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Cloning, expression, and purification of human TNF-α

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Background and Aims:TNF-α is a multifunctional cytokine produced by stimulated macrophages to control systems involved in cell proliferation, inflammation and immune regulation. Although normal serum level of TNF-α is very important to regulate immune responses, elevated serum level of TNF-α has been implicated in the pathogenesis of autoimmune diseases. Therefore, TNF-α is a suitable target and its specific inhibitors are effective therapeutic tools in the control and treatment of such diseases. To this end, production of TNF-α in large scale is of great importance providing a valuable tool in many research projects.
Methods: In the current work, we cloned human TNF-α coding region into the gluthatione S-transferase (GST) containing vector, pGEX-6p-1, using the cDNA library prepared from white blood cells. Following confirmation of the construct by sequencing, the plasmid was transformed into E.coli BL21 for expression of the GST-TNF-α fusion protein. Purification of the fusion protein was conducted using the affinity and size exclusion chromatographic methods.
Results: White blood cells were isolated from 5 ml blood collected from healthy voluntaries and used for total RNA extraction, which in turn used in reverse transcriptase PCR reaction to prepare cDNA library. The cDNA of interest was amplified by PCR and cloned in pGEX expression vector. The generated construct was transformed into E. coli BL21 for high level expression of the recombinant protein. SDS-PAGE and immunobloting experiments were used to detect the expression of the protein before purification steps by affinity and size exclusion chromatography.
Conclusions: In this work, a plasmid DNA construct was prepared by cloning TNF-α coding region in pGEX vector and used to successfully produce GST-hTNF-α fusion protein in E. coli. Affinity chromatography, followed by size exclusion chromatography was applied to prepare high purity human TNF-α which can be used as the target in variety of anti-TNF alpha drug development experiments.

Keywords: TNF-alpha; GST-fusion protein; Expression