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Tumor Necrosis Factor-α Signaling in Macrophages

Narayanan Parameswaran* and **Sonika Patial**
Department of Physiology and Division of Pathology, Michigan State University, East Lansing, Michigan, USA

Abstract

Tumor necrosis factor-α (TNFα) was cloned over 2 decades ago and its identification in part led to the discovery of a super family of tumor necrosis factors (TNFs) and their receptors. TNFα signals through two transmembrane receptors, TNFR1 and TNFR2, and regulates a number of critical cell functions including cell proliferation, survival, differentiation, and apoptosis. Macrophages are the major producers of TNFα and interestingly are also highly responsive to TNFα. Aberrant TNFα production and TNF receptor signaling have been associated with the pathogenesis of several diseases, including rheumatoid arthritis, Crohn's disease, atherosclerosis, psoriasis, sepsis, diabetes, and obesity. TNFα has been shown to play a pivotal role in orchestrating the cytokine cascade in many inflammatory diseases and because of this role as a "master-regulator" of inflammatory cytokine production, it has been proposed as a therapeutic target for a number of diseases. Indeed anti-TNFα drugs are now licensed for treating certain inflammatory diseases including rheumatoid arthritis and inflammatory bowel disease. In this review we discuss the discovery of TNFα and its actions especially in regulating macrophage biology. Given its importance in several human diseases, we also briefly discuss the role of anti-TNFα therapeutics in the treatment of inflammatory diseases.

Keywords

TNFα; disease; inflammation; macrophage; arthritis; inflammatory bowel disease

I. INTRODUCTION

It has been known for over a century that bacterial-derived endotoxins can cause hemorrhagic necrosis of tumors in humans. However, it was not until 1962 that O'Malley et al.[1] first demonstrated that this effect of endotoxins is indirect. They showed that serum from animals treated with lipopolysaccharides (LPS) could trigger hemorrhagic necrosis of tumors in animals not exposed to LPS. O'Malley et al. further proposed that the effect of endotoxin is mediated by a "tumor-necrotizing factor." One decade later, Carswell et al.[2] showed that the serum of bacillus Calmette-Guérin–infected mice treated with endotoxin contained a substance, which they named "tumor necrosis factor" (TNF) and further demonstrated that the actions of TNF on tumor necrosis were similar to that of the endotoxin. They demonstrated that the TNF-positive serum was equally effective to that of endotoxin in causing hemorrhagic necrosis of methylcholanthrene A-induced fibrosarcoma (Meth A). In addition, they showed that TNF can cause necrosis of several transplanted tumors similar to that of Meth A.[2]

*Address all correspondence to Narayanan Parameswaran, PhD, 2201 Biomedical Physical Sciences Building, Department of Physiology, Michigan State University, East Lansing, MI 48824; Tel.: 517-884-5115; Fax: 517-355-5125; paramesw@msu.edu.

One decade after these observations, the cDNA for human and murine TNFα were cloned and expressed in *Escherichia coli*.[3,4] Human TNFα was also purified to homogeneity from the cell culture supernatants of the HL-60 cell line as a protein with a molecular weight of 17,000 by Aggarwal et al.[5] Interestingly, in some independent studies Beutler and colleagues identified TNF as a factor that induces LPS-induced wasting or cachexia in mice.[6,7] Subsequent studies have demonstrated that TNF is a prototypic member of a large superfamily known as the TNF/TNFR superfamily, which now comprises more than 40 members. Considerable advances have been made in our understanding of the biology and the clinical role of TNFα. In this review, we focus mainly on the role of TNFα in macrophage biology and how these studies are relevant to the pathogenesis of many inflammatory diseases.

II. STRUCTURE AND SYNTHESIS OF TNFα

The TNFα gene is present as a single copy gene on human chromosome 6 (murine chromosome 17).[8] The gene consists of four exons and three introns. Interestingly, more than 80% of the mature TNFα sequence is encoded in the fourth exon. Exons I and II mainly contain the leader peptide sequence. Messenger RNA for TNFα is expressed in a wide range of cells, including monocytes and macrophages. TNFα gene expression is regulated at the transcriptional level by several factors, including nuclear factor kappa b (NFκB) and nuclear factor activated T cells (NF-AT). TNFα production is also regulated at the translational level via the UA-rich sequence in the 3′ untranslated region of human TNFα mRNA[8,9] Human TNFα is expressed as a 27-kDa (233 amino acid) protein that is then proteolytically cleaved to a 17-kDa (157 amino acid) molecule. The 76-amino-acid presequence in the 27-kDa protein is highly conserved, and seems to serve to anchor the precursor protein to the membrane. This membrane integrated 27-kDa TNFα (mTNFα) undergoes proteolytic cleavage by a metalloprotease TNFα-converting enzyme (TACE), resulting in the 17-kDa soluble TNFα or sTNFα.[10,11] The 17-KDa TNFα protomers are composed of two antiparallel β-pleated sheets with antiparallel β-strands, which form a jelly-roll β-structure. It is believed that mTNFα and sTNFα regulate biological responses at autocrine/paracrine and endocrine levels, respectively.[12,13]

III. TNFα SIGNAL TRANSDUCTION

TNFα is a pleiotropic cytokine produced by many different types of cells in the body. However, cells of the monocytic lineage—such as macrophages, astroglia, microglia, Langerhans cells, Kupffer cells, and alveolar macrophages—are the primary synthesizers of TNFα.[14,15] TNFα acts through two transmembrane receptors: TNF receptor 1 (TNFR1), also known as p55 or p60, and TNF receptor 2 (TNFR2), also known as p75 or p80 (Fig. 1). TNFR1 and TNFR2 both contain four cysteine-rich repeats in their extracellular domains, forming elongated shapes that interact with the lateral grooves of TNFα trimer formed between two of its three protomers.[16] TNFR1 is constitutively expressed in most mammalian tissues, whereas the expression of TNFR2 is highly regulated and is typically expressed in the cells of the immune system. Binding of TNFα onto TNFR1 is considered to be an irreversible mechanism, whereas binding of TNFα onto TNFR2 has both rapid on and off kinetics. Therefore, it has been suggested that TNFR2 might act as a “ligand passer” to TNFR1 in some cells, increasing the local concentration of TNFα at the cell surface through rapid ligand binding and dissociation.[17]

TNFα binds to both TNFR1 and TNFR2 with high affinity. There is, however, some species specificity in terms of the receptor subtype and TNFα binding. It has been shown that human TNFα binds only to the mouse TNFR1.[18,19] There are also other unique differences between TNFR1 and TNFR2. For example, the cytoplasmic portion of TNFR1, but not TNFR2,

contains a death domain. In addition, TNFR2 can be fully activated only by mTNFα and not by sTNFα.[20] Both TNFR1 and TNFR2 can be cleaved from the cell surface by the matrix metalloproteinase family of enzymes in response to an inflammatory signal. Interestingly, these cleaved extracellular domains of the TNF receptors retain the ability to bind TNFα, and thus function as endogenous inhibitors of TNFα.[21]

Activation from TNFR1 is responsible for a large number of inflammatory responses classically attributed to TNFα. This has Volume 20, Number 2, 2010 been extensively demonstrated by experiments using receptor-specific antibodies,[22,23] receptor-specific ligands,[24,25] and TNFR1- or TNFR2-deficient mice.[14,26,27] TNFα trimer binds to the extracellular domain of TNFR1, releasing the inhibitory protein, silencer of death domains (SODD), from the intracellular domain of TNFR1. The intracellular domain of the oligomerized TNFR1 is then bound by an adaptor protein TNF receptor-associated death domain (TRADD),[28] which recruits additional adaptor proteins: receptor interacting protein-1 (RIP-1), a serine/threonine kinase,[29] and TNFR-associated factor 2 (TRAF2), an E3 ubiquitin ligase (Fig. 1).[30] This complex is then internalized and the TRADD-RIP-1-TRAF2 complex is released from TNFR1. These adapter proteins are then involved in activating key signaling pathways. RIP-1 recruitment of MEKK-3 and transforming growth factor-beta (TGFβ)-activated kinase (TAK1) subsequently activates the IKK (inhibitor of κB kinase) complex. The IKK complex then phosphorylates (primarily by IKKβ) IκBα, as well as other IκB proteins, which then leads to the ubiquitination and degradation of IκBα. This then results in the release of NFκB subunits that are bound to IκBα under unstimulated conditions. The free NFκB subunits translocate into the nucleus and evoke gene transcription.[31–36]

In addition to RIP1-MEKK3-TAK1–mediated activation of the NFκB pathway, TRAF2 has also been shown to activate NFκB by binding to the IKK complex[37] and by recruiting inhibitor of cellular apoptosis proteins (cIAP)-1 and cIAP-2. These caspase inhibitors also possess ubiquitin ligase activity by which they play a role in IκB degradation.[31] Interestingly, it has been shown that TNF induction of NFκB activation in macrophages can be mediated by c-Src, a nonreceptor tyrosine kinase.[38] Therefore, there are many variations of the signaling mediators for TNFα-induced signaling pathways, depending on the cell type. In this regard, we recently showed that TNFα-induced NFκB activation in a macrophage cell line is dependent on G-protein coupled receptor kinases-2 and -5.[39] G-protein coupled receptor kinases were originally discovered for their role in the desensitization of G-protein coupled receptors.[40] In a recent study, we found that GRK2 and GRK5 can directly interact with and phosphorylate IκBα and mediate TNFα-induced NFκB activation in Raw264.7 macrophages (Fig. 1). We also found that IKKβ was dispensable in this particular cell line because knock-down of IKKβ did not affect TNFα-induced IκBα phosphorylation, whereas knockdown of GRK2 and GRK5 blocked TNFα-induced IκBα phosphorylation and NFκB activation. It is not clear if this role of GRK2 and 5 is specific for macrophages or for this particular macrophage cell line. Sorriento et al.[41] recently reported that GRK5 is a negative regulator of NFκB signaling in endothelial cells, and this role of GRK5 is independent of its kinase activity in an endothelial cell line. Thus, the role of GRKs in NFκB signaling may depend on the cellular context.

Stimulation of TNFR also activates a MAP3K called apoptosis-signaling kinase-1 (ASK-1)[42] that associates with TRAF2 in the TRADD-RIP-1-TRAF2 complex, activating MAP2Ks, MEK-4, and MEK-6, which in turn activate c-Jun N-terminal kinases (JNKs) and p38 MAPK.[43] JNK and p38 MAPK subsequently activate transcription of many genes via transcription factors such as AP-1. In addition to JNK and p38 MAPKs, TNFR also activates the ERK signaling pathway via activation of TPL2-MEK-ERK pathway.[35] Stimulation of the TPL2 pathway involves the IKKβ-mediated phosphorylation of NFκB1 p105, which is

stoichiometrically associated with TPL2 under unstimulated conditions. IKKβ phosphorylation of p105 leads to ubiquitination and partial degradation of p105, which then releases TPL2. The free TPL2 then activates the MEK-ERK signaling.[44–47] In addition to these signaling pathways, TNFR1 activation is also involved in pro-apoptotic signaling via the Fas-associated death domain. Micheau et al.[48] showed that TNFR1-induced pro-apoptotic signaling is mediated by the formation of two distinct signaling complexes. Complex-I is formed rapidly at the plasma membrane and consists of TNFR1, TRADD, RIP, TRAF2, and c-IAP1, and this complex triggers NFκB response without affecting apoptosis. However, a second complex is formed that lacks the TNFR1 but consists of FADD and procaspases-8 and -10. This complex is formed in the cytoplasm and initiates apoptosis if the NFκB signaling does not induce antiapoptotic proteins such as $FLIP_L$.

Although TNFR1 is responsible for most cellular responses to TNFα (including cytotoxicity, cell growth, NFκB activation, and upregulation of adhesion and cytokine genes), TNFR2 signaling has been reported to be important for proliferation of lymphoid cells. In some cell types TNFR2 has also been shown to be important for cytotoxicity as well as NFκB activation.[49–54] Using TNFR1 knockout mice, Pfeffer et al.[14] demonstrated that TNFR1 plays a crucial role in endotoxemia from lipopolysaccharides or from *Staphylococcus aureus* enterotoxin B. The same research group, however, showed that TNFR1 knockout mice succumb to infection from *Listeria monocytogenes* because of severely impaired bacterial clearing. Using a similar model, Rothe et al.[26] showed that although the TNFR1 knockout mice are resistant to the lethal effects of low doses of LPS after D-galactosamine sensitization, they remain susceptible to high dose LPS. In another study using TNFR2 knockout mice, Erickson et al.[27] showed that the TNFR2 knockout mice show normal T-cell development. However, TNF-induced tissue necrosis is markedly inhibited in these mice, suggesting that TNFR2 plays an important role in TNF-mediated necrotic effects. Using fibroblasts from TNFR1 and TNFR2 knockout mice, Kalb et al.[55] demonstrated that TNFR1 and TNFR2 activate signaling pathways with different kinetics. Therefore, depending on the cell type, TNFR1 and TNFR2 may have distinct, as well as overlapping, roles in signal transduction and gene expression.

IV. ROLE OF MACROPHAGES IN INFLAMMATION AND DISEASE

Macrophages are innate immune cells that form the first line of defense against invading pathogens. In addition, these cells also play a crucial role in tissue homeostasis, coordination of adaptive immune responses, inflammation, and repair.[57] Elie Metchnikoff first coined the term “macrophage” to describe large mononuclear phagocytic cells.[56] Cells related to macrophages have also been found in early life forms. In addition, some protozoans also have certain features similar to that of mammalian macrophages.[57] Among the mononuclear phagocytic system macrophages are the major differentiated cell types. Macrophages also exhibit significant structural and functional heterogeneity and are distributed throughout the body.[57] They are present in the liver, lungs, lymphoid organs, gastrointestinal tract, central nervous system, serous cavities, bone, synovium, and skin, and thus participate in a variety of physiological and pathophysiological processes.[57]

Macrophages represent a major defense system against invasion of the host by a range of microorganisms including that of bacteria, viruses, fungi, and protozoa.[57] They are involved in the recognition, phagocytosis, and destruction of the organisms. In addition, macrophages are also involved in antigen presentation and secretion of a wide variety of products, including enzymes, enzyme inhibitors, cytokines, chemokines, complement components, coagulation factors, and arachidonic acid intermediates.[57] Interestingly, apart from secretion of a repertoire of cytokines/chemokines, macrophages also respond to these products in an autocrine/paracrine manner, thus accentuating the inflammatory response. Because of these

various functions of macrophages, these cells have been implicated in a number of disease processes including rheumatoid arthritis, autoimmune and primary immunodeficiency diseases, Alzheimer's, wound healing processes, and atherosclerosis, as well as in tumor biology. Because of this crucial role in various physiological and pathophysiological processes, mechanisms by which macrophages respond to extracellular stimuli have been examined at great depths. In this regard, several studies have determined the specific role of TNFα signaling mechanisms and their biological consequences with respect to macrophage biology.

V. REGULATION OF MACROPHAGE BIOLOGY BY TNFα

A. Role of TNFα in Inflammation

TNFα is a powerful pro-inflammatory agent that regulates many facets of macrophage function. It is rapidly released after trauma, infection, or exposure to bacterial-derived LPS and has been shown to be one of the most abundant early mediators in inflamed tissue.[58] Among its various functions is its pivotal role in orchestrating the production of a pro-inflammatory cytokine cascade. TNFα is thus considered to be a "master regulator" of pro-inflammatory cytokine production.[59] In addition to pro-inflammatory cytokines, TNFα also increases lipid signal transduction mediators such as prostaglandins and platelet activating factor.[60] Based on these roles, TNFα has been proposed as a central player in inflammatory cell activation and recruitment and is suggested to play a critical role in the development of many chronic inflammatory diseases (Fig. 2).[61]

B. Role of TNFα in Macrophage Activation

Toll-like receptors induce the production of TNFα from macrophages that, in addition to other factors, activates macrophages. Exogenous addition of TNFα, however, activates macrophages only after priming with interferon gamma (IFNγ). TNFα and IFNγ exhibit a cross-talk at the level of TNFR1 to induce activation of macrophages. It has been shown that TNFα induces a stronger activation of NFκB in the presence of IFN-γ. IFN-γ signaling causes nuclear localization of STAT1α that precludes it from being recruited at TNFR1, leading to an enhanced TNFα-induced NFκB activation.[62] Activated macrophages can migrate to sites of inflammation, where they encounter pathogens and lyse them. This is accomplished by an increased production of toxic oxygen species and via induction of inducible nitric oxide synthase (iNOS) to produce nitric oxide (NO). In a recent study, Magez et al.[63] demonstrated that the control of *Trypanosoma congolense* infection is dependent upon macrophage- and neutrophil-derived soluble TNFα. They also showed that intact TNFR1 signaling via nitric oxide pathway was essential for this event. Using an *in vivo* approach, Salkowski et al.[64] demonstrated that LPS-induced iNOS expression in the liver is, in part, dependent on TNFR signaling in the macrophages.

Studies on TNFα signaling in macrophages have mostly focused on the acute and transient activation of signal transduction pathways and transcription factors such as NFκB. However, a recent study investigated the responses of primary macrophages during a 2-day period after TNFα stimulation.[65] The results from this study showed that TNFα induces an autocrine loop that is characterized by a low and sustained production of IFN-β. IFN-β was found to act synergistically with canonical TNFα signal to induce a sustained expression of genes encoding inflammatory molecules and a delayed expression of genes encoding interferon-response molecules. These molecules are then thought to prime macrophages for enhanced responses to subsequent challenge with microbial products or cytokines. This feed-forward loop plays an important role in sustaining inflammation.[65]

C. Role of TNFα in Resolution of Inflammation

In addition to its role in the initiation and perpetuation of inflammation, TNFα has also been shown to be important in the resolution of inflammation. In a study by Michlweska et al.,[66] the authors examined the role of TNFα on LPS-mediated efferocytosis of neutrophils by human monocyte-derived macrophages. They demonstrated that LPS-mediated inhibition of efferocytosis of neutrophils by macrophages is mediated via TNFα. Because efferocytosis plays a crucial role in clearing neutrophils, this role of TNFα is especially important in the resolution of inflammation.

D. Role of TNFα in Proliferation, Apoptosis, and Differentiation of Macrophages

Studies have shown that the long-term survival of macrophages is dependent on autocrine signaling by TNFα.[67] Because TNFα mediates many of the pathological effects of LPS-TLR4 in conditions such as septic shock, it is suggested that prolonged macrophage survival mediated by TNFα plays an important role in sepsis.[68] In an effort to determine the role of TNFR in Fas-induced apoptosis, Takada et al.[69] generated macrophage cell lines derived from wild-type mice as well as from TNFR1, TNFR2, and TNFR1/2 genetically deleted mice. The authors demonstrated that both TNFR1 and TNFR2 were required for Fas-induced macrophage apoptosis because both TNFR1 and TNFR2 deleted cells were resistant to anti-Fas-induced apoptosis. A similar cross-talk mechanism was also shown for another member of the TNF family, namely RANKL (receptor activator for NF-κB ligand). Takada and Aggarwal[70] showed that RANKL signaling in macrophages is modulated by TNF receptors. In contrast to its reported roles in macrophage apoptosis, Guilbert et al.[71] showed that TNFα increases the proliferation of growth-competent macrophages in the presence of macrophage colony-stimulating factor-1 (M-CSF). TNFα has also been shown to enhance the production of macrophages *in vitro* from primitive mouse hematopoietic progenitor cells.[72]

In addition to these roles, Witsell and Schook[73] demonstrated that TNFα has macrophage differentiation capabilities. TNFα gene transcripts are expressed during differentiation of bone marrow-derived macrophages. To test the importance of this, these authors used an antisense approach and blocked TNFα gene expression during differentiation. They found that in the absence of TNFα (in the differentiating macrophages), the cells followed a proliferative program instead of going through the differentiation program. These results suggest that “autocrine” TNFα effects are important in promoting the macrophage differentiation. Taken together, these results suggest that similar to other cell types, TNFα plays an important role in the proliferation, apoptosis, and differentiation of macrophages.

E. Role of TNFα in Atherosclerosis

There is now strong evidence for the role of macrophage-derived TNFα in the development of atherosclerosis.[74–77] Because macrophage scavenger receptor plays an important role in foam cell formation, Hsu et al. examined the effect of TNFα on macrophage scavenger receptor expression. They found that TNFα downregulates macrophage scavenger receptor gene expression and protein via transcriptional and post-transcriptional processes.[78] In another study, TNFα-induced MAPK pathway was shown to be important in the transcriptional regulation of macrophage scavenger receptor and increase in the receptor expression. However, long-term treatment with TNFα led to downregulation of the scavenger receptor and foam cell formation potentially via a post-transcriptional mechanism.[79] Because macrophage scavenger receptor and foam cell formation play critical roles in the pathogenesis of atherosclerosis, these studies further implicate an important role of macrophage TNFα in atherosclerosis. In inflammatory models, TNFα has also been shown to be important in the upregulation of adhesion molecules that are critical in

extravasation of monocytes. Studies have shown that this role of TNFα is mediated via the NFκB pathway.[80]

F. Role of TNFα in Osteoclastogenesis

Osteoclasts derived from the monocyte/macrophage lineage are an important cell type that regulates bone formation and remodeling under normal conditions, as well as in pathologic bone loss seen in diseases such as rheumatoid arthritis. Members of the TNF superfamily, especially TNFα and RANKL, are intricately involved in osteoclast differentiation and function as well as in bone destruction through osteoclast activation.[81] In this regard, RANK-knockout mice show severe osteopetrosis with total occlusion of the bone marrow space within the endosteal bone. These mice also lack osteoclasts but have normal osteoclast progenitors.[82] There is strong evidence that TNFα can induce formation of osteoclasts via a mechanism that is independent of RANKL signaling.[83] In addition, TNFα has been shown to promote bone resorption *in vitro* and *in vivo* and induces secretion of RANKL in osteoblastic cells.[84–89] TNFα has also been shown to be an important mediator of LPS-induced osteoclastogenesis.[90] Furthermore, TNFα alone or in combination with interleukin 1 (IL-1) increases osteoclast numbers seen at sites of bone resorption.[91] TNFα also increases the numbers of circulating osteoclast precursors by promoting their proliferation and differentiation in the bone marrow.[92] In addition, TNFα, in the presence of M-CSF has also been reported to directly act on osteoclast precursors and induce osteoclast differentiation independent of RANKL.[93] Activation of p38 MAP kinase plays an important role in TNF-induced osteoclast differentiation.[94] Moreover, TNFα has been shown to promote the survival of differentiated osteoclasts, which generally have a short life span. This function of TNFα seems to be dependent on the phosphatidylinositol 3-kinase, Akt, and MEK/ERK signaling pathways.[95] TNFα-induced osteoclast formation has also been shown to be dependent on TRAF2.[96]

In contrast, Lam et al.[97] have shown that although TNFα alone fails to induce differentiation of murine osteoclast precursors, priming with RANKL (with a concentration that is insufficient to induce osteoclastogenesis by itself) dramatically enhances TNFα-induced osteoclast differentiation. Moreover, TNFα, in association with RANKL, activates these cells to resorb bone, causing joint erosions.[98] Taken together, these results suggest that TNFα (alone or together with RANKL) does play an important physiological role in the development and differentiation of the musculoskeletal system. In light of the fact that TNFα levels are increased in inflammatory musculoskeletal diseases, TNFα has been proposed to mediate local bone destruction in these diseases.[99]

The physiological functions of TNFα in macrophages are numerous; thus, any aberration in its production or signaling in part plays a crucial role in the pathogenesis of many inflammatory diseases. Therefore, it is not surprising that TNFα and its receptors have been targeted for therapeutic development for the treatment of inflammatory diseases.

VI. THERAPEUTIC AGENTS TARGETING TNFα IN INFLAMMATORY DISEASE

Even though TNFα is important for normal homeostatic mechanisms including host defense, dysregulated production of TNFα has been found in several inflammatory diseases. In addition, TNFα-activated macrophages are the principal components of the immunopathology of many autoimmune diseases.[100] Thus, macrophage-derived TNFα is now implicated in a number of diseases, including rheumatoid arthritis, inflammatory bowel disease, psoriatic arthritis, ankylosing spondylitis, juvenile chronic arthritis, atherosclerosis, and sepsis (Fig. 3).[101–104] After a number of laboratory-based studies and human clinical

trials, five drugs (TNFα blocking agents) are currently licensed for treating some of these diseases, including rheumatoid arthritis and Crohn's disease. These five drugs are as follows: 1) etanercept is a recombinant human soluble fusion protein of TNFR2 coupled to the Fc portion of IgG[105]; 2) infliximab is an anti-TNF human-murine chimeric IgG1 monoclonal antibody[106]; 3) adalimumab is a human anti-human TNFα antibody that was produced by phage display[107]; (4) certolizumab pegol is a PEGylated TNFα antibody[108]; and 5) golimumab is a human anti-TNFα IgG1κ monoclonal antibody that can be administered by the patient.[109]

A. TNFα Blockers in Rheumatoid Arthritis

TNFα is considered to be the key inflammatory cytokine in rheumatoid arthritis and is found in high levels in patients with the disease. Its dysregulated secretion by macrophages is involved in both inducing and maintaining synovitis. It has been shown that TNFα activates p38 and ERK MAPKs in the synovial macrophages.[110] TNFα also affects the bone marrow, causing anemia in patients with rheumatoid arthritis.[111] Based on extensive studies on the role of TNFα in rheumatoid arthritis, TNFα was targeted for the treatment of this disease. Since their first license for clinical use in 1998, TNFα antagonists have been used with good therapeutic benefits. Several studies suggest that anti-TNFα therapeutics may work by affecting signaling pathways resulting in cell cycle arrest, apoptosis, and suppression of cytokine production. For instance, etanercept and infliximab have been shown to induce apoptosis in monocytes and macrophages both in synovial fluid and peripheral blood *in vivo*.[112] Furthermore, treatment with TNFα blockers reduces the number of infiltrating synovial granulocytes and macrophages as well as reduces the expression of chemokines IL-8 and monocyte chemotactic protein-1.[113]

B. TNFα Blockers in Inflammatory Bowel Disease, Ankylosing Spondylitis, and Psoriasis

Similar to its role in rheumatoid arthritis, TNFα has also been shown to be crucial in the pathogenesis of inflammatory bowel disease.[114–116] Clinical studies demonstrate that infliximab is effective in the treatment of patients with Crohn's disease and ulcerative colitis.[117,118] Infliximab is also effective in mucosal healing in patients who are refractory to conventional treatment using corticosteroids and/or immunosuppressive agents.[119,120] In patients with ankylosing spondylitis, both etanercept and infliximab have been shown to be effective in inducing and maintaining remission.[121–129] Because psoriasis is an inflammatory skin disorder, patients with psoriasis also develop inflammatory arthritis.[130,131] Clinical studies have shown that anti-TNFα agents are effective in treating the dermatological, as well as the articular, components of psoriasis.

C. TNFα in Other Diseases

TNFα has been shown to play an important role in the pathogenesis of neurological diseases. Microglial cells (from monocytic lineage) in the central nervous system are one of the major producers of TNFα and participate in a number of pathophysiological conditions in the brain.[132] With regard to disease pathogenesis, TNFα in the brain has been shown to have both harmful and beneficial effects, thus making it a difficult therapeutic target in neurological diseases.[133–137] In one study in humans, neutralization of TNFα did not benefit patients with relapsing-remitting multiple sclerosis and, in fact, was shown to increase the disease process.[138] Therefore, further extensive studies on the "basic science" aspects of TNFα in the central nervous system are essential before it can be targeted for treating neurological diseases.

There is accumulating evidence that patients with rheumatoid arthritis and other chronic inflammatory diseases have increased incidence of cardiovascular disease. TNFα has been implicated in this process, and clinical trials have shed some evidence that the TNFα

blocking in rheumatoid arthritis patients ameliorates the cardiovascular risk.[139] TNFα has also been implicated in diseases of other systems such as respiratory and renal disease and there is some evidence that the TNFα blocking agents may be effective in some of these conditions.[140,141] Further research in both basic science as well as clinical research is essential before exploring the therapeutic potential of anti-TNFα agents in other diseases such as cancer, diabetes, obesity, and so forth.

VII. PERSPECTIVES AND CONCLUSIONS

Although actions of TNFα have been known for more than a century, TNFα was identified only in 1975 and cloned a decade later. The last 2 decades of active research on TNFα have not only unraveled a TNF/TNFR superfamily, but have also led to seminal advances in our understanding of the role of macrophage-derived TNFα in various inflammatory disease processes. Importantly, the discovery of TNFα and its current clinical use represent a classic example of how laboratory-based research can be translated into clinical medicine for treating inflammatory disease. Current biotherapies in rheumatoid arthritis target TNFα by repetitively administering recombinant proteins to block TNFα. However, it is necessary to target specific cells and tissues and to avoid the frequent administration of recombinant proteins. Gene therapy and targeting TNFα at the RNA level seem to be promising approaches. These techniques can be used for the specific suppression of immune system targets and also for replacing the frequent administration. In one such study, murine macrophages transfected with siRNA against TNFα showed marked inhibition of TNFα secretion. In addition, TNFα siRNA delivered *in vivo* using liposomes caused a 50% to 70% decrease in articular and systemic TNFα secretion, suggesting some promise in these methods for future therapeutics.[142] Further research on the most efficient ways to block TNFα selectively is needed.

Of further importance is the knowledge that we have obtained from clinical studies with regard to the mechanisms of TNFα actions, which helps return clinical medicine back to laboratory-based research to answer some critical and unexpected clinical findings. In this regard, it should be noted that more that one third of patients suffering from any disease against which anti-TNFα drugs have been approved do not benefit clinically from anti-TNFα treatment. Future studies are thus needed to investigate if these differences are due to different mechanisms of disease pathogenesis in different patients or due to the presence of other regulators in the pathogenesis of disease. This can be achieved by laboratory-based research in which we can further investigate the molecular mechanisms of these disease processes as well as the mechanisms of “normal” TNFα signaling. In understanding the normal regulators of the signaling process, especially in macrophages, we can then begin to understand if aberrant regulation of these “regulators” contributes to the heterogeneity in disease process. This will not only help us to understand the overall mechanisms of TNFα signaling in normal and inflammatory diseases, but it will also lead to identification of potential therapeutic targets that may be superior to currently available therapeutics. As noted above, TNFα is equally essential in maintaining a normal homeostasis even though its aberrant production and signaling can lead to inflammatory disease process. Therefore, selective targeting of the “pathogenic” signaling pathway may render better outcomes than are currently available.

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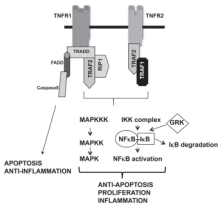

FIGURE 1.
Signaling from TNFα receptors.

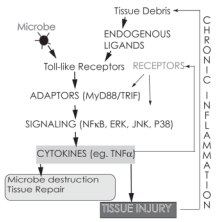

FIGURE 2.
TNFα plays a central role in the perpetuation of inflammation in chronic diseases.

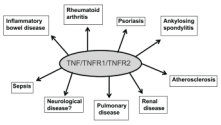

FIGURE 3.

TNFα and its receptors play a major role in a number of inflammatory diseases. Anti-TNFα agents are clinically used to treat some of these diseases.