EXPRESSION OF SINGLE-CHAIN FV AGAINST ELECTRONEGATIVE LDL IN *PICHIA PASTORIS*

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Oxidative modification of low-density lipoproteins (LDL) is an essential step in atherogenesis, generating electronegative LDL (LDL^-), which has chemotactic, cytotoxic and immunogenic properties. The aim of this study is the generation of anti-LDL^- mAbs and their expression as single-chain FV (scFv). LDL^- was isolated from human blood plasma and used as an antigen for immunization of Balb/c mice. Upon screening, two different hybridomas were selected based on their ability to recognize LDL^- and not native LDL. The cDNAs that code for V_H and V_L were obtained by RT-PCR using specific immunoglobulin primer libraries. The VH and VL genes were cloned and sequenced. Hybridoma 1A3 uses a VH segment from J558.84 and a JH2 segment and 8.24/Jk5 VL, while 2C7 harbor Vmu 3.2 (J558)/Jh4 VH segments and a 8.24/Jk5 VL. Oligonucleotides were synthetized and those gene segments were cloned in *Pichia pastoris* immunoglobulin expression vector. This study is the first step to the expression of anti-LDL^- scFv. This recombinant protein has a great potential for clinical diagnostic applications, including *in vitro* immunoassays and clot imaging reagents.

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