Prokaryotic chimer protein consisting of multiple M_2e of Influenza A virus and *Leishmania major* HSP70 induced specific immune responses in mice

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Introduction

Developing an effective human influenza vaccine is a major concern due to stable antigenic variation of surface glycoproteins. Conserved antigens are new vaccine candidates because it is not necessary to match the prepared vaccine annually with circulating strains. The extracellular domain of the M_2 protein (M_2e) consists of N-terminal 24 residue which shows remarkable conservation among all subtypes of influenza A viruses. This peptide could induce antibodies with inhibitory activity against influenza virus replication *in vivo* to diminish the influenza related diseases. To improve the immunogenicity of this peptide, we fused three tandem repeats of M_2e to *Leishmania major* HSP70 $_{(221\text{-}604)}$ and evaluate immune responses in mice.

Fig 1: Schematic model of the three tandem repeats of M_2e

Materials and Methods

Three overlapping peptides covering M2e with appropriate linker were designed using bioinformatics sites, synthesized and cloned in puc57 vector and then subcloned into prokaryotic expression vector pet28a and pet28-hsp. Chimer protein (3M2e-hsp70) and 3M2e peptide were produced in E.coli and purified using Ni-NTA columns. Fifteen microgram of the purified proteins dissolved in 100 micro liter phosphate buffered saline were injected to female Balb/C mice intramuscularly in three periods with fifteen days apart. All animals were left to bleed before and after immunization. Specific anti-M2 antibodies were measured by indirect ELISA.

Results

The accuracy of cloning process was confirmed by restriction enzyme analysis, colony PCR and DNA sequencing. The recombinant proteins were successfully expressed in E.coli BL21 cells as probed in western blotting using mAb specific to M2e. The sera of immunized animals were analyzed by ELISA. The results showed that 3M2e-hsp chimer protein induced high level of anti-M2 antibodies compared to control mice as shown in fig 2. IgG isotyping ELISA revealed that chimer protein induced both IgG1 and IgG2a simultaneously indicating the prevalence of a mix of Th1/Th2 responses.
The tertiary structure of chimer protein was predicted and analyzed using Swiss-PdbViewer and WebLab Viewer. The prediction results showed that fusing of HSP to the c-terminal of 3M2e peptide doesn't mask the predominant epitopes.

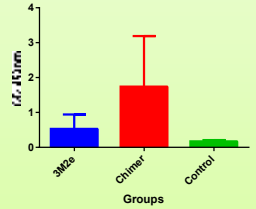

Fig 2. Antibody responses in chimer and 3M2e proteins immunized groups

Conclusions

Influenza A vaccine based on M_2e protein has limited potency. Hence, optimal approaches to enhance immunogenicity of M_2e protein immunization remain to be established. Applying heat shock proteins as adjuvant may play a crucial role in integrating innate and adaptive immunity. They have a function in intracellular protein folding, assembly and transport. On the other hand, the degree of epitope density of M_2e has been shown to be a critical factor influencing the magnitude of epitope-specific responses. In the present study we increased density of M_2e and fused it to HSP as adjuvant and showed that fusion peptide induced both sides of immune responses and might protect mice against lethal challenge.