Original Article

Design, Cloning, and Expression assay of the *NP* Gene in a Bicistronic Vector Harboring the Mouse *IL-18* Gene: Potential Implications for Type A Influenza Vaccine Investigations

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Abstract

Objective: Vaccination is the most effective tool to prevent influenza virus morbidity and mortality. Despite surface antigen mutability, the influenza virus inner proteins are remarkably conserved among various strains. The high similarity of NP protein sequences among various strains of influenza type A from one side, and the potency of IL-18 molecular adjuvant in shifting immune response toward Th1 from the other side, has persuaded us to evaluate the possibility to clone and express the influenza type A NP gene in a bicistronic vector that harbored the mice *IL-18* gene. We sought to assess their immunogenic activities in a susceptible mouse model.

Methods: Initially, we extracted genomic RNA from the influenza virus PR8. After cDNA synthesis, the target gene encoding NP was amplified by PCR after which the NP gene was cloned in the pJET1.2/blunt TA vector. The accuracy of cloned gene was confirmed by PCR, enzymatic digestion and sequencing. The pJET1.2-NP plasmid and pIRES-Igk/mIL18/Fc plasmids were simultaneously digested by *BstXI/NotI* enzymes. We inserted the digested NP fragment into the pIRES-Igk/mIL18/Fc plasmid using T4 ligase. Transformation into DH5α and colony selection was done. Gene cloning was confirmed by PCR, enzymatic double-digestion and sequencing. Eventually, by transfection of the constructed mIL-18-pIRES2-NP plasmid into BHK-21 and RAW264.7 cell lines, we assessed the expressions of *NP* and *IL-18* by indirect immunofluorescence and ELISA, respectively.

Results: Electrophoresis of the PCR product, enzymatic digestion, and sequencing showed that the influenza *NP* gene successfully cloned into pIRES-Igk/mIL18/Fc to generate the mIL-18-pIRES2-NP plasmid. Indirect immunofluorescence and ELISA indicated the successful expressions of *NP* and mIL-18 from the produced plasmid in eukaryotic cell lines.

Conclusion: The results of the present study confirm expressions of *NP* and *IL-18* genes from mIL-18-pIRES2-NP. This is a proper candidate for *influenza A* gene vaccine in future investigations.

Keywords: Influenza, Cloning, *NP* gene, Mouse *IL-18*, Bicistronic vector

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طراحی، همسانه‌سازی و بررسی بیان ژن NP در ناقل دوسیسترونی واجد ژن اینترلوکین ۱۸ موشی با هدف استفاده در مطالعات واکسن آنفلوانزا

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