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Background: The use of new nano materials in biology and medicine has been the focus of interest in recent years. The unique physico-chemical properties of carbon nanotubes have made them an excellent candidate in biology and medicine. One of the key advantages of carbon nanotubes in biomedical applications is that they can be easily internalized by cells, and therefore can act as delivery vehicles for a variety of molecules relevant to therapy and diagnosis. Carbon nanotubes can be single-walled or multi-walled and are now produced in substantial quantities for a variety of commercial applications. The clinical applications of using carbon nanotubes in medicine will depend on the outcomes of efficacy and Immunotoxicological studies, which will provide the necessary risk-to-benefit assessments for carbon-nanotube based materials. Materials and Methods: The objective of the present study was to evaluate the *in vitro* inflammatory properties of single walled carbon nanotubes functionalized with polyethylene glycol (PEG-SWNT). Human monocytic cell line THP-1 was cultured with various concentrations of PEG-SWNT in different time points and the expression of several innate immunity receptor genes was analyzed by real time quantitative PCR method. In this study the expression of several pattern recognition receptors (PRRs) including TLR2, TLR4, CD14 and adaptor protein MyD88 was analyzed in the PEG-SWNT treated THP-1 cells. Results: According to our results a significant up-regulation of TLR2, TLR4, CD14 and MyD88 was observed when THP-1 cells were treated with various concentrations (10 μg/ml, 50 μg/ml and 100 μg/ml) of PEG-SWNT. However, when the cells were treated with 200μg/ml PEG-SWNT, the expression of PRRs was down-regulated which might suggest that, higher concentration of PEG-SWNT is toxic for the cells. Conclusion: the results presented in this study shows that PEG-SWNT could trigger cellular inflammatory responses as was shown by the expression analysis of genes involved in innate immunity and inflammation.
Keywords: Single Walled Carbon Nanotubes, Polyethylene Glycol PEG-SWNT, THP-1 Cells

287. Gene Expression Analysis of Toll-Like Receptor 2 And 4 (TLR2 And TLR4) In Peripheral Blood Mononuclear Cells (PBMCs) of Mycobacterium Avium Subsp. Paratuberculosis Infected Cows with Quantitative Real-Time PCR (qPCR)

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Background: Mycobacterium avium subsp. paratuberculosis (M. ptb) is the causative agent of Johne's disease, a chronic granulomatous enteritis in ruminants, characterized by thickening of the intestinal wall and progressive wasting leading to death of the animal. Current estimates indicate that Johne's disease is one of the most costly infectious diseases of livestock in many countries including Iran. The immune response to mycobacterial infection involves phagocytosis of organisms by mononuclear phagocytes and sequestration within phagosomes. The capacity of the organism to prevent macrophage activation and phagosome maturation and to attenuate induction of a Th1 immune response appears to largely determine its pathogenicity. Our current understanding of the biology of M. ptb infection has been hindered by limited knowledge of the host factors involved in the immune response to the organism and the lack of appropriate molecular tools to dissect the host–pathogen interaction. Recognition by the host of molecular patterns displayed by microbes is essential to development of an effective immune response. Pattern recognition receptors (PRRs) are the main sensors of pathogen and danger signals in innate immunity. They are mainly expressed by macrophages and dendritic cells of different organs. Toll like receptors (TLRs) are the most studied and best characterized PPRs which are responsible for sensing pathogen associated molecular patterns (PAMPs). Recent studies have focused on the role of the TLRs family of cell membrane receptors in initiating cell signaling associated with mycobacterial infections. Activation of TLRs has also been shown to coordinate the adaptive immune response to microorganisms. Materials and Methods: In this study, we aimed on the expression levels of bovine TLR2 and TLR4 transcripts in the peripheral blood mononuclear cells (PBMC) of cows infected with M.ptb by Real-time quantitative PCR (qPCR). Blood samples were taken from five cows which were positive in two consecutive ELISA test for paratuberculosis. As for the control, blood samples of five ELISA negative cows were also taken. After isolation of peripheral blood mononuclear cells (PBMC), total RNA was isolated and cDNA was synthesized using Oligo dT. primers. Then, the primer pairs for TLR2, TLR4 as target genes and GAPDH and β-actin as housekeeping and calibrator genes were optimized in a gradient RT-PCR experiment. Subsequently, qPCR analysis was set up to quantify and compare the relative expression levels of TLR2 and TLR4 transcripts in PBMC of paratuberculosis positive and negative cows. Results: Statistical analysis using one way T-test showed a highly significant up-regulation of TLR2 and TLR4 expression in PBMC of paratuberculosis infected cows as compared to the negative control group ($p<0.001$). Conclusion: the results presented in this study, can shed more lights to the insight mechanisms behind the molecular immuopathogenesis of paratubeculosis which might eventually pave the way for designing novel and more effective therapeutic as well as preventive strategies.
Keywords: TLR2, TLR4, PBMCs, Mycobacterium Avium Subsp, qPCR

288. Characterization of Recombinant Structural Subunit of Colonization Factor6as a Candidate Vaccine against Enterotoxigenic *Escherichia Coli*

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Background: Enterotoxigenic *Escherichia coli* (ETEC) is recognized as one of the common agent of diarrhea in children under 5 years in developing countries and an important agent for traveler's diarrhea. Enterotoxin and colonization factors(CFs) are the major virulence mechanisms in ETEC .CFs are surface proteins that mediate adherence to host small intestinal. CFs highly immunogenic and appear to be protective antigens in human. One of the most prevalent of the ETEC CF is CS6. CS6 consist of two structural subunit (CssA, CssB) that are appeared to be necessary for attachment to intestinal cells. In the present research, the CssB protein is expressed in *E. coli* and the purified protein was investigated to its immunological effect as a vaccine candidate. Materials and Methods: Information about CssB gene was obtained from gene bank. A sequence encoding the CssB gene was designed using E.coli codon bias. The optimized gene was synthetized and cloned into expression vector, pET28a .The CssB gene was expressed in *E. coli* BL21 (DE3) cells. The recombinant protein was confirmed with Western blot. The protein was purified with Ni-NTA affinity chromatography. The immunization studies were conducted in mice with purified CssB. ELISA test was performed on mice sera for determination of antibody production. Results: ELISA results showed high titer of antibody production in mice. Pretreatment of the ETEC cells with immunized mice antisera remarkably decreased their adhesion properties and blocked their binding to Caco-2 cells. Conclusion: Recombinant protein CssB is an immunogen protein molecule and one of the important components in vaccine strategies against ETEC.
Keywords: Enterotoxigenic *Escherichia coli*, Colonization factor, Recombinant protein, Vaccine candidate

289. Nanobody: Nanotechnology in Nature

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In all cameldis found two types of antibodies: The classical antibody of 2 H and 2 L chains and the HCAbs (unique for camelids). A H-chain of a HCAb lacks one of the constant domain of a H-chain of a classical antibody. The N-terminal domain of the HCAb, the Variable domain contains a few amino acid substitutions compared to the variable domain of a classical antibody, therefore named it a 'VHH'. This two chains antibody called heavy chains antibody (HCAb). The antigen binding site of HCAb so called nanobody. The recombinant, monoclonal Nanobody is well