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Short Communication

Preparation and Expression of M2 Gene Plasmid DNA for Potential use in Influenza A Vaccine Production

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Influenza is an enveloped virus belonging to the orthomyxoviridae family with eight segments of negative single-stranded RNA encoding at least 10 distinct proteins. The virus causes a respiratory disease in human with high economical damages. Vaccination is the most cost-effective method for preventing influenza (1, 2). Although most of the influenza vaccines targeted HA, responsible for receptor binding of the virus, the principal deficiency of these vaccines is their highly variable viral target antigens. The accumulation of point mutations is antigenic drift that leads minor and continuous antigenic changes which makes the adaptation of new virus vaccine a necessity for prevention of new epidemics (3, 4). So, it is necessary to produce new upgraded vaccines to prevent influenza epidemic in humans as suggested by the World Health Organization. On the other hand, pandemic influenza is considered as a global concern, could be initiated by a new influenza subtype attains the ability to spread between humans. Considering low effectiveness of anti-influenza drugs in reducing symptoms and duration of the disease, which takes too long to prepare a well match vaccine in industrial scale, administration of a universal vaccine encoding conserved but less antigenic proteins is more promising way to control seasonal and even a threatening pandemic Influenza (5, 6).

The transmembrane M2 protein is conserved among Influenza A viruses and has potential to be considered as a universal vaccine. It is encoded by a spliced the 7th segment RNA, M gene, which codes also for M1 protein (7, 8). The mature M2 homotetramer protein has pH-inducible ion channel activity for uncoating of viral RNP and plays an important role in viral replication (9, 10). It has been shown that anti-M2 antibodies are cross-protective among different subtypes, limit virus replication and reduce morbidity and mortality in mice. Although in natural infection the level of these antibodies are very low or even undetectable (11).

In the present study, we provided a preliminary preparation of plasmid DNA-based vaccine encoding the conserved M2 against influenza A. The expression of full-length M2 protein was determined in the eukaryotic cells.

Human influenza virus A/New Caledonia/20/99(H1N1) was inoculated into the Madin Darby canine kidney (MDCK) cell, provided by the Pasteur Institute of Iran. The cells were trypsinized after 18 hours and suspended in 1X PBS. Total RNA was extracted from the cell pellet using easy-RED^{TM} Total RNA was extracted, according to the procedures of iNtRON Biotechnology, South Korea and cDNA synthesis was performed using random hexamer. The M2 cDNA template was amplified by PCR using the specific primers as follow,

M2-forward:
*CTCGAG*ACCATGAGTCTTCTAACCG and
M2-rev:
*GGATCC*TTACTTCAACTCTATGCTGAC.

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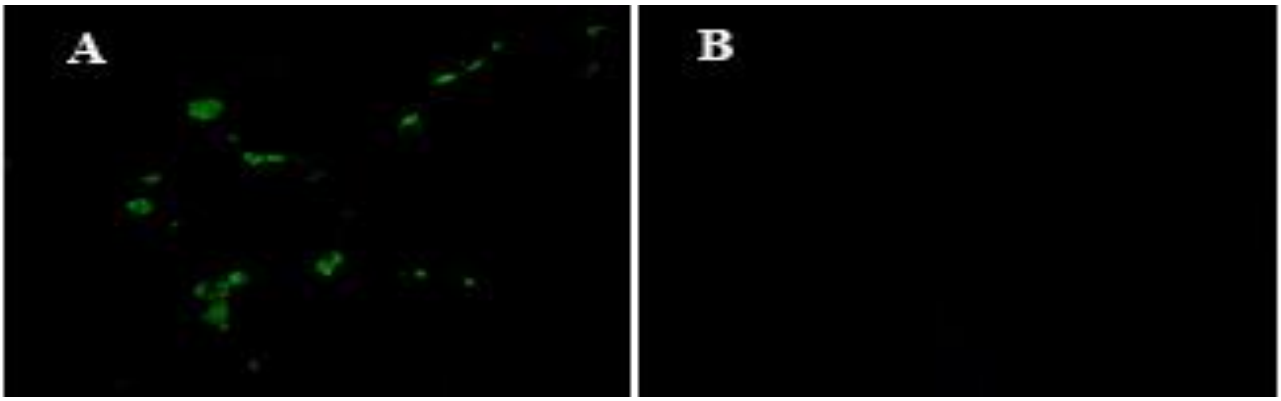


Fig. 1. Immunofluorescent staining .M2 expression was detected by Immunofluorescence microscopy in transfected COS-7 cell (A). Untransfected COS-7 cells were used as negative control (B).

The restriction sites for XhoI and BamHI were considered in forward and reverse primers, respectively. Amplification was performed in a thermal cycler using the standard procedures. The PCR product of 300-bp fragment was electrophoresed on 2% agarose gel and purified using Gel extraction kit (Qiagen).The purified M2 amplicon was cloned intopGEM-T easy vector using T4 DNA ligase (Promega, A 1360) and transformed into Top10F'competent Ecoli cells. The recombinant plasmid was confirmed by PCR, digestion and sequencing (not shown). The target gene was cutfrom verified plasmid and subcloned into pcDNA3.1/ Hygro (Invitrogen, Grand Island, NY), downstream of CMV promoter. Plasmid DNA was amplified in E. coli Top10F' and purified by plasmid mini preparations (iNtRON Biotechnology, Inc.).

Transfection of plasmid into COS-7 cells was performed using Lipofectamine 2000 (Invitrogen).

Expression of M2 protein in eukaryotic cells was determined using immunofluorescent assay and western blot analysis. For immunostaining, the transfected COS-7 cells were fixed in pre-cooled acetone/methanol at -20°C for 15 minutes and stained immediately by the indirect immunofluorescence technique. Monoclonal mouse anti-influenza M2 antibody (ab5416, Abcam, Cambridge, MA) and FITC conjugated goat anti- mouse IgG (Razi Biotech, Iran) were used as the primary and secondary antibody, respectively. Uninfected COS-7 cells were used as negative control. The stained slides were observed for fluorescence using a microscope equipped with epifluorescence (Fig.1).

To confirm M2 protein expression, the lysate of transfected cells were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoreses (SDS-PAGE) and the separated proteins were electrotransferred to nitrocellulose membranes using electro-blotting system (Bio-Rad) according to the standard protocols (12). The expressed proteins were probed using ab5416 monoclonal antibody followed by peroxidase-conjugated goat anti-mouse immunoglobulin (Dako, Glostrup, Denmark) as the secondary antibody. The reaction was visualized using DAB reagents (Amersham Bioscience, Piscataway, NJ, USA). An expected 15 KDa band corresponding to the full-length M2 protein was observed (not shown).

M2 is an evolutionary constant protein and its potential to induce extensively protective humoral immunity against influenza virus infection has been analyzed in some anti-viral studies (6, 8, 10). DNA vaccines often produce effective humoral and cellular immune responses that protect humans against multiple infectious agents in clinical models, although their potency needs further improvement (13, 14).

The above results suggest that recombinant plasmid pcDNA3.1/M2 could potentially be used as a DNA vaccine candidate. Our work is a preliminary study for planning further research on the immune effect of M2 protein as

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a novel vaccine for prevention of Influenza epidemics.

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