TUMOR NECROSIS FACTOR ALPHA (TNF-α)

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Abstract

Tumor necrosis factor alpha (TNF, TNFSF2) is an important multifunctional cytokine that regulates inflammation, host defense, immune responses, and apoptosis. TNF exists as a membrane-bound 26kDa, type II integral membrane protein, and a 17kDa soluble homotrimeric TNF that is generated via proteolytic cleavage by TNF-α converting enzyme (TACE, ADAM17). Both the membrane-bound and soluble forms of TNF can interact with either the 55kDa, type I tumor necrosis factor receptor (TNFRSF1A, TNFR1) or the 75kDa, type II TNF receptor (TNFRSF1B, TNFR2). TNFR2, however, may be preferentially activated by membrane-bound TNF rather than soluble TNF, which may lead to qualitatively distinct TNF responses. The main biologic function of TNF is to induce inflammation via the upregulation of gene transcription, primarily through the NF-κB and AP-1 signaling pathways, which leads to the expression of a large number of genes. TNF plays an important role in innate immunity and host defense against bacterial, fungal, and parasitic pathogens and is particularly important for host defense against intracellular organisms, such as Mycobacterium tuberculosis. TNF has also been implicated in the pathogenesis of pulmonary diseases, such as idiopathic pulmonary fibrosis, emphysema, sarcoidosis, acute lung injury, bacterial pneumonia, and asthma.

Introduction

Tumor necrosis factor alpha (TNF, TNFSF2) is the prototypical member of a large superfamily of at least 17 related cytokines that play key roles in regulating inflammation, host defense, adaptive immunity, apoptosis, autoimmunity, and organ development. Although the antitumor activity of TNF was initially recognized in the nineteen century when tumor regression was observed in the setting of acute bacterial infection, it was not until 1984 that the protein was purified and the cDNA was cloned. TNF is recognized as an important mediator of inflammatory and immune responses as well as apoptosis. TNF also plays a central role in the pathogenesis of several inflammatory diseases, including rheumatoid arthritis and inflammatory bowel disease. Furthermore, the proinflammatory effects of TNF have served as the rationale behind the introduction of anti-TNF therapies for the treatment of rheumatoid arthritis and Crohn’s disease, which have included a recombinant soluble human TNFR2-Ig fusion protein and an anti-TNF monoclonal antibody.

Structure of the TNF Gene and Protein

The TNFSF2 gene resides on human chromosome 6p21.3 and on murine chromosome 17, where it is preceded by the TNFSF1 gene that encodes lymphotoxin-α (LTA, LT, TNF-β). Human TNFSF2 is a single-copy gene of 3.6 kb that is composed of four exons and three introns. Expression of the TNFSF2 gene can be regulated at both the transcriptional and the translational level. The TNFSF2 promoter is complex and contains multiple transcription factor binding sites, including those for nuclear factor kappa B (NF-κB), activator protein 1 (AP-1), AP-2, specificity protein-1 (SP-1), Eq6-transformation specific (ETS), nuclear factor of activated T cells (NF-AT), activating transcription factor-2 (ATF-2), and Krox-24. A cyclic AMP response element (CRE), a purine-rich box (PU.1), and a MHC class II-like ‘Y box’ are also present. Translational regulation may also occur via the UA-rich sequence in the 3’ untranslated region of human TNF mRNA, which may destabilize the transcript.

The crystal structure for soluble TNF protein was initially solved in 1989 and has been obtained at high resolution. TNF exists as a compact trimer in the shape of a cone or bell that is composed of three monomers, which are intimately associated about a threefold axis of symmetry. Each of the TNF monomers exists as two elongated, antiparallel β-pleated sheets, each with eight, antiparallel β-strands that form a ‘jelly-roll’ topology. Further insights have been gained by analyses of the interaction between lymphotoxin and the extracellular domain of TNFR1, which forms a complex composed of three receptor molecules bound symmetrically to one lymphotoxin trimer. The receptors, which exist in a rod-shaped configuration, align along their long parallel axis and interact with the lateral grooves of the trimeric ligand. Although TNF receptors were thought to undergo ligand-dependent trimerization, pre-ligand-binding assembly domains have been identified in the extracellular domains of TNFR1 and TNFR2 that mediate specific ligand-independent assembly of receptor trimers.

Regulation of TNF Production and Activity

Macrophages are a primary source of TNF production. TNF can also be produced by many cell types, including T and B cells, mast cells, fibroblasts, keratinocytes, osteoblasts, smooth muscle cells,
endothelial cells, epithelial cells, and tumor cells (Figure 1). TNF production can be stimulated by a wide variety of mediators, including bacterial products (e.g., lipopolysaccharide), cytokines (e.g., IL-1, TNF, and interferon-γ), viruses (e.g., HIV and influenza A), parasites (e.g., Plasmodium and Entamoeba), complement (e.g., C5a), immune complexes, phorbol ester, calcium ionophore, and UV irradiation. In particular, large quantities of TNF can be produced in response to bacterial lipopolysaccharide, which contributes to the pathogenesis of septic shock. Alternatively, suppressors of TNF production include cytokines (e.g., IL-4, IL-6, IL-10, and TGF-β), cyclic AMP, cyclic nucleotide phosphodiesterase inhibitors (e.g., pentoxifylline and rolipram), cyclosporine A, dexamethasone, and viruses (e.g., Epstein–Barr virus).

Human TNF exists in either membrane-bound or soluble forms. TNF is initially expressed as a 26 kDa, type II integral membrane protein that is arranged as a stable homotrimer. The 17 kDa soluble homotrimeric TNF is generated from the membrane-bound form via proteolytic cleavage between Ala 76 and Val 77 by TNF-α converting enzyme (TACE, ADAM17), which is a member of the ADAM (a disintegrin and metalloprotease) family. ADAMs are a large family of type I transmembrane proteins that contain multiple functional domains, including a prodomain (that maintains the enzyme in an inactive state via a cysteine switch mechanism), a zinc-dependent catalytic domain, a disintegrin–cysteine-rich domain, an epidermal growth factor-like repeat, a transmembrane domain, and an intracytoplasmic tail. Both the membrane-bound and the soluble forms of TNF can interact with either the 55 kDa, type I tumor necrosis factor receptor (TNFRSF1A, TNFR1) or the 75 kDa, type II TNF receptor (TNFRSF1B, TNFR2). TNFR2, however, may be preferentially activated by membrane-bound TNF rather than soluble TNF, which may lead to qualitatively distinct TNF responses.

**Biological Functions of TNF**

TNF is a multifunctional, proinflammatory cytokine that mediates pleiotropic biological functions, especially host defense. The broad spectrum of TNF-mediated biological functions is a direct consequence of its ability to induce the expression of a large number of gene products. Genes or proteins that can
be induced by TNF include transcription factors (e.g., c-fos and c-jun), cytokines (e.g., IL-1, TNF, IL-6, and IL-8), chemokines (e.g., eotaxin), growth factors (e.g., platelet-derived growth factor and granulocyte-macrophage colony-stimulating factor), adhesion molecules (e.g., ICAM-1, VCAM-1, and E-selectin), receptors (e.g., IL-2Rz and EGF receptor), complement (e.g., C3), major histocompatibility complex proteins (e.g., MHC class I and MHC class II), acute phase reactants (e.g., haptoglobin), and enzymes (e.g., nitric oxide synthase, manganese superoxide dismutase, and matrix metalloproteases).

TNF was initially identified via its ability to induce cytotoxicity against tumor cell lines and to mediate tumor necrosis in certain animal models. TNF was also found to be identical to the protein cachectin, which mediates fever and muscle wasting in cancer patients and weight loss, anorexia, and lethality in mice. TNF has been identified as playing an important role in innate immunity and host defense against bacterial, fungal, and parasitic pathogens. In particular, TNF plays a key role in host defense against intracellular pathogens, such as *Mycobacterium tuberculosis*, Leishmania major, and *Listeria monocytogenes*. This is exemplified by the association between anti-TNF therapy and the reactivation of latent tuberculosis. Similarly, anti-TNF therapy has been associated with infections caused by *Histoplasma capsulatum*, Aspergillus fumigatus, and L. monocytogenes. Tissue macrophages and T lymphocytes produce TNF in response to invasion by intracellular pathogens, which then mediates cytokine and chemokine production and the subsequent recruitment of activated leukocytes to the site of infection. In later stages of infection, TNF enhances antigen presentation and T-cell costimulation, thereby promoting the induction of acquired immunity.

Another important function of TNF is the induction of apoptosis, which typically occurs in the absence of concomitant signaling by NF-κB. This is exemplified by transgenic mice deficient in NF-κB signaling, which undergo embryonic lethality as a consequence of TNF-mediated liver cell apoptosis. The biologic role of TNF-mediated apoptosis, however, requires further clarification.

Insights into the biological function of TNF have also come from studies utilizing transgenic models. TNF knockout (TNF−/−) mice develop normally and have no structural or morphological abnormalities. TNF−/− mice are highly susceptible to challenge with infectious agents (e.g., *Candida albicans* and *L. monocytogenes*), are resistant to lethality from minute doses of lipopolysaccharide following sensitization with D-galactosamine, demonstrate low toxicity to TNF, have defective granuloma formation, do not form germinal centers after immunization, and display reduced immunoglobulin responses to thymus-dependent antigens. Furthermore, the function of macrophages, neutrophils, and T cells derived from TNF−/− mice is normal. TNF has been implicated in the pathogenesis of diabetes and may be an important mediator in obesity because TNF−/− mice are protected from obesity-induced insulin resistance.

Insights into TNF function have also been derived from studies of transgenic mice lacking the two TNF receptors, TNFR1 and TNFR2. For example, TNF may play an important role in the regulation of central nervous system injury and function. TNF may have a neuroprotective function because neuronal damage secondary to ischemic and epileptic injury is exacerbated in TNFR1/TNFR2 double knockout mice. Similarly, in a model of experimental autoimmune encephalitis, TNF−/− mice develop severe autoimmune-mediated demyelination, which can be attenuated by TNF administration.

**TNF Receptors and Signaling Pathways**

The main biologic function of TNF is to induce inflammation via the upregulation of gene transcription, primarily through the NF-κB and AP-1 signaling pathways. This is accomplished by signaling through two members of the TNF receptor superfamily, TNFR1 and TNFR2. TNFR1 is constitutively expressed by most cell types, whereas TNFR2 has a highly regulated pattern of cellular expression. Binding of TNF to TNFR1 mediates the translocation of TNFR1 to lipid rafts, where it recruits the adaptor protein, TNF receptor-associated death domain (TRADD), via a direct interaction with the TNFR1 death domain. TRADD then serves as an assembly platform to mediate the recruitment of other adaptor proteins, such as receptor-interacting protein (RIP) and TNFR-associated factor-2 (TRAF2), which in turn recruit I-κB kinases and thereby activate the NF-κB pathway (Figure 2). TNFR1 and RIP within the lipid rafts are then ubiquitinated, which leads to their degradation via the proteasome pathway. TNFR1 signaling through TRAF2 can also activate the c-Jun N-terminal kinase (JNK) pathway through the MAPK kinase, mitogen-activated protein kinase kinase-7 (MKK7), which results in the phosphorylation of c-Jun, with resultant increases in AP-1 activity.

TNF signaling can also induce apoptosis, primarily via TNFR1-mediated signaling. TNF-mediated apoptosis requires the blockade of the NF-κB pathway because NF-κB activation induces the expression of several antiapoptotic genes, including c-FLIP.
(cellular FADD-like interleukin-1-converting enzyme-inhibitory protein) and the cellular inhibitor of apoptosis proteins (IAPs), cIAP1 and cIAP2. Two models have been proposed for TNF-mediated apoptosis. One model proposes two sequential signaling complexes, with the initial formation of a TNFR1, TRADD, RIP1, and TRAF2 signaling complex I at the plasma membrane, followed by a cytoplasmic complex II composed of TRADD, RIP1, FADD (Fas-associated death domain), and caspase-8 that mediates cell death in the absence of NF-κB activation. The second model proposes the ligand-dependent formation of a TNFR1-associated death-inducing signaling complex (DISC) that is dependent on receptor internalization within endocytic vesicles, termed TNF receptosomes. In this model, receptor internalization allows the recruitment of TRADD, FADD, and caspase-8 to form a TNFR1-associated DISC within TNF receptosomes (Figure 2). Caspase-8 is rapidly activated and initiates apoptosis. TNF receptosomes then fuse with trans-Golgi vesicles to form multivesicular endosomes, allowing the caspase-8-mediated activation of acid sphingomyelinase and cathepsin D cascades, which can induce apoptosis via Bid cleavage and caspase-9 activation.

**TNF and Pulmonary Disease**

TNF has been implicated in the pathogenesis of a variety of diseases, including rheumatoid arthritis,
inflammatory bowel disease, endotoxin-mediated septic shock, HIV infection, diabetes, graft-versus-host disease, allograft rejection, and neoplastic disease. TNF may also play a role in the pathogenesis of pulmonary diseases, including pulmonary fibrosis, bacterial pneumonia, emphysema, sarcoidosis, acute lung injury, and asthma. Evidence of a role for TNF in pulmonary fibrosis derives from murine models in which overexpression of TNF in the lung produced a lymphocytic and fibrosing alveolitis that resembled human idiopathic pulmonary fibrosis. Similarly, TNF receptor knockout mice are protected from the fibroproliferative effects of inhaled asbestos fibers. TNF has also been shown to mediate anti-inflammatory and antifibrotic effects in murine models of bleomycin-induced lung injury and fibrosis, consistent with a complex role for TNF in the pathogenesis of pulmonary fibrosis.

TNF knockout mice display impaired bacterial killing and increased mortality, consistent with a role for TNF in host defense against bacterial pneumonia. TNF has also been implicated in the pathogenesis of cigarette smoke-induced emphysema because TNF-mediated processes were responsible for 70% of the airspace enlargement in a murine model, which may be a consequence of enhanced neutrophil recruitment. Similarly, overexpression of TNF in murine type II alveolar epithelial cells has been associated with severe airspace enlargement and pulmonary hypertension. TNF may also contribute to weight loss and cachexia associated with severe chronic obstructive pulmonary disease.

TNF has also been implicated in the pathogenesis of pulmonary sarcoidosis and the acute respiratory distress syndrome. Patients with progressive or severe sarcoidosis demonstrate increased spontaneous TNF production from alveolar macrophages, whereas elevated TNF release from alveolar macrophages may correlate with disease progression. TNF may play an important role in acute lung injury because TNF or TNFR1 knockout mice demonstrated attenuated lung neutrophil recruitment and microvascular permeability in a murine model of hemorrhage-induced acute lung injury. Lastly, although clinical evidence suggests a possible role for TNF in the pathogenesis of asthma, investigations utilizing murine models of allergic asthma have produced varied results. The results of future clinical trials will clarify whether a role exists for anti-TNF therapies in the treatment of these pulmonary diseases.


Further Reading


