10 TNF Blockade: An Inflammatory Issue

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Abstract. Tumor necrosis factor (TNF), initially discovered as a result of its antitumor activity, has now been shown to mediate tumor initiation, promotion, and metastasis. In addition, dysregulation of TNF has been implicated in a wide variety of inflammatory diseases including rheumatoid arthritis, Crohn's disease, multiple sclerosis, psoriasis, scleroderma, atopic dermatitis, systemic lupus erythematosus, type II diabetes, atherosclerosis, myocardial infarction,

osteoporosis, and autoimmune deficiency disease. TNF, however, is a critical component of effective immune surveillance and is required for proper proliferation and function of NK cells, T cells, B cells, macrophages, and dendritic cells. TNF activity can be blocked, either by using antibodies (Remicade and Humira) or soluble TNF receptor (Enbrel), for the symptoms of arthritis and Crohn's disease to be alleviated, but at the same time, such treatment increases the risk of infections, certain type of cancers, and cardiotoxicity. Thus blockers of TNF that are safe and yet efficacious are urgently needed. Some evidence suggests that while the transmembrane form of TNF has beneficial effects, soluble TNF mediates toxicity. In most cells, TNF mediates its effects through activation of caspases, NF-κB, AP-1, c-jun N-terminal kinase, p38 MAPK, and p44/p42 MAPK. Agents that can differentially regulate TNF expression or TNF signaling can be pharmacologically safe and effective therapeutics. Our laboratory has identified numerous such agents from natural sources. These are discussed further in detail.

10.1 Introduction

Tumor necrosis factor (TNF)-α and TNF-β, produced primarily by monocytes and lymphocytes, respectively, were first isolated in 1984, as cytokines that kill tumor cells in culture and induce tumor regression in vivo (Aggarwal et al. 1984). Intravenous administration of TNF to cancer patients produced numerous toxic reactions, including fever (Kurzrock et al. 1985). In animal studies, TNF has been shown to mediate endotoxin-mediated septic shock (Beutler et al. 1985). Other reports have indicated that dysregulation of TNF synthesis mediates a wide variety of diseases, including rheumatoid arthritis and inflammatory bowel disease (also called Crohn's disease) (Fig. 1).

10.2 TNF Cell Signaling

TNF is a transmembrane protein with a molecular mass of 26 kDa that was originally found to be expressed in macrophages and has now been found to be expressed by a wide variety of cells. In response to various stimuli, TNF is secreted by the cells as a 17-kDa protein through a highly regulated process that involves an enzyme: TNF-activating converting enzyme (TACE).

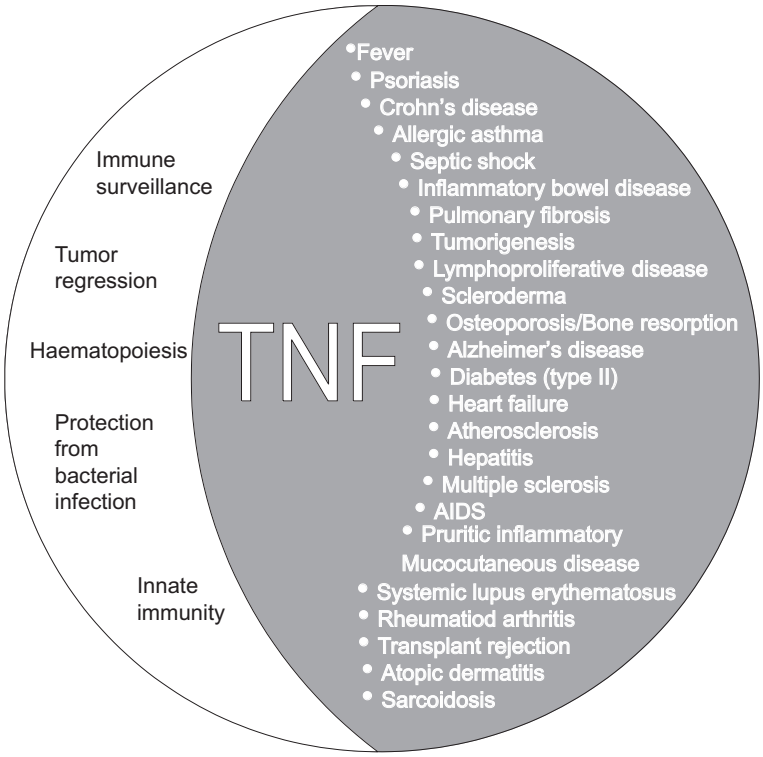

Fig. 1. TNF synthesis mediates a wide variety of diseases including rheumatoid arthritis and inflammatory bowl disease

TNF mediates its effects through two different receptors (Fig. 2): TNF receptor I (also called p55 or p60) and TNF receptor II (also called p75 or p80). While TNF receptor I is expressed on all cell types in the body, TNF receptor II is expressed selectively on endothelial cells and on cells of the immune system. TNF binds to two receptors with comparable affinity. Why there are two different receptors for TNF is incompletely understood. Evidence related to differential signaling (Aggarwal et al. 2002), ligand passing (Tartaglia et al. 1993), binding to soluble TNF vs transmembrane TNF (Grell et al. 1995) has been presented.

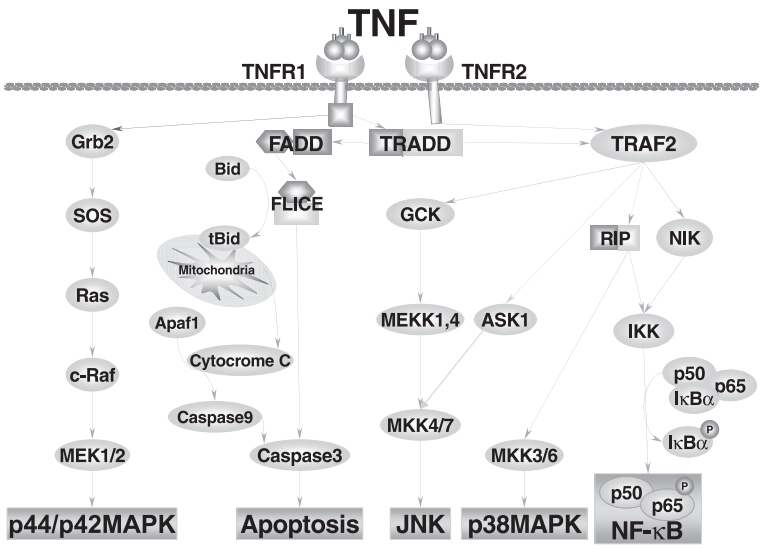

Fig. 2. TNF cell signaling pathway

The cytoplasmic domain of the TNF receptor I has a death domain, which has been shown to sequentially recruit TNF receptor-associated death domain (TRADD), Fas-associated death domain (FADD), and FADD-like ICE (FLICE) (also called caspase-8), leading to caspase-3 activation, which in turn induces apoptosis by inducing degradation of multiple proteins (Nagata and Golstein 1995). TRADD also recruits TNF receptor-associated factor (TRAF2), which through receptor-interacting protein (RIP) activates IκBα kinase (IKK), leading to IκBα phosphorylation, ubiquitination, and degradation, which finally leads to NF-κB activation. NF-κB activation is followed by the expression of various genes that can suppress the apoptosis induced by TNF. Through recruitment of TRAF2, TNF has also been shown to activate various mitogen-activated protein kinases (MAPK), including the c-jun N-terminal kinases (JNK) p38 MAPK and p42/p44 MAPK. TRAF2 is also essential for the TNF-induced activation of AKT, another cell-survival signaling pathway. Thus TNFRI activates both apoptosis and cell survival signaling pathways simultaneously.

In contrast to TNFR1, the cytoplasmic domain of TNFR2 lacks the death domain and binds TRAF1 and TRAF2 directly. Through activation of JNK, TNF activates AP-1, another redox-sensitive transcription factor. Gene-deletion studies have shown that TNFR2 can also activate NF-κB, JNK, p38 MAPK, and p42/p44 MAPK (Mukhopadhyay et al. 2001).

TNFR2 can also mediate TNF-induced apoptosis (Haridas et al. 1998). Because TNFR2 cannot recruit TRADD-FADD-FLICE, how TNFR2 mediates apoptosis is not understood. Various pieces of evidence suggest that homotrimeric TNF binds to homotrimeric TNF receptor to mediate its signals (Ameloot et al. 2001). TNF receptor deletion studies have provided evidence that this receptor communicates with receptors for other ligands, including receptor activator of NF-κB ligand (RANKL, a member of the TNF superfamily) and endotoxin (Takada and Aggarwal 2003b 2004).

Since its discovery, TNF has been linked to a wide variety of diseases. How TNF mediates disease-causing effects is incompletely understood. The induction of pro-inflammatory genes by TNF has been linked to most diseases. The pro-inflammatory effects of TNF are primarily due to its ability to activate NF-κB. Almost all cell types, when exposed to TNF, activate NF-κB, leading to the expression of inflammatory genes. Over 200 genes have been identified that are regulated by NF-κB activation (Kumar et al. 2004). These include cyclooxygenase-2 (COX-2), lipoxygenase-2 (LOX-2), cell-adhesion molecules, inflammatory cytokines, chemokines, and inducible nitric oxide synthase (iNOS). TNF mediates some of its disease-causing effects by modulating growth. For instance, for most tumor cells TNF has been found to be a growth factor (Sugarman et al. 1985). These include ovarian cancer cells, cutaneous T cell lymphoma (Giri and Aggarwal 1998) glioblastoma (Aggarwal et al. 1996), acute myelogenous leukemia (Tucker et al. 2004), B cell lymphoma (Estrov et al. 1993), breast carcinoma (Sugarman et al. 1987), renal cell carcinoma (Chapekar et al. 1989), multiple myeloma (Borset et al. 1994), and Hodgkin's lymphoma (Hsu and Hsu 1990). Various fibroblasts, including normal human fibroblasts, scleroderma fibroblasts, synovial fibroblasts, and periodontal fibroblasts, proliferate in response to TNF. Why on treatment with TNF some cells undergo apoptosis, others undergo proliferation, and most are unaffected is not understood. The differences are not due to lack of receptors or variations in their affinity.

10.3 Inhibitors of TNF Cell Signaling

Because of the critical role of TNF in mediating a wide variety of diseases, TNF has become an important target for drug development. Since TNF mediates its effects through activation of NF-κB, AP-1, JNK, p38 MAPK, p44/p42 MAPK, and AKT (Fig. 3); agents that can suppress these pathways have potential for therapy of TNF-linked diseases. Below is a review of inhibitors of such pathways.

10.3.1 NF-κB Blockers

Most diseases that have been linked to TNF have also been linked to NF-κB activation (Aggarwal 2003), indicating that TNF mediates its pathological effects through activation of NF-κB. Thus blockers of NF-κB have a potential for alleviating TNF-linked diseases. Several NF-κB blockers have been identified using targets that mediate the TNF-induced NF-κB activation pathway (Fig. 4). Various hormones and cytokines have also been described that can suppress TNF-induced NF-κB activation. These include IL-4, IL-13, IL-10, melanocyte-stimulating hormone (βMSH), luteinizing hormone (LH), human chorionic gonadotrophin (HCG), and IFN-α/β (Aggarwal et al. 2002). Other agents that suppress NF-κB activation include inhibitors of proteosomes, inhibitors

Signaling pathways activated by TNF

Inflammatory pathway	NF-κB
Stress pathway	JNK & p38
Cell survival pathway	PI-3K/Akt
Mitogenic pathway	MAPK/Erk
JAK/STAT pathway	
Apoptosis pathway	Caspases

Fig. 3. TNF mediates its effects through activation of NF-κB, AP-1, JNK, p38 MAPK, p44/p42 MAPK, and AKT

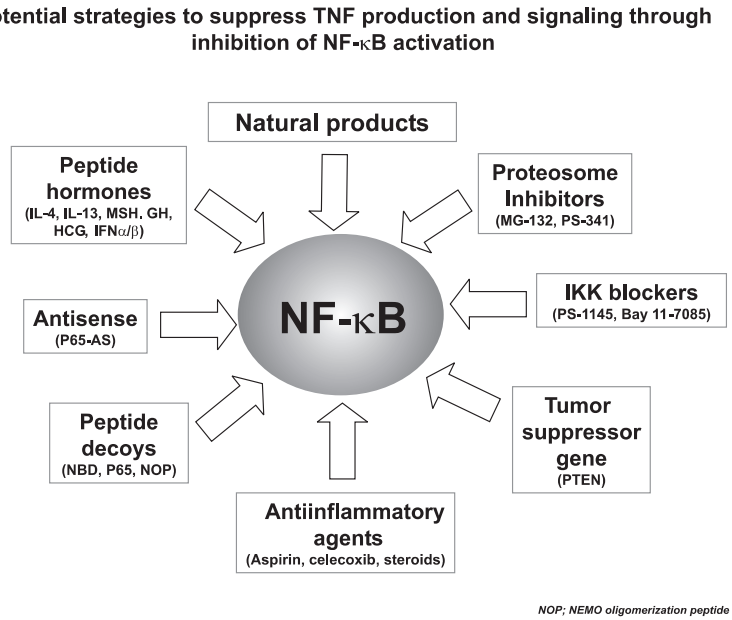

Fig. 4. Potential strategies to suppress TNF production and signaling through inhibition of NF-κB activation

of ubiquitination, inhibitors of the protein kinase that phosphorylates Iκbα (IKK), inhibitors of IKK activation (Fig. 5), decoy peptides of IKK and p65, antisense oligonucleotides, and Si RNA to p65. These inhibitors, although quite effective in suppressing NF-κB activation, have shown limited promise in the treatment of the disease for a variety of reasons. These include in vivo toxicity, lack of specificity, and poor bioavailability. Thus safer and efficacious blockers of TNF-induced NF-κB activation are needed. Our laboratory has described numerous agents derived from natural sources that can suppress NF-κB activation induced by TNF quite effectively. Through suppression of NF-κB, these inhibitors can also suppress expression of TNF. These polyphenols (e.g., curcumin, a diferuloylmethane) have been tested in animals and found to exhibit good activity in suppression of tumorigenesis, Alzheimer disease, diabetes, arthritis, Crohn's disease, and cardiovascular disease.

Development of IKK blockers

Compound	IC50 (in vitro)	IC50 (in vivo)	Investigator
Bayer Yakuhin			
BY-4j	8.5 nM	0.06uM	Murata T, 2004
Millennium			
PS1145/MNL120	20 nM	3 uM	Hideshima T, 2002
Celgene			
SPC839	62 nM	10 uM	Palanki MS, 2003
BMS			
BMS345541	300 nM	1-5 uM	Burke et al, 2003
Pharmacia/Pfizer			
SC-514	3-12 uM	100 uM	Kishore N, 2003
Wyeth-Ayerst			
WAY-169916	1 uM	N. A.	Steffan R, 2004

SPC839 is now called AS602868

Fig. 5. Development of IKK blockers

10.3.2 AP-1 Blockers

Although TNF is a potent activator of AP-1, no specific blocker of AP-1 activation has yet been described. However, most phytochemicals that suppress NF-κB activation also suppress AP-1 activation (Manna and Aggarwal 2000; Manna et al. 2000; Torii et al. 2004).

10.3.3 Suppression of TNF-Induced P38 MAPK Activation

TNF is one of the most potent activators of p38 MAPK (Lee et al. 1994). This activation is specifically inhibited by SB203580 (Henry et al. 1998; Kumar et al. 1997). Campbell et al. (2004) examined the role of p38 MAPK in the regulation of TNF in primary human cells relevant to inflammation, e.g., macrophages and rheumatoid synovial cells (Campbell et al. 2004). Using a dominant-negative variant (D168A) of p38 MAPK and a kinase inhibitor, SB203580, they confirmed in primary human macrophages that p38 MAPK regulates TNF production using

a posttranscriptional mechanism requiring the 3′ untranslated region of the gene. However, in LPS-activated primary human macrophages, they found p38 MAPK modulation of TNF transcription was mediated through p38 MAPK regulation of NF-κB. Interestingly, this mechanism was not observed in rheumatoid synovial cells. It is important to note that the dominant negative mutant of p38 MAPK, but not SB203580, was effective at inhibiting spontaneous TNF production in these ex vivo rheumatoid synovial cell cultures. These data indicate that there are potential major differences in the role of p38 MAPK in inflammatory signaling that have a bearing on the use of this kinase as a target for therapy. These results also indicate that this kinase is a valid target in rheumatoid disease. Vanden Berghe et al. (1998) found that p38 and p44/p42 MAPK pathways are required for NF-κB p65 transactivation mediated by TNF. De Alvaro et al. (2004) showed that TNF produces insulin resistance in skeletal muscle by activation of IKK in a p38 MAPK-dependent manner. Similarly, Hernandez et al. (2004) showed that Rosiglitazone ameliorates insulin resistance in brown adipocytes of Wistar rats by impairing TNF induction of p38 and p42/p44 mitogen-activated protein kinases.

10.3.4 Suppression of TNF-Induced JNK Activation

TNF is one of the most potent activators of JNK (Chen and Goeddel 2002). This activation is specifically inhibited by SP600125. Bennett et al. (2001) reported the identification of an anthrapyrazolone series that significantly inhibits JNK1, -2, and -3 (K(i) = 0.19 μM). SP600125 is a reversible ATP-competitive inhibitor with greater than 20-fold selectivity vs a range of kinases and enzymes tested. In cells, SP600125 dose dependently inhibited the phosphorylation of c-Jun and the expression of the inflammatory genes COX-2, IL-2, IFN-gamma, and TNF and prevented the activation and differentiation of primary human CD4 cell cultures. In animal studies, SP600125 blocked LPS-induced expression of TNF and inhibited anti-CD3-induced apoptosis of CD4(+) CD8(+) thymocytes. Our study supports targeting JNK as an important strategy in inflammatory disease, apoptotic cell death, and cancer. Ishii et al. (2004) found that inhibition of JNK activity improves ischemia/reperfusion injury in rat lungs.

10.3.5 Suppression of TNF-Induced P42/p44 MAPK Activation

Besides p38 MAPK and JNK, TNF is also potent activator of p42/p44 MAPK, also called ERK1/2 (Johnson and Lapadat 2002). This activation is inhibited by PD98059 (Kumar et al. 1998b). TNF-induced extracellular signal-regulated kinase (ERK) activation is required for proliferation of most cells. Murakami-Mori et al. (1999) found that ERK1/2 in Kaposi sarcoma (KS) cells was significantly activated by TNF through tyrosine/threonine phosphorylation. A selective inhibitor for ERK1/2 activator kinases, PD98059, profoundly inhibited not only the activation of ERK1/2, but also TNF-induced KS cell proliferation. They therefore proposed that the TNFR-I-ERK1/2 pathway plays a pivotal role in transmitting to KS cells the mitogenic signals of TNF. They found that actinomycin D treatment of KS cells selectively abolished expression of MADD, a novel TNFR-I-associated death domain protein. TNF-alpha failed to induce ERK1/2 activation in the actinomycin D-treated cells. MADD may couple TNFR-I with the ERK1/2 signaling pathway required for KS cell proliferation. Using similar inhibitors, Goetze et al. (1999) found that TNF-induced migration of vascular smooth muscle cells is MAPK-dependent. Tran et al. (2001) found that MAPK/ERK overrides the apoptotic signaling from Fas, TNF, and TRAIL receptors. They observed that a number of FasR-insensitive cell lines could redirect the proapoptotic signal to an anti-apoptotic ERK1/2 signal, resulting in inhibition of caspase activation. They determined that similar mechanisms are operational in regulating the apoptotic signaling of other death receptors. Activation of the FasR, TNF-R1, and TRAIL-R rapidly induced ERK1/2 activation, an event independent of caspase activity. Whereas inhibition of the death receptor-mediated ERK1/2 activation was sufficient to sensitize the cells to apoptotic signaling from FasR and TRAIL-R, cells were still protected from apoptotic TNF-R1 signaling. The latter seemed to be attributable to the strong activation of the anti-apoptotic factor NF-κB, which remained inactive in FasR or TNF-related apoptosis-inducing ligand receptor (TRAIL-R) signaling. However, when the cells were sensitized with cycloheximide, which is sufficient to sensitize the cells also to apoptosis by TNF-R1 stimulation, Tran et al. noticed that adenovirus-mediated expression of constitutively active MKK1 could rescue the cells from apoptosis induced by the re-

ceptors by preventing caspase-8 activation. Taken together, these results showed that ERK1/2 has a dominant protecting effect over apoptotic signaling from the death receptors. This protection, which is independent of newly synthesized proteins, acts in all cases by suppressing activation of the caspase effector machinery.

10.3.6 Suppression of TNF-Induced AKT Activation

TNF is also a potent activator of AKT. Ozes found that NF-κB activation by TNF requires the Akt serine-threonine kinase (Ozes et al. 1999). Pastorino found TNF induced the phosphorylation of BAD by Akt at serine 136 in HeLa cells under conditions that are not cytotoxic. BAD phosphorylation by TNF was dependent on phosphatidylinositide-3-OH kinase (PI3K) and was accompanied by the translocation of BAD from the mitochondria to the cytosol (Pastorino et al. 1999). Blocking the phosphorylation of BAD and its translocation to the cytosol with the PI3 K inhibitor wortmannin activated caspase-3 and markedly potentiated the cytotoxicity of TNF. Transient transfection with a PI3K dominant-negative mutant or a dominant-negative mutant of the serine-threonine kinase Akt, the downstream target of PI3K, and the enzyme that phosphorylates BAD similarly potentiated the cytotoxicity of TNF. By contrast, transfection with a constitutively active Akt mutant protected against the cytotoxicity of TNF in the presence of wortmannin. Phosphorylation of BAD prevents its interaction with the antiapoptotic protein Bcl-XL. Transfection with a Bcl-XL expression vector protected against the cytotoxicity of TNF in the presence of wortmannin.

Yang et al. (2004) reported the discovery of a small-molecule Akt pathway inhibitor, Akt/protein kinase B signaling inhibitor-2 (API-2), by screening the National Cancer Institute Diversity Set. API-2 suppressed the kinase activity and phosphorylation level of Akt. The inhibition of Akt kinase resulted in suppression of cell growth and induction of apoptosis in human cancer cells. API-2 was highly selective for Akt and did not inhibit the activation of PI3K, phosphoinositide-dependent kinase-1, protein kinase C, serum- and glucocorticoid-inducible kinase, protein kinase A, signal transducer and activators of transcription 3, ERK-1/2, or JNK. Furthermore, API-2 potently inhibited tumor growth in nude mice

of human cancer cells in which Akt is aberrantly expressed/activated but not of those cancer cells in which it is not.

10.4 Role of TNF in Skin Diseases

Although it suppresses the proliferation of cells, TNF was also found to induce the proliferation of dermal fibroblasts (Berman and Wietzerbin 1992). The TNF-induced JNK pathway has been found to be dysregulated in patients with familial cylindromatosis in whom CYLD, a tumor suppressor, is mutated. Such patients have an autosomal dominant predisposition to multiple tumors of the skin of the arms and legs (Reiley et al. 2004). Dysregulation of TNF has been linked with several skin diseases, including rheumatoid arthritis, atopic dermatitis, scleroderma, psoriasis (Kane and FitzGerald 2004), systemic sclerosis, Crohn's disease, pyoderma gangrenosum, sarcoidosis, and cutaneous T cell lymphoma.

Furthermore, keratinocyte-derived TNF acts as an endogenous tumor promoter and can also regulate AP-1 activity in mouse epidermis. Arnott found that TNFR2 cooperated with TNFR1 to optimize TNFR1-mediated TNF bioactivity on keratinocytes in vitro. They found that expression of both TNF-receptor subtypes is essential for optimal skin tumor development and provided some rationale for the use of TNF antagonists in the treatment of cancer (Arnott et al. 2004).

Because keloids, which are characterized as an overexuberant healing response, represent an inflammatory response, it is logical to assume that cytokines play a part in orchestrating keloid pathology. Messadi identified differences in the expression of NF-κB and its related genes between keloid and normal skin fibroblasts (Messadi et al. 2004). They showed that TNF upregulated 15% of NF-κB signal pathway-related genes in keloid fibroblasts compared to normal skin. At the protein level, keloid fibroblasts and tissues showed higher basal levels of the TNF-receptor-associated factors TRAF1, TRAF2-TNF-α, inhibitor of apoptosis (c-IAP-1), and NF-κB, compared with normal skin fibroblasts. Keloid fibroblasts showed a constitutive increase in NF-κB-binding activity both with and without TNF-α treatment. It is possible that NF-κB and its targeted genes, especially the antiapoptotic genes, could play

a role in keloid pathogenesis; thus targeting NF-κB could help in developing therapeutic interventions for the treatment of keloid scarring.

TNF receptor-associated periodic syndrome (TRAPS) is an autosomal dominant inherited condition characterized by periodic fever and pain. TRAPS is a model for a novel pathogenic concept in which a TNF receptor fails to be shed; thus suggesting that medications targeting TNF may be effective in TRAPS (Masson et al. 2004).

That TNF signaling plays a role in skin is also evident from molecular imaging of NF-κB, a primary regulator of stress response. Carlsen developed transgenic mice that express luciferase under the control of NF-κB, enabling real-time noninvasive imaging of NF-κB activity in intact animals (Carlsen et al. 2004). We showed that, in the absence of stimulation, strong, intrinsic luminescence is evident in lymph nodes in the neck region, thymus, and Peyer's patches. Treating mice with stressors such as TNF-α, IL-1α, or LPS increases the luminescence in a tissue-specific manner, with the strongest activity observable in the skin, lungs, spleen, Peyer's patches, and the wall of the small intestine. Liver, kidney, heart, muscle, and adipose tissue exhibit less intense activities. Exposure of the skin to a low dose of UV-B radiation increases luminescence in the exposed areas. In ocular experiments, when LPS and TNF-α were injected into NF-κB-luciferase transgenic mice, a 20- to 40-fold increase in NF-κB activity occurred in the lens, as well as in other LPS- and TNF-α-responsive organs. Peak NF-κB activity occurred 6 h after injection of TNF-α and 12 h after injection of LPS.

Mice exposed to 360 J/m(2) of UV-B exhibited a 16-fold increase in NF-κB activity 6 h after exposure, much like TNF-α-exposed mice. Thus, in NF-κB-luciferase transgenic mice, NF-κB activity also occurs in lens epithelial tissue and is activated when the intact mouse is exposed to classical stressors. Furthermore, as revealed by real-time noninvasive imaging, induction of chronic inflammation resembling rheumatoid arthritis produces strong NF-κB activity in the affected joints. These investigators used this model to demonstrate regulation by manipulating vitamin A status in mice. NF-κB activity is elevated in mice fed a vitamin A-deficient (VAD) diet and suppressed by excess doses of retinoic acid. They thus demonstrated the development and use of a versatile model for monitoring NF-κB activation both in tissue homogenates and in intact

animals after the use of classical activators, during disease progression and after dietary intervention.

10.5 Bright Side of TNF

Two TNF-neutralizing agents are licensed in the US. Infliximab has been licensed for the treatment of Crohn's disease and is used with methotrexate for the treatment of rheumatoid arthritis (Hanauer 2004). Etanercept is a soluble TNF receptor type 2 that is licensed for the treatment of rheumatoid arthritis, including the juvenile form, and, more recently, was licensed for the treatment of psoriatic arthritis (Baraliakos and Braun 2004). Anti-TNF does not cure rheumatoid arthritis or Crohn's disease, but blocking TNF may reduce the inflammation caused by too much TNF.

10.6 Dark Side of TNF Blockers

Serious infections, including sepsis and fatal infections, have been reported in patients receiving TNF-blocking agents. Many of the serious infections in patients treated with anti-TNF have occurred in patients on concomitant immunosuppressive therapy that, in addition to their Crohn's disease or rheumatoid arthritis, could predispose them to infections. Caution should be exercised when considering the use of anti-TNF in patients with a chronic infection or a history of recurrent infection. Anti-TNF should not be given to patients with a clinically important, active infection. The companies that manufacture TNF blockers have indicated serious side effects of these drugs (Table 1).

Some patients who took Remicade developed symptoms that can resemble a disease called lupus. Lupus-like symptoms may include chest discomfort or pain that does not go away, shortness of breath, joint pain, or a rash on the cheeks or arms that gets worse in the sun.

Although TNF blocking therapy is currently used, a great deal of caution is needed. Mohan's group described the clinical features of leukocytoclastic vasculitis (LCV) associated with the use of TNF-alpha blockers (Mohan et al. 2004). They identified 35 cases of LCV, 20 following etanercept administration and 15 following infliximab admin-

Table 1. Side effects associated with administration of TNF blockers in human

Infections	
Granulomatous infection	Wallis et al. 2003
Opportunistic infection	Slifman et al. 2003 Lee et al. 2002
Heart failure	
Hematopoiesis	
Allergic reactions	
Nervous system disorders	
Lymphoma	Wolfe and Michaud 2004 Sandborn et al. 2004
Lupus-like symptoms	
Leukocytoclastic vasculitis (LCV)	Mohan et al. 2004 Devos et al. 2003

istration. Seventeen of the 35 (48.5%) were biopsy-proven cases, and the others had skin lesions that were clinically typical for LCV. Twenty-two of 35 (62.8%) patients had complete or marked improvement of skin lesions upon stopping the TNF-alpha blocker. Three patients who had received etanercept had continuing lesions despite discontinuation of the drug; one of these patients improved when switched to infliximab. One patient who received infliximab was reported to have continuing lesions despite discontinuation of the drug and treatment with prednisone and antihistamines. Six patients experienced a positive rechallenge (recurrence of LCV on restarting therapy with a TNF-α blocker) and three patients a negative rechallenge phenomenon. LCV lesions improved in patients despite continuing use of concomitant medications reportedly associated with LCV.

Various adverse cutaneous reactions to anti-TNF-α monoclonal antibody have been reported. In clinical studies with infliximab (Remicade), adverse drug reactions were most frequently reported in the respiratory system and in the skin and appendages. Devos described six patients receiving anti-TNF-α therapy (infliximab) for Crohn's disease or rheumatoid arthritis who showed adverse cutaneous reactions and were diagnosed with LCV, lichenoid drug reaction, perniosis-like erup-

tion (two patients), superficial granuloma annulare, and acute folliculitis (Devos et al. 2003).

Wallis's group examined the relationship between the use of TNF antagonists and onset of granulomatous infection using data collected through the Adverse Event Reporting System of the US Food and Drug Administration for January 1998–September 2002 (Wallis et al. 2003). Granulomatous infections were reported at rates of approximately 239 per 100,000 patients who received infliximab and approximately 74 per 100,000 patients who received etanercept, indicating a significant difference between the two drugs. Tuberculosis was the most frequently reported disease, occurring in approximately 144 and approximately 35 per 100,000 infliximab-treated and etanercept-treated patients, respectively. Candidiasis, coccidioidomycosis, histoplasmosis, listeriosis, and other infections caused by nontuberculous mycobacteria were reported with significantly greater frequency among infliximab-treated patients. Seventy-two percent of these infections occurred within 90 days after starting infliximab treatment, and 28% occurred after starting etanercept treatment ($p < 0.001$). These data indicate a risk of granulomatous infection that was 3.25-fold greater among patients who received infliximab than among those who received etanercept. The clustering of reports shortly after initiation of treatment with infliximab is consistent with reactivation of latent infection.

Because of the potential for a decrease in host resistance to infectious agents due to treatment with anti-TNF agents, Slifman evaluated cases of opportunistic infection, including those caused by *Listeria monocytogenes*, in patients treated with these products (Slifman et al. 2003). The FDA Adverse Event Reporting System, a passive monitoring system, was reviewed to identify all reports of adverse events (through December 2001) associated with *L. monocytogenes* infection in patients treated with infliximab or etanercept. Fifteen cases associated with infliximab or etanercept treatment were identified. In 14 of these cases, patients had received infliximab. The median age of all patients was 69.5 years (range, 17–80 years); 53% were women. Six deaths were reported. Among patients for whom an indication for use was reported, nine patients (64%) with rheumatoid arthritis and five patients (36%) with Crohn's disease (information was not reported for one patient). All patients for whom information was reported were receiving concurrent

immunosuppressant drugs. Thus postlicensure surveillance suggests that *L. monocytogenes* infection may be a serious complication of treatment with TNF-neutralizing agents, particularly infliximab.

Lee also sought to identify cases of opportunistic infection, including histoplasmosis, in patients treated with these products (Lee et al. 2002). The US Food and Drug Administration's passive surveillance database for monitoring postlicensure adverse events was reviewed to identify all reports received through July 2001 of histoplasmosis in patients treated with either infliximab or etanercept. Ten cases of *Histoplasma capsulatum* infection were reported: nine associated with infliximab and one associated with etanercept. In patients treated with infliximab, manifestations of histoplasmosis occurred within 1 week to 6 months after the first dose and typically included fever, malaise, cough, dyspnea, and interstitial pneumonitis. Of the ten patients with histoplasmosis, nine required treatment in an intensive care unit and one died. All patients had received concomitant immunosuppressive medications in addition to infliximab or etanercept, and all resided in *H. capsulatum*-endemic regions. Thus postlicensure surveillance suggests that acute life-threatening histoplasmosis may complicate immunotherapy with TNF-α antagonists, particularly infliximab. Histoplasmosis should be considered early in the evaluation of patients who reside in *H. capsulatum*-endemic areas in whom infectious complications develop during treatment with infliximab or etanercept.

The risk of lymphoma is increased in patients with rheumatoid arthritis, and some studies suggest that methotrexate and anti-TNF therapy might be associated independently with an increased risk of lymphoma. However, data from clinical trials and clinical practice do not provide sufficient evidence concerning these issues because of small sample sizes and selected study populations. Wolfe and Michaud (2004) determined the rate of and standardized incidence ratio (SIR) for lymphoma in patients with rheumatoid arthritis in general and in these patients by treatment group. Additionally, we sought to determine predictors of lymphoma in rheumatoid arthritis. We prospectively studied 18,572 patients with rheumatoid arthritis who were enrolled in the National Data Bank for Rheumatic Diseases (NDB). Patients were surveyed biannually, and potential lymphoma cases received detailed follow-up. The SEER (Survey, Epidemiology, and End Results) cancer data resource was used

to derive the expected number of cases of lymphoma in a cohort that was comparable in age and sex.: The overall SIR for lymphoma was 1.9 [95% confidence interval (95% CI), 1.3–2.7]. The SIR for biologic use was 2.9 (95% CI, 1.7–4.9) and for the use of infliximab (with or without etanercept) was 2.6 (95% CI, 1.4–4.5). For etanercept, with or without infliximab, the SIR was 3.8 (95% CI, 1.9–7.5). The SIR for MTX was 1.7 (95% CI, 0.9–3.2), and was 1.0 (95% CI, 0.4–2.5) for those not receiving MTX or biologics. Lymphoma was associated with increasing age, male sex, and education. Although the SIR is greatest for anti-TNF therapies, differences between therapies were slight, and confidence intervals for treatment groups overlapped. The increased lymphoma rates observed with anti-TNF therapy may reflect channeling bias, whereby patients with the highest risk of lymphoma preferentially receive anti-TNF therapy. Current data are insufficient to establish a causal relationship between the treatments and the development of lymphoma.

Sandborn and Loftus also noted that patients with moderate to severely active Crohn's disease treated with infliximab may have a small but real risk of developing severe infections, opportunistic infections, and non-Hodgkin's lymphoma (Sandborn et al. 2004).

10.7 Identification of Novel Blockers of TNF

Small molecules such as plant-derived phytochemicals, which are safer and yet effective in suppressing both production and action of TNF, should be explored. Inasmuch as TNF expression in most cells is regulated by the transcription factor NF-κB, agents that suppress this factor will also block TNF production. Phytochemicals such as curcumin, resveratrol, betulinic acid, ursolic acid, sanguanarine, capsaicin, gingerol, anethole, and eugenol, have been shown to suppress NF-κB (Aggarwal et al. 2004; Dorai and Aggarwal 2004; Han et al. 2001; Kumar et al. 1998a; Murakami et al. 2003; Shishodia et al. 2003; Takada and Aggarwal 2003a), and thus can suppress TNF production (Lukita-Atmadja et al. 2002) (Table 2). Such agents should be further explored.

Table 2. Phytochemicals known to suppress NF-κ B activation induced by inflammatory agents

Polyphenols	Terpenes
Amentoflavone	Andalusol
Apigenin	Anethol and analogs
Anethole	Artemisinin
Arctigenin and demethyltraxillagenin	Avicins
Baicalein and its derivatives	Betulinic acid
Bakuchiol (Drupanol)	Celastrol
Cannabinol	Costunolide
Capsaicinoids	Ergolide
Carnosol	Excisanin A
Catalposide	Foliol
Catechin and theaflavins	Germacranolides
Curcumin	Ginkgo biola ext.
Emodin	Ginsenoside Rg3
Flavopiridol	Guaianolides
Genistein	Helenalin
Glossogyne tenuifolia	Hypoestoxide
Hematein	Kamebacetal A
HMP	Kamebakaurin
Hypericin	Kaurenic acid
Isomallotochromanol and isomallotochromene	Linearol
Luteolin	Oleandrin
Nordihydroguaiaretic	Oxoacanthospermoldes
Acid	Parthenolide
Panduratin A	Pristimerin
Pycnogenol	Triptolide (PG 490)
Rhein	Ursolic acid
Rocaglamides	Alkaloids
Sanggenon C	Cepharanthine
Sphondin	Conophylline
Silymarin	Morphine and its analogs
Saucerneols	Tetrandine
Sauchinone and Manassantins	Sinomenine A
Wedelolactone	Benz[α]phenazine
Yakuchinones A and B	Lapachone
Benzopyrene	Caffeic acid phenethyl ester
Rotenone	CAPE
Chlorophyll catabolite	Phenolics
Pheophorbide A	Ethyl gallate
Iridoid glycoside	Saponin
Aucubin	Calagualine
Others	Stilbene
α-Lipoic acid	Resveratrol and analogues
Astaxanthin	
Germinated barley	
S-allylcysteine	
Vitamin C	
Vitamin E	

10.8 Conclusions

Extensive research in the last few years has clearly proven that TNF is a pro-inflammatory cytokine and thus is involved in the pathogenesis of variety of diseases. Suppression of TNF is a double-edged sword. While unquestionably TNF plays a critical role in inflammation, suppression of TNF will mitigate both its beneficial and its harmful effects. These studies show that safer modulators of TNF are needed.

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