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Abstract (poster session)

Recombinant system for expression of cholera toxin B subunit in Escherichia coli

M. Bustanshenas*, B. Bakhshi, M. Ghorbani, M. Atyabi, D. Norouzian (Tehran, IR)

Background & Objectives: Cholera toxin (CT) is the key virulence factor of Vibrio cholerae, which is encoded by the ctxAB operon, which resides in the genome of a filamentous bacteriophage (CTX) that specifically infects V. cholerae. The symptoms of cholera are mainly caused by cholera toxin (CT), B subunit of which binds to the GM1 ganglioside and promote the endocytosis of CT. The aim of this study was to clone and express ctxB and to purify the recombinant protein using pAE as an efficient expression vector. Materials & Methods: The recombinant pAE-CTB was transformed to the competent E.coli BL21 to express CTB protein. The system was induced by IPTG after which cells were harvested from LB medium by centrifugation and analyzed by 15% SDS-PAGE. Western blotting performed using cholera toxin-specific antibody. Recombinant CTB was expressed in this system with 6XHis tag at N-terminus and was purified through Ni2+-charged column chromatography. Concentration of protein measured with Bradford assay. The functionality of the CTB pentamers was assessed by GM1-ELISA assay. Results: SDS-PAGE analysis showed the expression of rCTB in the system and western blot analysis confirmed the presence of recombinant CTB in blotting membranes. Recombinant CTB was able to bind GM1 in a dose-dependent manner. Some part of rCTB may be expressed in the inclusion bodies so we also lubricated the inclusion bodies. Conclusion: Our results confirmed that although expressed in the inclusion bodies, 6XHis-tagged rCTB was properly refolded, easily purified, and as expected was free of possible CTA contaminants. This will enable us to study CTB immunological properties, oral tolerance, its use as mucosal adjuvant or in vaccine development.