

Grams II, Raymond W.

TNF: Structure and Function

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TNF- α is a cytokine released from activated macrophages and mast cells. It was identified a little over three decades ago as a protein responsible for lysis of certain types of cells, with specificity for tumor cells. Despite hopes as an anti-tumor agent, significant cytotoxic effects have excluded TNF- α from targeted therapies against multiple diseases such as osteoporosis, atherosclerosis, cancer, allograft rejection, and autoimmune disorders. In order to understand the pleiotropic effects expressed by TNF- α , researchers have attempted to map the functional regions of this gene hoping to discover the amino acid residues related to receptor binding and differentiation, and cytotoxicity. Although many residues critical for biological function have been determined, the exact residues responsible for cytotoxicity and receptor binding affinity have not been located.

A relatively new, and thus unstudied area into the understanding of genes comes from the analyzation of the evolutionary pathways or changes that have taken place in that gene throughout its existence. In an effort to survive, genes such as TNF- α adapt to their circumstances such that they can combat the effects of their external environment, which environment also changes to combat these genetic adaptations.

In studying these pathways through the selection based program TreeSAAP along with the alignment and tree reconstruction programs of Clustal-X and PAUP*, I was able to identify amino acid properties that have been important to the function and optimality of the TNF- α gene protein. This study also allowed for the identification of functional sites corresponding to these properties and their evolutionary significance in time.

Of 31 amino acid properties tested, three properties exhibited high degrees of selection. Those properties are the following: alpha-helical tendencies, power to be at the C-terminus of the α -helix, and turn tendencies. Interestingly, all three of these properties are related to the conformational structure of the protein. When these properties were compared to their corresponding amino acid residues, they reflected specific sites outlined to be associated with binding affinity (e.g., AA residues 11-35) (Goh et al., 1991). These results denoted a direct correlation between conformation and binding affinity which could explain the differentiation between the receptors of TNF- α TNFR-1 and TNFR-2. Upon examination of amino acid residues, significant levels of selection were found within residue clusters of the transmembrane precursor which may provide insights into amino acid residues and properties responsible for the mechanism of secretion and the regulation of cleavage, which to this point have not been identified. Residues found to be under TNF- α tertiary structure present evidence that supports hypotheses resulting from site directed mutagenesis studies (Jones et al. 1992). Mutagenesis studies manifest allowable variance among functional domains near the apex of the trimer in accordance with residues found to be under selection in this study. Hydrophobic regions at the base of the protein structure only allow for slight conservative variation as manifested by selection acting on several of these residues. Highly conserved residues of the central region of the trimeric interface essential for overall biological activity remained largely conserved in all

mammalian taxa sampled relative to all physicochemical properties included. Selection leading to adaptation for increased binding affinity support Jones et al. (1992) that residues at the aqueous interface may be involved in the functional interaction of the TNF- α trimer with receptor proteins. The phylogenetic tree exhibited patterns of decreased conservation of TNF- α among organisms with shorter life spans (lagomorphs, rodents, etc.), possibly due to increased cell division, or generation time effects, though testing this hypothesis was beyond the scope of study.

Past studies have identified residues important for overall biological activity and regions believed to be responsible for binding and cytotoxicity in the TNF- α protein. Examining evolutionary pathways of this cytokine has identified specific amino acid properties under selection which reveal inherent functional and structural characteristics and specific amino acid residues of TNF- α . Differentiation among receptors determine functionality of TNF- α (and possibly pleiotropic effects), while binding affinity to receptors is a likely key to threshold mechanisms of the body. Residues found to be under selection show direct links to areas associated with binding affinity and interaction with receptors, while other areas may be used as targets for future site-directed mutagenesis studies.

This research was presented at the international conferences of Evolution and Molecular Evolution during the summer of 2003. In recent months additional sequences from TNF- α have been made available and the selection based models used in this study have been updated. In addition a sister gene to TNF- α , LTA, may provide a link to further understanding the mechanisms associated with TNF- α , as LTA binds to the same two receptors. With this new information available I plan to update and expand this study during the upcoming winter semester and if results confirm and further the conclusions of this past study, a publication will be written in fall of 2004. The overall hope and aim of this research is to understand the mechanisms surrounding TNF- α gene protein so as to further anti-cancer research and gene therapy with effective mutagens which retain cytotoxic action on tumors but do not allow for systemic toxicity.

References

- Goh, C.R., A.G. Porter. (1991). Structural and Functional Domains in Human Tumor Necrosis Factors. *Protein Engineering* 4:385-389.
- Jones, E.Y., D.I. Stuart, et al. (1992). Crystal Structure of TNF. Pp. 93-127 in *Tumor Necrosis Factors*. Marcel Dekker, New York.
- Swofford, D. (2002). PAUP*. Phylogenetic Analysis Using Parsimony (* and other methods), version 4.0b10. Sunderland, Mass., Sinauer Associates.
- Thompson, J.D., T.J. Gibson, et al. (1997). "The ClustalX-Windows interface: Flexible strategies for multiple sequence alignment aided by quality analysis tools." *Nucleic Acids Research* 25: 4876-4882.
- Woolley, S., J. Johnson, et al. (2003). "TreeSAAP: Selection on Amino Acid Properties using phylogenetic trees." *Bioinformatics*.