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Tumor necrosis factor

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Abstract

Tumor necrosis factor (TNF) is a critical cytokine, which contributes to both physiological and pathological processes. This mini-review will briefly touch the history of TNF discovery, its family members and its biological and pathological functions. Then, it will focus on new findings on the molecular mechanisms of how TNF triggers activation of the NF-κB and AP-1 pathways, which are critical for expression of proinflammatory cytokines, as well as the MLKL cascade, which is critical for the generation of ROS in response to TNF. Finally, this review will briefly summarize recent advances in understanding TNF-induced cell survival, apoptosis and necrosis (also called necroptosis). Understanding new findings and emerging concepts will impact future research on the molecular mechanisms of TNF signaling in immune disorders and cancer-related inflammation.

Keywords

TNF; NF-κB/AP-1; Apoptosis/necrosis; Cancer and inflammation

1. Introduction

Since its discovery, TNF has been the center of study for its roles in normal physiology, acute inflammation, chronic inflammation, autoimmune disease and cancer-related inflammation. In addition, TNF receptor 1 (TNFR1)-mediated apoptosis, necrosis (also called necroptosis) and survival in TNF signaling have drawn much attention, leading to numerous new findings and emerging new concepts. This mini-review briefly summarizes the discovery and definition of TNF, its sources and its biological and pathological functions as well as the molecular mechanisms of its actions.

2. Synonyms

Tumor necrosis factor alpha (TNFα) is also known as endotoxin-induced factor in serum, cachectin, and differentiation inducing factor.

3. Definition

The tumor necrosis factor (TNF) family includes TNF alpha (TNFα), TNF beta (TNFβ), CD40 ligand (CD40L), Fas ligand (FasL), TNF-related apoptosis inducing ligand (TRAIL), and LIGHT (is homologous to lymphotoxins, exhibits inducible expression, and competes with HSV glycoprotein D for *H*VEM, a receptor expressed by T lymphocytes), some of the

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most important cytokines involved in physiological processes, systematic inflammation, tumor lysis, apoptosis and initiation of the acute phase reaction.

4. Discovery

More than 100 years ago, Dr. Williams B. Coley used crude bacterial extracts to treat tumor patients. He found that the bacterial extracts had an ability to induce tumor necrosis. While tumors were regressive, patients receiving bacterial extracts also suffered from a severe systematic inflammatory reaction. One of the major inflammatory stimulators now known to cause this reaction was identified in 1975, when a protein factor in the serum of endotoxin-treated animals was found to cause lysis in tumor cells and was therefore named “tumor necrosis factor”. In 1984, the TNF gene was isolated and characterized. About the same time, another gene encoding a protein, which was purified from T lymphocytes and was named T lymphotoxin alpha (TLα) in 1968, was also isolated and characterized. These two genes were found to belong to the same family. Thus, TNF was named “TNFα”, while TLα was called “TNFβ”. In 1985, Nobel Prize winner Dr. Bruce Beutler and his colleagues purified a protein called cachectin from the supernatant of endotoxin-treated macrophages. This protein induced wasting (cachexia) and septic shock in murine recipients. Cachectin and TNF were later revealed to be the same protein.

5. Sources

TNFα is mainly produced by macrophages, whereas TNFβ is mainly produced by T lymphocytes. Other cells can also express TNFα and TNFβ at low levels.

6. Genes and proteins

The human and murine TNFα genes are located on chromosome 6 and chromosome 17, respectively, and are preceded by the TNFβ gene. Both genes are present in a single copy, are approximately 3 kilobases in size and contain 4 exons. Several DNA binding sites for the transcription factor nuclear factor kappa B (NF-κB) have been identified within the promoter region of the TNFα gene; therefore, it appears that the expression of TNFα is NF-κB dependent. A DNA binding site for the high mobility group 1 (HMG1) protein is located in the promoter region of the TNFβ gene.

Two forms of TNFα exist: the membrane-bound form (mTNFα) and the soluble form (sTNFα). Human mTNFα contains 157 amino acids (aa) and a 76 aa leader sequence, while mouse mTNFα contains 156 aa plus a 79 aa leader sequence. During synthesis, TNFα translocates to the cell membrane where the TNFα converting enzyme (TACE) sheds the mTNFα into sTNFα. In contrast to TNFα, TNFβ only exists in the soluble form (sTNFβ).

TNF is conserved among species. For example, human TNF is 80% homologous to mouse TNF. The homology between TNFα and TNFβ in both species is approximately 30%.

7. Biological and pathological functions

TNF exerts many important physiological and pathological actions. TNF causes tumor cell necrosis (a process that involves cell swelling, organelle destruction, and finally cell lysis) and apoptosis (a process that involves cell shrinking, the formation of condensed bodies, and DNA fragmentation). Moreover, studies in TNFα- or TNFR-deficient mice have revealed that TNF plays an important role in the regulation of embryo development and the sleep-wake cycle, and that TNF is important for lymph node follicle and germinal center formation as well as host defense against bacterial and viral infection. TNF has been shown

to be an endogenous pyrogen that causes fever. Chronic exposure to a low dose of TNF may cause cachexia, wasting syndrome, and depression.

Additionally, TNF is a key mediator of both acute and chronic systematic inflammatory reactions. TNF not only induces its own secretion, but it also stimulates the production of other inflammatory cytokines and chemokines. TNF is a vital player in animal models of endotoxin-induced septic shock, and in chemotherapy-induced septic shock in late-stage lung cancer patients. TNF plays a central role in autoimmune diseases such as rheumatoid arthritis (RA), inflammatory bowel diseases including Crohn's disease and ulcerative colitis, multiple sclerosis, systemic lupus erythematosus and systemic sclerosis.

Finally, TNF has emerged as an important risk factor for tumorigenesis, tumor progression, invasion and metastasis. TNF is a key intermediary of cancer-associated chronic inflammation. Longterm usage of aspirin decreases TNF secretion and significantly reduces incidence of human colorectal colon cancer. Furthermore, TNF is frequently detected in biopsy samples from human breast, ovarian and renal cancers, as well as in adjacent stromal cells. It is also evident that TNF mediates the development of human malignant mesothelioma in response to environmental exposure to asbestos, and it has been shown that asbestos is not pathogenic in the absence of TNFR [1]. In other experimental murine cancer models, TNF has been shown to promote early stage liver and skin cancer, and enhance colon cancer metastases to the lung. This tumor promotion effect of TNF has also been observed in drosophila. For example, in the absence of the drosophila tumor suppressor *Scribble (Scrib)* gene, oncogenic protein Ras^{v12} can switch off the tumor suppressive activity of TNF, leading to tumor progression and invasion [2].

8. TNF and anti-TNF therapies in autoimmune disease and cancer

TNF has never been considered as a systemic anti-cancer drug because it has been implicated in a wide spectrum of systemic disorders. However, a high dose of regional TNF not only causes necrosis and the subsequent destruction of tumor blood vessels, but also enhances efficacy of chemotherapy. Therefore, TNF has been used for regional treatment of metastatic melanoma and advanced soft tissue sarcoma.

Conversely, due to the crucial role of TNF in the pathogenesis of autoimmune disorders and cancer-associated chronic inflammation, anti-TNF treatment has been developed as a therapy for rheumatoid arthritis, inflammatory bowel disease, cancer-related cachexia, leukemia and ovarian cancer. The strategies developed to block TNF signaling either utilize anti-TNF antibodies (Remicade/Infliximab, Adalimumab/Humira and Golimumab) to neutralize sTNF, or Fc fragments of human immunoglobulin 1 fused with extracellular domains of TNFR1 or TNFR2 (Certolizumab/Cimzia and Etanercept/Enbrel) to block the interaction of TNF with TNFRs.

Overall, anti-TNF therapy has been very successful in ameliorating symptoms of autoimmune disorders. However, some side effects have been reported. For example, patients receiving anti-TNF regiments exhibit unexpected toxicities, and anti-TNF therapy may enhance the risk of tuberculosis infection. Most ominously, patients receiving anti-TNF therapy may have an increased risk of developing breast cancer, lung cancer, lymphoma, and skin cancer. Yet, the epidemiological review of many clinical trials involving anti-TNF therapy for RA did not find any link between such therapy and an increased risk of cancer. Nevertheless, the potential pro-oncogenic effect of anti-TNF therapy requires further investigation.

9. Molecular mechanisms

The molecular mechanisms behind TNF's actions have been extensively investigated. Particularly, scientists have been keen to elucidate how TNF triggers activation of the Ikappa B (IκB) kinase (IKK)/NF-κB and mitogen-activated protein kinase (MAPK)/AP-1 pathways (Fig. 1), which are essential for the expression of pro-inflammatory cytokines, and to understand how TNF induces apoptosis and necrosis (Fig. 1). TNF binds to its receptors TNFR1 and TNFR2, which can either be membrane bound or soluble. TNFR1 and TNFR2 each interact with both mTNFα and sTNFα. However, TNFR1 signaling is strongly activated by both mTNFα and sTNFα, while TNFR2 signaling can only be efficiently activated by mTNFα. TNFR1 is ubiquitously expressed while TNFR2 is mainly expressed on lymphocytes and endoepithelial cells. Upon ligation, either TNFR1 or TNFR2 forms a homodimer, but interestingly they do not form a TNFR1/TNFR2 heterodimer. TNFR1 contains a death domain, which allows it to interact with other death domain-containing adaptor proteins, whereas TNFR2 lacks a death domain.

9.1. Activation of the IKK/NF-κB and MAPK/AP-1 pathways

When TNFR1 binds to TNF, its conformation is changed such that its death domain can interact with TNFR-associated factor containing death domains (TRADD), which in turn recruits TNFR-associated factors (TRAFs) including TRAF2 and TRAF5, as well as the cellular inhibitor of apoptosis proteins 1 and 2 (c-IAP1/2) to form the TNF receptor signaling complex (TNF-RSC) (Fig. 1). Not only are c-IAP1/2 important for activation of IKK, but they are also critical for activation of components along the MAPK pathway, including JNK and p38 [3].

Within the IKK pathway, a linear ubiquitin assembly complex (LUBAC) containing HOIL-1, HOIP and Sharpin has recently been identified [4-6]. LUBAC is recruited to the TNF-RSC by TRADD, TRAF2/5 and c-IAP1/2 (Fig. 1). Importantly, LUBAC is not only required for the stabilization of TNF-RSC, but it also adds linear ubiquitin chains to both the receptor-interacting protein 1 (RIP1) and the regulatory subunit of IKK, IKKγ (also called NEMO), thus bringing both RIP1 and IKKγ to TNF-RSC (Fig. 1). This results in the formation of the IKKγ/IKKα/β complex and the TAK1 (TGFβ-activated kinase 1)/TAB1/2 (TAK1 binding protein 1 and 2) complex. Intriguingly, c-IAP1/2, but not TRAF2, are E3 ligases that catalyze lysine (K) 11-, 48-, or 63-linked polyubiquitination of RIP1 and c-IAP1/2 themselves, and this ligase activity is required for the recruitment of LUBAC to c-IAP-generated ubiquitin chains [4,7].

The polyubiquitinated RIP1 triggers activation of TAK1, which in turn activates IKKα and IKKβ. Although both IKKα and IKKβ phosphorylate IκBα, IKKβ is the major kinase leading to ubiquitination and degradation of IκBα, and subsequently leading to NF-κB translocation to the nucleus where it initiates the transcription of more than 200 NF-κB-dependent genes, including cell survival genes, pro-inflammatory cytokines, chemokines, growth factors and TNFα itself. TAK1 also activates JNK and p38, resulting in the respective phosphorylation of c-Jun and ATF2, and the subsequent formation of a c-Jun/ATF2 heterodimer called activating protein 1 (AP-1) (Fig. 1). AP-1 is another critical transcription factor and has similar functions to NF-κB.

Interestingly, TNFR2 does not contain a death domain, but it is able to form a complex with TRAF2 and TRAF5, leading to the activation of NF-κB and AP-1 upon stimulation with TNF.

9.2. Induction of apoptosis

TNFR1 also uses TRADD to recruit the Fas-associated protein-containing death domain (FADD) and deubiquitinated RIP1 (Fig. 1). Both FADD and RIP1 interact with pro-caspase 8, resulting in its cleavage and activation. Activated caspase 8 in turn mediates the cleavage of the pro-apoptotic protein Bid generating a truncated form (tBid), which translocates to the mitochondria and decreases mitochondrial membrane potential resulting in cytochrome c release. Cytochrome c, together with the apoptotic protease activating factor 1 (Apaf1), binds to the initiator pro-caspase 9 forming an apoptosome complex, which activates other caspases including 3 and 7 resulting in cell apoptosis. The normal development of c-IAPs knockout mice, which show embryonic lethality, can be rescued via deletion of TNFR1 but not TNFR2, further indicating a critical role for TNFR1 in TNF-induced apoptosis [8].

9.3. Initiation of necrosis

Deubiquitinated RIP1 can also recruit the receptor-interacting protein 3 (RIP3), forming the RIP1-RIP3 necrosome. Within this necrosome, RIP1 and RIP3 phosphorylate each other, further stabilizing the complex. Moreover, the phosphorylation of RIP3 at serine residues 227 and 232 is essential for its interaction with and the phosphorylation of its substrate, the mixed lineage kinase domain-like protein (MLKL) [9,10]. Although it is currently unclear whether MLKL acts as a kinase, it is known to be a critical mediator of the interaction of the RIP1/RIP3 necrosome with the phosphoglycerate mutase family member 5 (PGAM5) [11]. There are two isoforms of PGAM5, PGAM5 long (PGAM5L) and PGAM5 short (PGAM5S). PGAM5L mediates the association of PGAM5S with the RIP1/RIP3/MLKL necrosome complex (Fig. 1). PGAM5S is phosphorylated and subsequently activated by RIP3. Activated PGAM5S dephosphorylates dynamin-related protein 1 (Drp1) leading to activation of Drp1 and subsequent necrosis [11].

Knockdown of MLKL, PGAM5 or Drp1 largely impairs the necrosis induced by TNF [9-11]. Additionally, MLKL is required for sustained JNK activation and the generation of reactive oxygen species (ROS) [10], which induce necrosis via the PGAM5/Drp1 cascade [11].

10. Conclusion

TNF is one of the most important pro-inflammatory cytokines, and plays a pivotal role in the pathogenesis of immune disorders and tumor development. Further understanding of TNF's actions and the mechanisms underlying TNF pathology will allow for the development of a new generation of anti-TNF therapies that will cause fewer side effects, yet still maintain high efficacy in the treatment of immune disorders and cancer-related inflammation.

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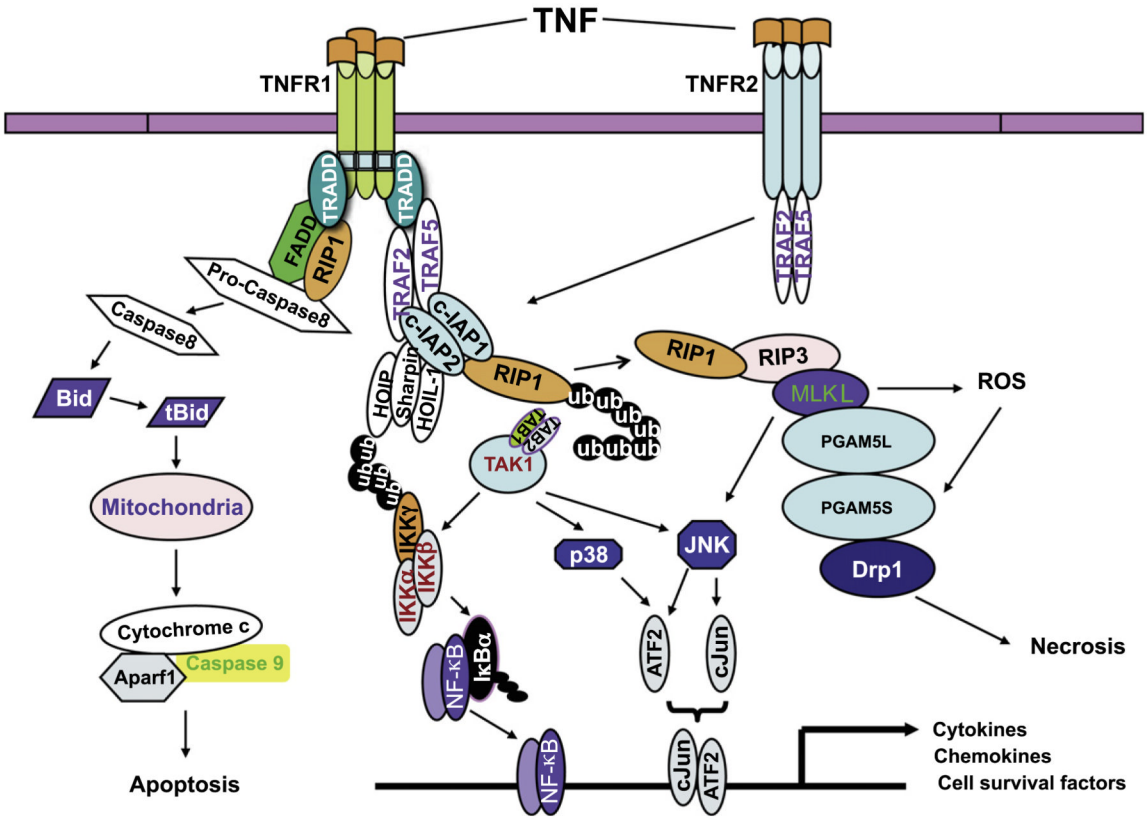

Fig. 1.

A model of TNFα signaling in inflammation, apoptosis and necrosis. TNF binds to TNFR1 and TNRF2. TNFR1 interacts with TRADD via its death domain (DD), in turn recruiting TRAF2, TRAF5 and c-IAP1/2 to form the TNF-RSC. RIP1 and LUBAC are then recruited to the TNF-RSC, leading to ubiquitination of RIP1 and IKKγ and formation of the IKKγ/IKK and TAK1/TAB1/TAB2 complexes. Polyubiquitinated RIP1 triggers TAK1 activation, which in turn activates IKKα/β, JNK and p38 leading to NF-κB and AP-1 activation, respectively. AP-1 and NF-κB bind to DNA binding sites located in the promoter regions of their target genes and initiate gene expression. TNFR1 also uses TRADD to recruit FADD and deubiquitinated RIP1. Both FADD and RIP1 use their DD to interact with DDs within the initiator pre-caspase 8, leading to its cleavage and activation. Activated caspase 8 triggers cleavage of Bid into tBid, which then enters mitochondria leading to a decrease in mitochondrial membrane potential and subsequent cytochrome c release. Cytochrome c, together with Aparf1 and caspase 9, forms an apoptosome complex that triggers apoptosis. In addition, deubiquitinated RIP1 interacts with RIP3 forming the RIP1-RIP3 necrosome, resulting in the mutual phosphorylation of RIP1 and RIP3. RIP3 then interacts with and phosphorylates MLKL, which recruits PGAM5 (PGAM5L and PGAM5S). RIP3 phosphorylates PGAM5S, which in turn dephosphorylates Drp1 leading to its activation and subsequent necrosis. MLKL is required for the generation of ROS, which then trigger necrosis via the PGAM5/Drp1 cascade. MLKL is also important for sustained JNK activation by TNF.