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Vaccine development

PROKARYOTIC CHIMER PROTEIN CONSISTING OF MULTIPLE M2E OF INFLUENZA A VIRUS AND LEISHMANIA MAJOR HSP70 INDUCED SPECIFIC IMMUNE RESPONSES IN MICE

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Objectives: 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.

Methods: Three overlapping peptides covering M_2e 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 ($3M_2e$-hsp70) and $3M_2e$ peptide were produced in *E.coli* and purified using Ni-NTA columns. The purified proteins in different formulation (Alum and/or CpG ODN motifs) 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-M_2 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 M_2e. The early ELISA results showed that $3M_2e$-hsp chimer protein induced high level of anti-M_2 antibodies compared to control mice. The immunized mice are going to challenge intranasally with mouse-adapted H1N1 and H3N2 Influenza viruses to evaluate the vaccine regimen efficacy in comparison to control groups.

Conclusion: 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 power immune responses and might protect mice against lethal challenge.