CHAPTER 5

Viral TNF Inhibitors as Potential Therapeutics

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Abstract

The immune system functions by maintaining a delicate balance between the activities of pro-inflammatory and anti-inflammatory pathways. Unbalanced activation of these pathways often leads to the development of serious inflammatory diseases. TNF (Tumor Necrosis Factor) is a key pro-inflammatory cytokine, which can cause several inflammatory diseases when inappropriately up-regulated. Inhibition of TNF activities by using modulatory recombinant proteins has become a successful therapeutic approach to control TNF activity levels but these anti-TNF reagents also have risks and certain limitations. Biological molecules with a different mode of action in regulating TNF biology might provide a clinically useful alternative to the current therapeutics or in some cases might be efficacious in combination with existing anti-TNF therapies. TNF is also a powerful host defense cytokine commonly induced in the host response against various invading pathogens. Many viral pathogens can block TNF function by encoding modulators of TNF, its receptors or downstream signaling pathways. Here, we review the known virus-encoded TNF inhibitors and evaluate their potential as alternative future anti-TNF therapies.

Introduction

Dynamic interactions are set into motion between the host and pathogens whenever they encounter each other. All successful pathogens, including viruses, bacteria and intracellular parasites have adapted diverse mechanisms to counteract the innate and adaptive responses mounted by the host. During this process many have evolved to express specific pathogen-encoded molecules that have regulatory roles in controlling the immune system of the infected host. These pathogen-derived molecules have often been well-honed by evolutionary selection pressures and can be attractive platform candidates as novel therapeutics to regulate the host immune system in diseases where exacerbated immune or inflammatory cascades have become pathologic to the host.

Pro-inflammatory cytokines like TNFα (here called TNF) play very important roles in orchestrating host defense against invading pathogens, but uncontrolled expression of these cytokines sometimes creates inflammatory diseases in humans if not properly regulated. Various anti-TNF therapeutics, such as neutralizing monoclonal antibodies or Fc fusions of TNF receptor ectodomains, have now entered into the arena of clinical usage to control inappropriate and excessive elaboration of TNF. Virus-encoded TNF inhibitors or modulators of TNF function can be exceedingly potent inhibitors of TNF pro-inflammatory activities. Here we discuss whether any of these virus-derived inhibitors might have potential clinical utility as an alternative strategy to dampen TNF-mediated pathologies.

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TNF and TNF-Mediated Signaling

TNF is first expressed as a membrane-bound ligand that can be cleaved and secreted as a nonglycosylated trimer of a 17-kDa protein. TNF is predominantly expressed from macrophages, monocytes, CD4+ and CD8+ T-cells, smooth muscle cells, activated NK cells, neutrophils and fibroblasts. TNF production is inducible by a number of diverse stimuli, such as interferons, IL-2 and IL-18. The initial precursor protein, 26 kDa pro-TNF, is translated, translocated to the endoplasmic reticulum, transported to the cell surface via the Golgi apparatus and is then presented on the cell membrane as a homo-trimeric complex. This cell-surface form of TNF can interact with TNF receptors of neighboring cells or it can be cleaved and released from the cell surface as a soluble trimeric ligand by the TNF converting enzyme (TACE). Cleavage and release from the cell surface appears to have some role for the biological properties of the TNF molecule in vivo, but both forms of the ligand can induce potent signaling activities following interaction with the two known TNF receptors in cell culture. For both the cell associated and secreted forms of TNF, ligand trimerization is required for biological activity. Either the cell bound or soluble TNF ligand binds to two structurally distinct receptors: Type I (TNFR1/p55) and Type II (TNFR2/p75), which are present on the membrane of all cell types except erythrocytes. The two receptors differ significantly in their binding affinities with TNF and other TNF-superfamily members, as well as differing in their intracellular signaling pathways. Both receptors have multiple cytoplasmic domains that control their signaling properties but TNFR1 also has an additional intracellular death domain (DD) for its diverse signaling events.

The trimeric TNF ligand binds to the extracellular domain of the receptors, via domains referred to as Cysteine-Rich Domains (CRDs), which induces conformational changes in the receptor and activates the intracellular signaling pathway, which itself can vary according to the cell type. Binding of TNF with TNFR1 leads to the release of the inhibitory protein silencer of death domains (SODD) from TNFR1 intracellular DD. Release of SODD allows binding of TRADD (TNFR1-associated death domain protein) to the DD, which can further activate either the apoptotic pathway, via the Fas-associated death domain (FADD) protein, or the pro-inflammatory pathway, via TNF receptor-associated factor 2 (TRAF2) and receptor-interacting protein (RIP), resulting in the activation of nuclear factor-κB (NF-κB) (Fig. 1). In contrast to TNF-RI, TNF-R2 is unable to activate the TRADD/FADD pathway and signals only through the TRAF2-associated pathway. Some studies have indicated the presence of cross-talk between the two receptors, which is likely to be responsible for the net response of a cell upon TNF stimulation. It is also possible that other cellular receptors can form complexes with TNF-receptors and thus add yet more levels of complexity in TNF-induced signaling.

PLAD Domain of TNFRs

The TNFR superfamily members are all Type I transmembrane proteins characterized by the presence of one to six hallmark CRDs. Many members of the TNFR superfamily (e.g., FAS, TNFR1 and TNFR2) exist as pre-assembled oligomers on the cell surface. This preligand assembly of TNFR oligomers is mediated by the preligand assembly domain (PLAD), which resides within the N-terminal cysteine-rich domain of the receptors and is not directly involved in ligand binding. PLAD-mediated preligand assembly has also been reported for TRAIL receptors and viral TNFR homologues. The PLAD domain of TNFR1 is critical in TNF responses, because mutation in the PLAD region reduces NF-κB activation and results in the TNFR-associated periodic syndrome, an autoinflammatory syndrome in man. Also, mutation in the PLAD region of FAS has been found to participate in pathogenesis of autoimmune lymphoproliferative syndrome (ALPS), a human genetic disease involving defective apoptosis, lymphocyte accumulation and autoimmunity. The mutant form of PLAD appears to inhibit the pre-assembly of FAS chain, thereby blocking the FAS intracellular signaling pathway. Recent evidence indicates that PLAD-mediated receptor association regulates cellular responses to TNF-like cytokines, especially in cells of the immune system such as CD4+ and CD8+ T-cells. Thus, targeting preligand assembly itself may offer new possibilities for therapeutic intervention in different pathological conditions involving hyperactive