Keywords: Monoclonal antibody, human IgG

4. **Generation of Random Mutant Library against ureC Subunit of Urease from *Helicobacter Pylori* by Error-prone PCR**

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Background: Introduction of antibody molecules and their fragments in research, diagnosis, and therapy has prompted the development of methods to improve their affinity and stability. Single-domain antibodies (VHHs) from naive libraries have dissociation constants (KDs) in the low range and thus, for most antibody applications, their intrinsic affinities need to be improved significantly. The process of somatic hypermutation can be mimicked by *in vitro* random mutagenesis. The most commonly used random mutagenesis method is error-prone PCR, which introduces random mutations during PCR by reducing the fidelity of DNA polymerase. Materials and Methods: Error-prone PCR was performed on the VHH gene obtained against ureC subunit of Urease from *Helicobacter pylori.*Higher concentrations of $MgCl_2$ (7 m*M*) and $MnCl_2$ (0.1, 0.3 mM) along with dITP (200Mm) was added to the reaction mixture in order to increase the error rate. To know the rate of mutation, PCR product were sequenced and compared with original VHH gene used as a template in PCR reaction. The PCR products as a mutant VHH library is then displayed on pComb3x phagmid. Result: Nucleotide sequence analysis compared with original VHH gene revealed that there is a direct correlation between rate of mutation and concentration of Mn,dITP. The rate of mutation observed in this research was 1-2

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