long term expression of target genes in cell lines. Materials and Methods: At the first step, a DNA fragment containing EGFP CDS, S/MAR elements and SV40 promoter was amplified using pGL268 pEpi-FGM18F plasmid as a template, and inserted into pTZ57R/T. Next this fragment was treated with *Nhe*I and inserted into the parental plasmid originated from pBAD/gIII A plasmid, between two ΦC31 recognition sites. Eventually after an induction of parental plasmid to produce integrase, minicircle DNA formation was resulted and checked in CHO cell line by measuring EGFP expression. Results and Conclusion: Data indicated that both parental plasmid and minicircle DNA carrying EGFP-S/MAR were constructed successfully. Moreover, generated minicircle retains functionally to produce EGFP in different passages and generations for at least 2 months. Thus construction of an efficient parental plasmid with S/MAR elements is applicable for long-term transfection in different cell lines in episomal state.

Keywords: Nonviral Minicircles, Gene Therapy, Construction, Transfection, Efficiency

283. Expression of Camelid-derived Heavy Chain Antibody (Nanobody) against *Clostridium botulinum* Neurotoxin E in Pichia pastoris

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Background: The methylotrophic yeast *Pichia pastoris* is one of the standard tools used in molecular biology for the generation of recombinant protein.As yeast, *P.pastoris* is a single-celled microorganism that can be easy manipulated and cultured. Being a eukaryote, Pichia pastoris is capable doing many of the post-translational modifications performed by higher eukaryotic cells such as proteolytic processing, proper folding, disulfide bond formation, and glycosylation. In the present work we have expressed and purified the camelid-derived heavy-chain antibody (nanobody) against Clostridium botulinum neurotoxin type E in Pichia pastoris. Consequently the structure and function of the protein so produced will be compared to that of previously E.coli expressed antibody particularly from affinity point of view . Materials and Methods: VHH gene was cloned into a high copy number vector, pPink-Hc and transferred first to *E. coli* top10. PichiaPink™ vector containing the VHH was linearized by cutting at a unique site to promote integration into the *Pichia pastoris* genome and transfered into competent cells. The transformed cell mixtures was spread on MD agar selection plates and incubated at 28°C for 6 days until colonies were formed. Recombinant VHH was expressed in pichia pastoris. BMGY and BMMY were used for expression of the protein of interest. Results: Recombinant VHH was expressed in pichia pastoris. Protein Expression was analyzed by SDS-PAGE. Conclusion: Many proteins that end up as inactive inclusion bodies in bacterial expression systems are produced as biologically active molecules in *P. pastoris*.

Keywords: Nanobody, *Clostridium botulinum*, Neurotoxin E, Pichia pastoris

284. Selection of Nanobodies form Naïve VHH Phage Library Derived from Camelied Heavy Chain Antibodies by Whole-cell Panning

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Background: Cholera is one of the major health threats in developing countries. Recent epidemics showed that effective diagnostic and treatment for *V.cholerea* is imperative. Antibodies are being used as tools for such diagnostic and therapeutic measures. They can specifically recognize and neutralize their target. Camels are able to produce antibodies that lack the light chains. These heavy chain antibodies have full capacity of antigen binding through three CDRs within their variable domain. Variable domains (VHH) or nanobodies can be easily mass produced in microorganism and are compatible with display technologies such as phage, ribosome and yeast display. The aim of this study was to select high affinity nanobodies from naïve library using phage display technology and cell panning. Materials and Methods: Lymphocytes were isolated from peripheral blood and total RNA was extracted and converted to cDNA. VHHs were amplified by two sets of primers using nested PCR. pComb3x phagmid and VHHs were digested using *SfiI* enzyme. After ligation recombinant phagmids were transferred into *E.coli* TG1 bacteria. VHH coding phage particles were produced by infecting transformed bacteria with M13K07 helper phages. 10^7 *V.cholerae* bacterium was coated in 96-well ELISA plate. Phage particles were added and high affinity VHH coding phages were isolated and propagated through five rounds of cell panning. Improvement of binding affinity was studied by polyclonal phage ELISA using *V.cholerae* bacterium as an antigen. Results: RNA extraction was resulted in 28s and 18s rRNA on 1% agarose gel. The 600, 700 and 900 bp amplicons were obtained from first PCR. The second PCR resulted in 400 bp VHHs. Ligation was confirmed with PCR on colonies and phagmids. Polycolonal phage ELISA showed increasing affinity after each panning and the highest affinity was obtained on fifth panning. Conclusion: VHHs selected by whole-cell panning from naïve library showed high affinity toward *V.cholerea* bacterium.

Keywords: Nanobodies, VHH Phage Library, Camelied Heavy Chain Antibodies, Whole-cell Panning

285. The Effect of Induced Hyperglycemia on the Expression Levels of TLR2 and TLR4 Genes in the Hippocampus of Male Wistar Rats **during a Time Course Induction of Diabetes Type 1**

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Background: Type 1 diabetes (T1D) is an organ-specific autoimmune disease that results from T cell-mediated destruction of insulin-producing pancreatic beta cells in genetically predisposed individuals. Numerous studies have shown that the inflammation induced by hyperglycemia is the main mechanism of the pathogenesis of diabetic neuropathy. The relationship between inflammation and diabetic neuropathy progression included the processes and complex molecular networks. There is compelling evidence that the innate immune system plays a key role in early mechanisms triggering diabetes. Toll like receptors (TLRs) are key molecules recognizing foreign and endogenous danger signals, activating and regulating innate immunity and inflammation, and finally inducing adaptive immunity. TLRs have been shown to play essential roles in infections, inflammatory diseases and cancer. A number of studies demonstrated that TLRs mediated innate immune responses could contribute to the induction of diabetes. Abundant evidence also suggests that TLRs are important players in neurodegenerative diseases, which involve many inflammatory components. Although many studies have shown that these genes are induced in diabetes, it is not clear whether these genes are involved in the development of diabetes.The role of inflammation in neurological diseases has been recently confirmed. Materials and Methods: In this study the time course expression of the TLR2 and TLR4genes in hippocampal brain tissue of diabetic male Wistar rats were studied. Hyperglycemia was induced in male wistar rats with intraperitoneal (I.P.) injection of Streptozotocin. In different time points (4, 6, 8 and 20 weeks) post diabetes type 1 induction, rats were euthanized and hippocampal brain tissues was removed for further analysis. RNA was extracted from hippocampal brain tissues samples followed by cDNA synthesization using oligo-dT primers. Exon specific TLR2 and TLR4 primers were used to amplify rat TLR2 and TLR4 cDNA. After performing semiquantitative RT-PCR, the expression level of TLR4 mRNA was quantified by real time quatitative PCR (qPCR). Results: Up-regulation of TLR2 and TLR4 transcripts during the time course after diabetes induction as compared to the control group was shown. Conclusion: Our results demonstrate that the expression of TLRs may play a decisive role in the pathogenesis and expansion of diabetes. It is possible that the expression of TLRs can eventually lead to neurodegenerative disease such as Alzheimer. Therefore, studies on the precise role of TLRs in neurodegenerative disease may yieldpotential molecular targets for developing therapeutics for controland prevention of diabetic neurodegenerative disorders.

Keywords: Hyperglycemia, TLR2, TLR4, Wistar Rats, Diabetes Type 1

286. The Inflammatory Properties of Single Walled Carbon Nanotubes Functionalized with Polyethylene Glycol (PEG-SWNT) in Human Monocytic THP-1 Cells

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