Production and characterization of anti-TNF-α Single Chain Variable Fragment Antibody in Recombinant *Escherichia coli*

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Tumor necrosis factor-α (TNF-α), a pleiotropic cytokine primarily produced by activated machrophage, became a useful target of therapy for several autoimmune diseases. Genes for the single chain variable fragment (scFv) of a monoclonal antibody (mAb) against TNF-α were cloned and expressed in *Escherichia coli*. Fusion of myeloma cells and spleen cells from the immunized mice resulted in hybridoma cells which were cultured to obtain the anti-TNF-α mAb. The complemetary DNA was constructed by the reverse transcription-polymerase chain reaction. DNA sequence analysis identified that each variable region was composed of the heavy chain variable region (V_H) as a type of IgG1 and the light chain variable region (V_L) as a type of *k*. Overlap-extension PCR using a linker encoding polypeptide $(Gly_4Ser)_3$ led to combination of V_H and V_L genes. Anti-TNF-α scFv which was expressed insoluble in the recombinant *E. coli* was purified by using the poly Histidine tag and refolded *in vitro*. ELISA analysis determined the binding affinity of the refolded TNF-α scFv one third of the anti-TNF-α mAb.