Insight into the Structure of Tumor Necrosis Factor: A Protein of Immunological Importance

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Introduction

Tumor Necrosis Factor (also known as TNF-α) is a multifunctional cytokine produced by macrophages and monocytes. TNF has a range of functions in 'host defense' against various pathogens and also in inflammation and immune-modulating activities. TNF and TNF-receptor (TNFR) play major roles in maintaining human immune-system homeostasis. Upon binding with TNFR-1, the TNF-α may activate the nuclear factor kappa B (NF-κB), eventually resulting in apoptosis or cell death.

Both TNF-α and β interact with two cell surface receptors, 55-kDa TNF Receptor-type1 (TNF-R1) and 75-kDa TNF Receptor-type2 (TNF-R2). TNF-R1 is the primary signaling receptor for TNF-α. Even though TNF-R1 and TNF-R2 have relatively similar repetitive cysteine rich extracellular regions, their cytoplasmic domains are different from each other. The cytoplasmic Death Domain (DD) of TNF-R1 can form a signaling complex that activates nuclear factor-kappaB (NF-κB) and leads to apoptotic cell death, while TNF-R2 does not have a DD.

Results and Discussion

Structural Analysis of TNF-α Protein

ST5W is a hexamer and each chain is 148 residues long. This mutant protein is less toxic and has 11-fold lower binding affinity toward TNF-R1.

M35 has three amino acid substitutions: Leu29 is changed to Ser; Ser52 is changed to Ile; and Tyr56 is changed to Phe. Moreover, part of N-terminal region (seven amino acids residues) is omitted, that portion is "disordered" in wild type.

Residue mutation (Leu→Ser) occurs at position 29 of the protein. Consequently, a structural rearrangement in the loop is observed between residues 29-36, which results in additional inter and intra-subunit contacts; this in turn acts to improve the protein's receptor binding preference. The other two residue mutations (52 and 56) do not introduce any obvious conformational changes.

Structural Analysis of Mutant TNF-α Protein

ST5W is a homotrimer consisted of three identical subunits, each of which contains 151 amino acid residues.

1TNF is essentially a β-sheet protein with its antiparallel extended β-pleated sheet sandwich arranged in a "jelly roll" orientation. In 1TNF, the receptor-binding site is usually located at the "base" of the trimer.

Although the structures of the three subunits in 1TNF are mostly similar, they do exhibit some measurable degree of local variance.

Methods

The wild type TNF-α structure (PDB ID: 1TNF) and a TNF-α mutant (M35) (PDB ID: ST5W) are used.

For a receptor protein, the 55kDa tumor necrosis factor receptor TNF-R1 (PDB ID: 1EXT) is considered.

Modeling/simulation and visualization tasks are carried out using Accelrys Discovery Studio Visualizer 3.5.

Summary and Outlook

Binding of TNF with TNF-R1 triggers several complex signaling pathways that include cell survival, cell differentiation and cell death.

Interactions of TNF and TNF-R1 activate several adaptor proteins, TNFR-associated death domain (TRADD) and RIP (receptor-interacting protein). TRADD and RIP, along with TNF-R-associated factor 2 (TRAF2) trigger IkBa Kinase (IκK) and finally lead to the activation of NF-κB.

Even though TNF and TNF-R are broadly studied, some of their signaling mechanism and pathways are still unclear and unspecified.

As a natural extension of this study, in future we may take a look at the signal transduction pathways of TNF and, examine how several proteins work together to build the signaling cascade.

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