Background: Dendritic cells (DCs) remain as the most important model for in vitro assay of viral protein interaction with immunologic system. Adenoviral vectors expressing viral proteins due to their efficiency in DC transduction employed for this purpose. The results achieved by this method are usually controversial owing to some parameters like differences in procedures used for Adenovirus preparation, transduction and other variables. Here, we report an experiment in order to evaluate the suitable MOI of the Adenoviral vectors for better DC transduction. Materials and Methods: The adenoviruses expressing GFP or Luciferase had been prepared and finally titrated by commercially available quantification methods. After that, Adeno-GFP was employed to assess it's infectivity at different MOIs from 50-1000. The MOIs of 250 and 1000 were selected based on the results of GFP detection and viability test. Dendritic cells status, maturation markers and function were evaluated by MLR assay, IL-12 and TNF-α cytokine production method as well Flowcytometery of surface markers (CD40, CD86, MHC-II). Adeno-Luciferase was also employed as another control virus. Results: Virus titer in 1000 MOI was more effective in DC transduction than 250 MOI albeit more toxicity that led to cell death among more than half of the DCs population. Both adenoviral vectors (Adeno-GFP or Adeno-Luc) induced DCs maturation by increasing the expression of surface markers and secretion of IL-12 and TNF-α in comparison to control groups. The DCs transduced by adenoviral vectors (GFP or Luciferase) also induced more proliferation of spleen lymphocytes in MLR assay. Conclusions: Adenoviral vectors, even in low titer, infect DCs and infectivity is dose dependent in all experiments. At high MOI, due to toxicity, a large part of population was not viable. Treatment of DCs with 2 different Adenoviruses leads to their maturation which observed by changes in CD40, CD86 and MHC-II surface expression markers and function. Conclusively, for study of effect of virus protein on DCs, to avoiding the saturated maturation of DCs following Adenovirus transduction, it is important to perform various controls and titration studies prior to starting a research. Here we explain simple points to establish an informative comparative basic study on DCs by Adenoviruse expressing viral proteins in order to distinguish even fine differences among viral proteins.
Keywords: Adenoviral vector, Dendritic cells, DC Transduction

267. Inhibition Effect of Variable Domain of Heavy Chain Antibody (Nanobody) Against Urease Activity of *Helicobacter Pylori*

Safaee Ardekani L[1*], Mousavi Gargari S.L[1], Rajabi Bazl M[2]
[1]Department of biology, faculty of basic science, Shahed University,Tehran, Iran, [2] Deprtment of clinical biochemistry, Shahid Beheshti University of Medical Science, Tehran, Iran

Background: Antibody administration against urease has been studied as a new effective therapeutic strategy. *Helicobacter pylori* infection is associated with gastritis and in some cases with gastric and duodenal ulcers or even adenocarcinoma. Urease is an enzyme that helps *H.pylori* to colonize the epithelium in the acidic environment of stomach. This enzyme release NH_3 by hydrolyzing urea. Produced ammonia can neutralize acidic environment and enable bacteria to survive in stomach. Neutralizing this enzyme unable the bacterium to colonization in the stomach acidic environment and will ultimately results in its death. In the present study, production of variable domain of heavy chain antibody (nanobody) against of UreC recombinant protein and evaluation of inhibitory effect was aimed. Materials and methods: A nanobody library derived from cDNA pool of dromedary was generated and an anti-UreC nanobody successfully was selected. After purification of nanobody, urease inhibitory of nanobody was measured. For assessment of inhibition effect of nanobody, 10^9*H. pylori* bacteria mixed with different concentrations of nanobody and were incubated in microplate wells for 16 hour at 4°C. 100 µl PBS containing 500 mM urea and 0.2 g/l phenol red were added to wells and incubated for 3 hour at 37°C. Three controls including the culture media, *H. pylori* bacteria and nanodody were used. Colorimetric measurement was done every 30 min during 3 hours in OD_{550} nm. The inhibition percentage was calculated as follow, Inhibition percentage = [(the enzymatic activity of bacteria without nanobody–the enzymatic activity of bacteria with nanobody)/the enzymatic activity of bacteria without nanobody] × 100%. Results: *H. pylori* was treated with nanobody and the optical density of the mixture was determined at 550 nm by the indicator of phenol red that shows 35% inhibition percentage. Conclusions: The result of urease inhibitory test showed that extracted nanobody against UreC can successfully inhibit the surface urease activity of *H.pylori* and could be an alternative to antibiotic treatment.
Keywords: Nanobody, Urease Activity, *Helicobacter Pylori*

268. Cross-Reactions against Recombinant InvH Protein with Sera from Mice Infected with Different Strains of Salmonella

*Dehghani B, Rasooli I
Department of Biology, Shahed University, Tehran, Iran

Background: Salmonella is one of the most prevalent food-borne diseases over the world. Vaccination is generally accepted as the most practical measure in that is easy to apply and the most economic, however, present vaccines have limited efficacy. Except for *Salmonella arizonae*, *invH* is present in all *Salmonella* strains. No homologous sequences were detected in *Yersinia, Shigelia, Proteus*, and several strains of enteroinvasive and enteropathogenic *E. coli*. InvH protein was present in the peptidoglycan and role of this protein for efficient bacterial adherence and entry into epithelial cells is inevitable. This research suggests that Invh could be a candidate for a new generation vaccine and diagnostic measures against prevalent serotypes of *salmonella enterica*. Materials and Methods: Six groups of mice were infected with *S.enteritidis*, *S. typhi*, *S.paratyphi* serovars A, B, C and *E.coli*. One group of mice was immunized with recombinant InvH protein. Five micrograms per well of the recombinant protein was used to coat the surface of a 96-well microtiter plates. Serial dilutions of mice sera against *S.enteritidis*, *S. typhi*, *S.paratyphi* serovars A, B, C, E.Coli and Immunized mice were added to the wells. Results: Antibody response with sera from immunized mice or inoculated with *Salmonella* strains were significantly higher than *invH* deficient bacteria such as *E.coli* and control group. Conclusion: Cross reaction with sera of *Salmonella* strains inoculated mice is indicative of possessing by *Salmonella* strains of the surface protein, InvH, that can be employed in both prophylactic and diagnostic measures against *Salmonella enterica*.
Keywords: Cross-Reactions, InvH Protein, Sera, Salmonella

269. Immunization against *Salmonella enterica* Serovar Entritidis with Recombinant InvH Protein

*Dehghani B, Rasooli I
Department of Biology, Shahed University, Tehran, Iran

Background: *Salmonellaenteritidis*, is one of the main causes of food-borne illness. It grows under natural conditions. Epidemic of human infections mediated by *Salmonella enterica* serovar Enteritidis was witnessed by last two decades of the 20th century. Poultry and poultry products such as fowl, shell eggs are the most prevalent sources of infection of *SalmonellaEnteritidis* in humans. Acellular vaccines containing bacterial immunodominant components such as surface proteins may be potent alternatives to live attenuated vaccines in order to reduce salmonellosis risk to human health. *invH* gene, an important part of needle complex in type three secretion system (TTSS) plays important role in efficient bacterial adherence and entry into epithelial cells.
Materials and Methods: The *invH* gene was amplified and cloned in *Escherichia coli BL21*, the protein was expressed. The recombinant protein purified by nickel–nitrilotriacetic acid (Ni–NTA) affinity chromatography was injected into BALB/C mice to induce immunity. The sera collected after second and third immunizations were assessed for specific IgG by ELISA. The immunized and control mice were orally challenged with various doses of *Salmonella Enteritidis*. Results: The purified InvH provoked significant rise of IgG in mice. Active protection induced by immunization with InvH against variable doses of Salmonella enteritidis, indicated that the immunized mice were completely protected against challenge with 10^4 LD_{50}. Conclusion: RecombinantInvH protein can induce production of antibody in mice. Immunization with InvH protein can develop protection against infection with *Salmonella Enteritidis*.
Keywords: Immunization, *Salmonella enterica*, Recombinant InvH Protein

270. Short Exposure to Collagenase and Co-Culture with Mice Embryonic Pancreas Improve the Human Dermal Fibroblast Culture. Introducing a New andSimple Co-Culture System

Mostafazadeh A[1*], Pandamooz S[1], Hadipour A[2], Akhavan-Niaki H[1], Pourghasem M[1], Abedian Z[1], Motevallizadeh Ardekani A[1]