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Immunobioinformatic analysis of the chimeric model of influenza A M2e antigen fused with molecular adjuvant of FliC: Designing, construction & its expression in *E. coli*

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Influenza virus makes a large impact on public health. Annual influenza epidemics cause of death worldwide by almost 250 thousand is considered. Due to permanent mutations in the genome of the virus and the perpetual possibility of producing new viruses that occur as seasonal or pandemic flu, producing a vaccine for this virus is very important. According to the research and understanding of the genome of this virus and the use of genetic engineering techniques, universal vaccine produce is not out of reach. M2e is a conserved epitope that exists among the epitopes candidates for the vaccine against influenza. In addition to that this influenza virus region is antigenic, it is similar in the majority of flu strains and it is protected in some strains with minor differences in amino acid. It does not count appropriate stimulus to the immune system because this peptidic region is too short. For this reason, a molecular adjuvant called FliC was used. In this study, the piece consists of three sequence repeats of the M2e epitope attached to FliC, the molecular adjuvant, (3M2e.FliC) then transferred the recombinant plasmid to *E. coli* strains (BL21 and ER2566), we compare the protein expression in two strains. Immunoinformatics analyzes confirmed that in this recombinant protein, M2e and FliC epitopes are recognized by the immune system and they are existing at the protein surface and available for the immune system. From other activities performed in this study was Three-dimensional modeling of 3M2e.FliC recombinant protein that in this section, a new modeling method was introduced for recombinant protein modeling that provides better results than usual modeling methods.

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Design and evaluation of a multi-epitope universal peptide against influenza virus infection in BALB/c mice

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Human infection with the new subtype influenza A virus is associated with a high mortality and morbidity and causes worldwide pandemic. There is necessity to improve a universal vaccine against influenza pandemic and produce protective immunity by inducing strain-specific neutralizing antibodies to the viral hemagglutinin. For this purpose we have designed a novel multiple linear epitopes (B-cell, CTL and T_h) immunogenic based on the hemagglutinin proteins backbone containing human T cell epitopes for H1 & H3 subtype. In this study, we use the epitope-based vaccine design by using immunoinformatics approach in order to predict the binding of B-cell and T-cell epitopes (class I and class II human leukocyte antigen [HLA]). BCPREDS was used to predict the B-cell epitope. Propred, Propred I, netMHCpan and netMHCIIpan, were used to predict the T-cell epitope. All epitopes were checked by epitope mapping, NCBI ORF Finder, ExPASy, Swiss-Pdb Viewer and Protean. This sequence was cloned into the prokaryotic expression vector peT41a. BALB/c mice were immunized with different dosages of recombinant protein and the immune responses were determined in the form of protective response against influenza virus, antibodies titers (IgG1 and IgG2a), spleen cell lymphocyte proliferation and the levels of interferon-γ and interleukin-4 cytokines. We observed an increase in the number of influenza virus-specific IFNγ-secreting splenocytes, composed of populations marked by $CD4^+$ and $CD8^+$ T cells producing IFNγ or TNFα. Upon challenge with influenza virus, the vaccinated mice exhibited decreased viral load in the lungs and a delay in mortality. These findings suggest that human multi-epitope recombinant influenza virus proteins are a valid approach for a general T-cell vaccine to protect against influenza virus infection.

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