, E. M. (2003). Mechanisms of TNF-a- and RANKL-mediated osteoclastogenesis and bone resorption in psoriatic arthritis. *J Clin Invest 111*, 821−831.

Roach, D. R., Briscoe, H., Saunders, B., France, M. P., Riminton, S., & Britton, W. J. (2001). Secreted lymphotoxin-alpha is essential for the control of an intracellular bacterial infection. *J Exp Med 193*, 239−246.

Rothe, J., Lesslauer, W., Lotscher, H., Lang, Y., Koebel, P., Kontgen, F., et al. (1993). Mice lacking the tumour necrosis factor receptor 1 are resistant to IMF-mediated toxicity but highly susceptible to infection by *Listeria monocytogenes*. *Nature 364*, 798−802.

Roux-Lombard, P., Punzi, L., Hasler, F., Bas, S., Todesco, S., Gallati, H., et al. (1993). Soluble tumor necrosis factor receptors in human inflammatory synovial fluids. *Arthritis Rheum 36*, 485−489.

Rutgeerts, P., Sandborn, W. J., Feagan, B. G., Reinisch, W., Olson, A., Johanns, J., et al. (2005). Infliximab for induction and maintenance therapy for ulcerative colitis. *N Engl J Med 353*, 2462−2476.

Salfeld, J., Kaymakcalan, Z., Tracey, D., Robert, A., & Kamen, R. (1998). Generation of fully human anti-TNF antibody D2E7. *Arthritis Rheum 41*, S57.

Sandborn, W. (2005). New concepts in anti-tumor necrosis factor therapy for inflammatory bowel disease. *Rev Gastroenterol Disord 5*, 10−18.

Sandborn, W. J., Hanauer, S. B., Katz, S., Safdi, M., Wolf, D. G., Baerg, R. D., et al. (2001). Etanercept for active Crohn's disease: a randomized double-blind, placebo-controlled trial. *Gastroenterology 121*, 1088−1094.

Sands, B. E. (2004). Why do anti-tumor necrosis factor antibodies work in Crohn's disease? *Rev Gastroenterol Disord 4*, S10−S17.

Santora, L. C., Kaymakcalan, Z., Sakorafas, P., Krull, I. S., & Grant, K. (2001). Characterization of noncovalent complexes of recombinant human monoclonal antibody and antigen using cation exchange, size exclusion chromatography, and BIAcore. *Anal Biochem 299*, 119−129.

Sasso, E. H., Barber, C. V., Nardella, F. A., Yount, W. J., & Mannik, M. (1988). Antigenic specificities of human monoclonal and polyclonal IgM rheumatoid factors. The Cγ2-Cγ3 interface region contains the major determinants. *J Immunol 40*, 3098−3107.

Saxne, T., Palladino, M. J., Heinegard, D., Talal, N., & Wollheim, F. (1988). Detection of tumor necrosis factor alpha but not tumor necrosis factor beta in rheumatoid arthritis synovial fluid and serum. *Arthritis Rheum 31*, 1041−1045.

Scallon, B. J., Moore, M. A., Trinh, H., Knight, D. M., & Ghrayeb, J. (1995). Chimeric anti-TNF-alpha monoclonal antibody cA2 binds recombinant transmembrane TNF-alpha and activates immune effector functions. *Cytokine 7*, 251−259.

Scallon, B., Cai, A., Solowski, N., Rosenberg, A., Song, X. -Y., Shealy, D., et al. (2002). Binding and functional comparisons of two types of tumor necrosis factor antagonists. *J Pharmacol Exp Ther 301*, 418−426.

Schett, G., Hayer, S., Zwerina, J., Redlich, K., & Smolen, J. S. (2005). Mechanisms of disease: the link between RANKL and arthritic bone disease. *Nat Clin Practice Rheum 1*, 47−53.

Schett, G. (2006). Rheumatoid arthritis: inflammation and bone loss. *Wien Med Wochenschr 156*, 34−41.

Schiff, M. H., Burmester, G. R., Kent, J. D., Pangan, A. L., Kupper, H., Spencer-Green, G. T., et al. (2006a). Safety analyses of adalimumab (HUMIRA) in global clinical trials and US postmarketing surveillance of patients with rheumatoid arthritis. *Ann Rheum Dis 65*, 889−894.

Schiff, M., Keiserman, M., Codding, C., Songcharoen, S., Berman, A., Nayiager, S., et al. (2006b). The efficacy and safety of abatacept or infliximab in RA patients with an inadequate response to MTX: results from a 1-year double-blind, randomized, placebo-controlled trial. *Arthritis Rheum 54*, 92.

Schneider-Brachert, W., Tchikov, V., Neumeyer, J., Jakob, M., Winoto-Morbach, S., Held-Feindt, J., et al. (2004). Compartmentalization of TNF receptor 1 signaling: internalized TNF receptosomes as death signaling vesicles. *Immunity 21*, 415−428.

Schottelius, A. J. G., Moldawer, L. L., Dinarello, C. A., Asadullah, K., Sterry, W., & Edwards, C. K. (2004). Biology of tumor necrosis factor-alpha-implications for psoriasis. *Exp Dermatol 13*, 193−222.

Schreiber, S., Rutgeerts, P., Fedorak, R. N., Khaliq-Kareemi, M., Kamm, M. A., Boivin, M., et al. (2005). A randomized, placebo-controlled trial of certolizumab pegol CDP870 for treatment of Crohn's disease. *Gastroenterology 129*, 807−818.

Serbina, N. V., Salazar-Mather, T. P., Biron, C. A., Kuziel, W. A., & Pamer, E. G. (2003). TNF/iNOS-producing dendritic cells mediate innate immune defense against bacterial infection. *Immunity 19*, 59−70.

Shen, C., Assche, G. V., Colpaert, S., Maerten, P., Geboes, K., Rutgeerts, P., et al. (2005). Adalimumab induces apoptosis of human monocytes: a comparative study with infliximab and etanercept. *Aliment Pharmacol Ther 21*, 251−258.

Shen, C. P., Van Assche, G. P. M. D., Rutgeerts, P. P. M. D., & Ceuppens, J. L. P. M. D. (2006). Caspase activation and apoptosis induction by adalimumab: demonstration in vitro and in vivo in a chimeric mouse model. *Inflamm Bowel Dis 12*, 22−28.

Smeets, T. J. M., Kraan, M. C., van Loon, M. E., & Tak, P. -P. (2003). Tumor necrosis factor alpha blockade reduces the synovial cell infiltrate early after initiation of treatment, but apparently not by induction of apoptosis in synovial tissue. *Arthritis Rheum 48*, 2155−2162.

Smolen, J. S., Han, C., Bala, M., Maini, R. N., Kalden, J. R., van der Heijde, D., et al. (2005). Evidence of radiographic benefit of treatment with infliximab plus methotrexate in rheumatoid arthritis patients who had no clinical improvement: a detailed subanalysis of data from the anti-tumor necrosis factor trial in rheumatoid arthritis with concomitant therapy study. *Arthritis Rheum 52*, 1020−1030.

Smolen, J. S., van der Heijde, D. M., St. Clair, E. W., Emery, P., Bathon, J. M., Keystone, E., et al. (2006). Predictors of joint damage in patients with early rheumatoid arthritis treated with high-dose methotrexate with or without concomitant infliximab: results from the ASPIRE trial. *Arthritis Rheum 54*, 702−710.

Smookler, D. S., Mohammed, F. F., Kassiri, Z., Duncan, G. S., Mak, T. W., & Khokha, R. (2006). Cutting edge: tissue inhibitor of metalloproteinase 3 regulates TNF-dependent systemic inflammation. *J Immunol 176*, 721−725.

Smyth, M. J., Johnstone, R. W., Cretney, E., Haynes, N. M., Sedgwick, J. D., Korner, H., et al. (1999). Multiple deficiencies underlie NK cell inactivity in lymphotoxin-alpha gene-targeted mice. *J Immunol 163*, 1350−1353.

St. Clair, E. W., Wagner, C. L., Fasanmade, A. A., Wang, B., Schaible, T., Kavanaugh, A., et al. (2002). The relationship of serum infliximab concentrations to clinical improvement in rheumatoid arthritis: results from ATTRACT, a multicenter, randomized, double-blind, placebo-controlled trial. *Arthritis Rheum 46*, 1451−1459.

St. Clair, E. W., van der Heidje, D., Smolen, J. S., Maini, R., Bathon, J., Emery, P., et al. (2004). Combination of infliximab and methotrexate therapy for early rheumatoid arthritis: a randomized, controlled trial. *Arthritis Rheum 50*, 3432−3443.

Straub, R. H., Härle, P., Sarzi-Puttini, P., & Cutolo, M. (2006). Tumor necrosis factor-neutralizing therapies improve altered hormone axes: an alternative mode of antiinflammatory action. *Arthritis Rheum 54*, 2039−2046.

Suffredini, A. F., Reda, D., Banks, S. M., Tropea, M., Agosti, J. M., & Miller, R. (1995). Effects of recombinant dimeric TNF receptor on human inflammatory responses following intravenous endotoxin administration. *J Immunol 155*, 5038−5045.

Tak, P. P. (2005). Effects of infliximab treatment on rheumatoid synovial tissue. *J Rheumatol Suppl 74*, 31−34.

Tak, P. P., & Firestein, G. S. (1999). Apoptosis in rheumatoid arthritis. In J. D. Winkler (Ed.), *Apoptosis and Inflammation (Progress in Inflammation Research)* (pp. 149−162). Basel: Birkhäuser Publishing Ltd.

Tak, P. P., Smeets, T. J. M., Daha, M. R., Kluin, P. M., Meijers, K. A. E., Brand, R., et al. (1997). Analysis of the synovial cell infiltrate in early rheumatoid synovial tissue in relation to local disease activity. *Arthritis Rheum 40*, 217−225.

Tak, P. P., Taylor, P. C., Breedveld, F. C., Smeets, T. J., Daha, M. R., Kluin, P. M., et al. (1996). Decrease in cellularity and expression of adhesion molecules by anti-tumor necrosis factor alpha monoclonal antibody treatment in patients with rheumatoid arthritis. *Arthritis Rheum 39*, 1077−1081.

Takayanagi, H., Iizuka, H., Juji, T., Nakagawa, T., Yamamoto, A., Miyazaki, T., et al. (2000). Involvement of receptor activator of nuclear factor κB ligand/osteoclast differentiation factor in osteoclastogenesis from synoviocytes in rheumatoid arthritis. *Arthritis Rheum 43*, 259−269.

Takemura, S., Braun, A., Crowson, C., Kurtin, P. J., Cofield, R. H., O'Fallon, W. M., et al. (2001). Lymphoid neogenesis in rheumatoid synovitis. *J Immunol 167*, 1072−1080.

Tas, S. W., Remans, P. H. J., Reedquist, K. A., & Tak, P. P. (2005). Signal transduction pathways and transcription factors as therapeutic targets in inflammatory disease: towards innovative antirheumatic therapy. *Curr Pharm Dis 11*, 581−611.

Taylor, P. C., Peters, A. M., Paleolog, E., Chapman, P. T., Elliott, M. J., McCloskey, R., et al. (2000). Reduction of chemokine levels and leukocyte traffic to joints by tumor necrosis factor alpha blockade in patients with rheumatoid arthritis. *Arthritis Rheum 43*, 38−47.

ten Hove, T., van Montfrans, C., Peppelenbosch, M. P., & van Deventer, S. J. H. (2002). Infliximab treatment induces apoptosis of lamina propria T lymphocytes in Crohn's disease. *Gut 50*, 206−211.

Terslev, L., Torp-Pedersen, S., Qvistgaard, E., Kristoffersen, H., Rogind, H., Danneskiold-Samsoe, B., et al. (2003). Effects of treatment with etanercept (Enbrel, TNRF:Fc) on rheumatoid arthritis evaluated by Doppler ultrasonography. *Ann Rheum Dis 62*, 178−181.

Thorbecke, G., Shah, R., Leu, C., Kuruvilla, A., Hardison, A., & Palladino, M. (1992). Involvement of endogeneous tumor necrosis factor alpha and transforming growth factor beta during induction of collagen type II arthritis in mice. *Proc Natl Acad Sci U S A 89*, 7375−7379.

Tutuncu, Z., Kavanaugh, A., Zvaifler, N., Corr, M., Deutsch, R., & Boyle, D. (2005). Fcgamma receptor type IIIA polymorphisms influence treatment outcomes in patients with inflammatory arthritis treated with tumor necrosis factor alpha-blocking agents. *Arthritis Rheum 52*, 2693−2696.

Ulfgren, A. K., Lindblad, S., Klareskog, L., Andersson, J., & Andersson, U. (1995). Detection of cytokine producing cells in the synovial membrane from patients with rheumatoid arthritis. *Ann Rheum Dis 54*, 654−661.

Ulfgren, A. K., Andersson, U., Engström, M., Klareskog, L., Maini, R. N., & Taylor, P. C. (2000). Systemic anti-tumor necrosis factor alpha therapy in rheumatoid arthritis down-regulates synovial tumor necrosis factor alpha synthesis. *Arthritis Rheum 43*, 2391−2396.

Utz, J. P., Limper, A. H., Kalra, S., Specks, U., Scott, J. P., Vuk-Pavlovic, Z., et al. (2003). Etanercept for the treatment of stage II and III progressive pulmonary sarcoidosis. *Chest 124*, 177−185.

Valencia, X., Stephens, G., Goldbach-Mansky, R., Wilson, M., Shevach, E. M., & Lipsky, P. E. (2006). TNF downmodulates the function of human CD4+ CD25hi T-regulatory cells. *Blood 108*, 253−261.

Van den Brande, J., Braat, H., van den Brink, G., Versteeg, H., Bauer, C., Hoedemaeker, I., et al. (2003). Infliximab but not etanercept induces apoptosis in lamina propria T-lymphocytes from patients with Crohn's disease. *Gastroenterology 124*, 1774−1785.

van der Laken, C. J., Voskuyl, A. E., Roos, J. C., Stigter van Walsum, M., de Groot, E. R., Wolbink, G., et al. (2006). Imaging and serum analysis of immune complex formation of radiolabeled infliximab and anti-infliximab in responders and non-responders to therapy for rheumatoid arthritis. *Ann Rheum Dis 66*, 253−256.

Van Deventer, S. (1997). Tumour necrosis factor and Crohn's disease. *Gut 40*, 443−448.

van Lent, P. L., Blom, A. B., Grevers, L., Sloetjes, A., & van den Berg, W. B. (2006). TLR-4 Induced FcγR expression potentiates early onset of joint inflammation and cartilage destruction during immune complex arthritis: tlr4 largely regulates FcγR expression by IL-10. *Ann Rheum Dis 66*, 334−340.

van Lieshout, A. W. T., Barrera, P., Smeets, R. L., Pesman, G. J., van Riel, P. L. C. M., van den Berg, W. B., et al. (2005). Inhibition of TNFalpha during maturation of dendritic cells results in the development of semi-mature cells: a potential mechanism for the beneficial effects of TNFalpha blockade in rheumatoid arthritis. *Ann Rheum Dis 64*, 408−414.

van Vollenhoven, R., Harju, A., Brannemark, S., & Klareskog, L. (2003). Treatment with infliximab (Remicade) when etanercept (Enbrel) has failed or vice versa: data from the STURE registry showing that switching tumour necrosis factor alpha blockers can make sense. *Ann Rheum Dis 62*, 1195−1198.

Vigna-Pérez, M., Abud, M. C., Portillo, S. H., Alvarado, S. B., Cuevas, O. E., Moreno, V. R., et al. (2005). Immune effects of therapy with Adalimumab in patients with rheumatoid arthritis. *Clin Exp Immunol 141*, 372−380.

Vis, M., Havaardsholm, E. A., Haugeberg, G., Uhlig, T., Voskuyl, A. E., van de Stadt, R. J., et al. (2006). Evaluation of bone mineral density, bone metabolism, osteoprotegerin and receptor activator of the NFκB ligand serum levels during treatment with infliximab in patients with rheumatoid arthritis. *Ann Rheum Dis 65*, 1495−1499.

Voon, D. C., Subrata, L. S., Karimi, M., Ulgiati, D., & Abraham, L. J. (2004). TNF and phorbol esters induce lymphotoxin-beta expression through distinct pathways involving Ets and NF-kappaB family members. *J Immunol 172*, 4332−4341.

Walsh, N. C., Crotti, T. N., Goldring, S. R., & Gravallese, E. M. (2005). Rheumatic diseases: the effects of inflammation on bone. *Immunol Rev 208*, 228−251.

Ware, C. F. (2005). Network communications: lymphotoxins, LIGHT, and TNF. *Annu Rev Immunol 23*, 787−819.

Ware, C., Crowe, P., Grayson, M., Androlewicz, M., & Browning, J. (1992). Expression of surface lymphotoxin and tumor necrosis factor on activated T, B, and natural killer cells. *J Immunol 149*, 3881−3888.

Wee, S., Pascual, M., Eason, J. D., Schoenfeld, D. A., Phelan, J., Boskovic, S., et al. (1997). Biological effects and fate of a soluble, dimeric, 80-Kda tumor necrosis factor receptor in renal transplant recipients who receive OKT3 therapy 1. *Transplantation 63*, 570−577.

Wegener's Granulomatosis Etanercept Trial (WGET) Research Group. (2005). Etanercept plus standard therapy for Wegener's granulomatosis. *N Engl J Med 352*, 351−361.

Weinblatt, M. E., Keystone, E. C., Furst, D. E., Moreland, L. W., Weisman, M. H., Birbara, C. A., et al. (2003). Adalimumab, a fully human anti-tumor necrosis factor alpha monoclonal antibody, for the treatment of rheumatoid arthritis in patients taking concomitant methotrexate: the ARMADA trial. *Arthritis Rheum 48*, 35−45.

Weinblatt, M. E., Kremer, J. M., Bankhurst, A. D., Bulpitt, K. J., Fleischmann, R. M., Fox, R. I., et al. (1999). A trial of etanercept, a recombinant tumor necrosis factor receptor:Fc fusion protein, in patients with rheumatoid arthritis receiving methotrexate. *N Engl J Med 340*, 253−259.

Weir, N., Athwal, D., Brown, D., Foulkes, R., Kollias, G. l., Nesbitt, A., et al. (2006). A new generation of high-affinity humanized PEGylated Fab' fragment anti-tumor necrosis factor-(alpha) monoclonal antibodies. *Therapy 3*, 535−545.

Weisman, M. H., Moreland, L. W., Furst, D. E., Weinblatt, M. E., Keystone, E. C., Paulus, H. E., et al. (2003). Efficacy, pharmacokinetic, and safety assessment of adalimumab, a fully human anti-tumor necrosis factor-alpha monoclonal antibody, in adults with rheumatoid arthritis receiving concomitant methotrexate: a pilot study. *Clin Ther 25*, 1700−1721.

Weyand, C., Seyler, T., & Goronzy, J. (2005). B cells in rheumatoid synovitis. *Arthritis Res Ther 7*, S9−S12.

Williams, R., Feldmann, M., & Maini, R. (1992). Anti-tumor necrosis factor ameliorates joint disease in murine collagen-induced arthritis. *Proc Natl Acad Sci U S A 89*, 9784−9788.

Williams-Abbott, L., Walter, B. N., Cheung, T. C., Goh, C. R., Porter, A. G., & Ware, C. F. (1997). The lymphotoxin-alpha (LTalpha) subunit is essential for the assembly, but not for the receptor specificity, of the membrane-anchored LTalpha 1beta 2 heterotrimeric ligand. *J Biol Chem 272*, 19451−19456.

Wolbink, G. J., Vis, M., Lems, W., Voskuyl, A. E., De Groot, E., Nurmohamed, M. T., et al. (2006). Development of antiinfliximab antibodies and relationship to clinical response in patients with rheumatoid arthritis. *Arthritis Rheum 54*, 711−715.

Wolfe, F., & Michaud, K. (2004). Lymphoma in rheumatoid arthritis: the effect of methotrexate and anti-tumor necrosis factor therapy in 18,572 patients. *Arthritis Rheum 50*, 1740−1751.

Wu, Q., Sun, Y., Wang, J., Lin, X., Wang, Y., Pegg, L. E., et al. (2001). Signal via lymphotoxin-betaR on bone marrow stromal cells is required for an early checkpoint of NK cell development. *J Immunol 166*, 1684−1689.

Yang, X., Tisch, R., Singer, S., Cao, Z., Liblau, R., Schreiber, R., et al. (1994). Effect of tumor necrosis factor alpha on insulin-dependent diabetes mellitus in NOD mice.I. The early development of autoimmunity and the diabetogenic process. *J Exp Med 180*, 995−1004.

Yao, Z., Li, P., Zhang, Q., Schwarz, E. M., Keng, P., Arbini, A., et al. (2006). Tumor necrosis factor-α increases circulating osteoclast precursor numbers by promoting their proliferation and differentiation in the bone marrow through up-regulation of c-Fms expression. *J Biol Chem 281*, 11846−11855.

Zeltser, R., Valle, L., Tanck, C., Holyst, M. M., Ritchlin, C., & Gaspari, A. A. (2001). Clinical, histological, and immunophenotypic characteristics of injection site reactions associated with etanercept: a recombinant tumor necrosis factor alpha receptor: Fc fusion protein. *Arch Dermatol 137*, 893−899.

Zhou, H. (2005). Clinical pharmacokinetics of etanercept: a fully humanized soluble recombinant tumor necrosis factor receptor fusion protein. *J Clin Pharmacol 45*, 490−497.

Zhou, H., Buckwalter, M., Boni, J., Mayer, P., Raible, D., Wajdula, J., et al. (2004). Population-based pharmacokinetics of the soluble TNFr etanercept: a clinical study in 43 patients with ankylosing spondylitis compared with post hoc data from patients with rheumatoid arthritis. *Int J Clin Pharmacol Ther 42*, 267−276.

Zhou, H., Jang, H., Fleischmann, R. M., Bouman-Thio, E., Xu, Z., Marini, J. C., et al. (2007). Pharmacokinetics and safety of golimumab, a fully human anti-TNF-a monoclonal antibody, in subjects with rheumatoid arthritis. *J Clin Pharmacol 47*, 383−396.

Ziolkowska, M., Kurowska, M., Radzikowska, A., Luszczykiewicz, G., Wiland, P., Dziewczopolski, W., et al. (2002). High levels of osteoprotegerin and soluble receptor activator of nuclear factor B ligand in serum of rheumatoid arthritis patients and their normalization after anti-tumor necrosis factor á treatment. *Arthritis Rheum 46*, 1744−1753.

Zwerina, J., Hayer, S., Tohidast-Akrad, M., Bergmeister, H., Redlich, K., Feige, U., et al. (2004). Single and combined inhibition of tumor necrosis factor, interleukin-1, and RANKL pathways in tumor necrosis factor-induced arthritis: effects on synovial inflammation, bone erosion, and cartilage destruction. *Arthritis Rheum 50*, 277−290.