OBTAINING OF INFLUENZA A MRNA-VACCINES ENCODING ARTIFICIAL ANTIGENS USING MODIFIED ANALOGS OF NUCLEOTIDES

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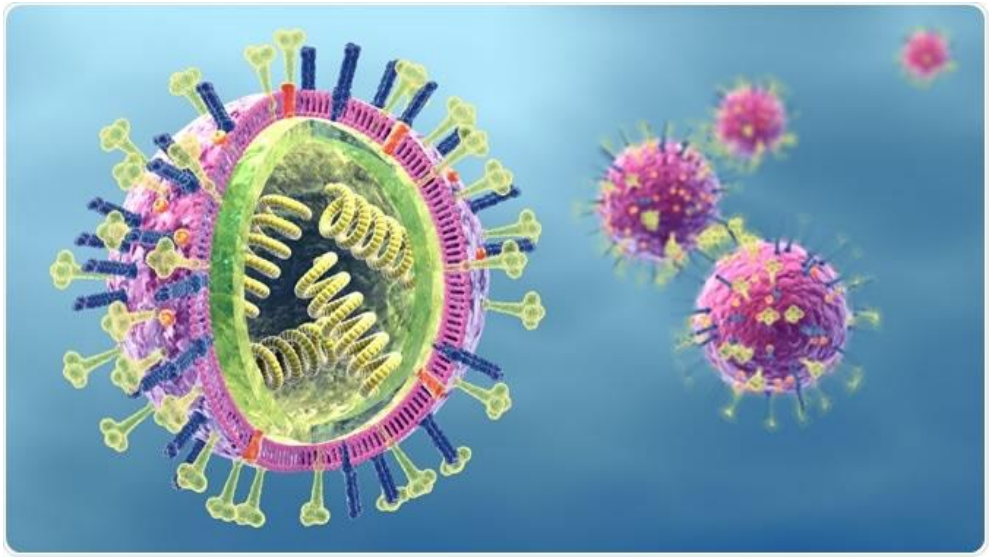
Introduction

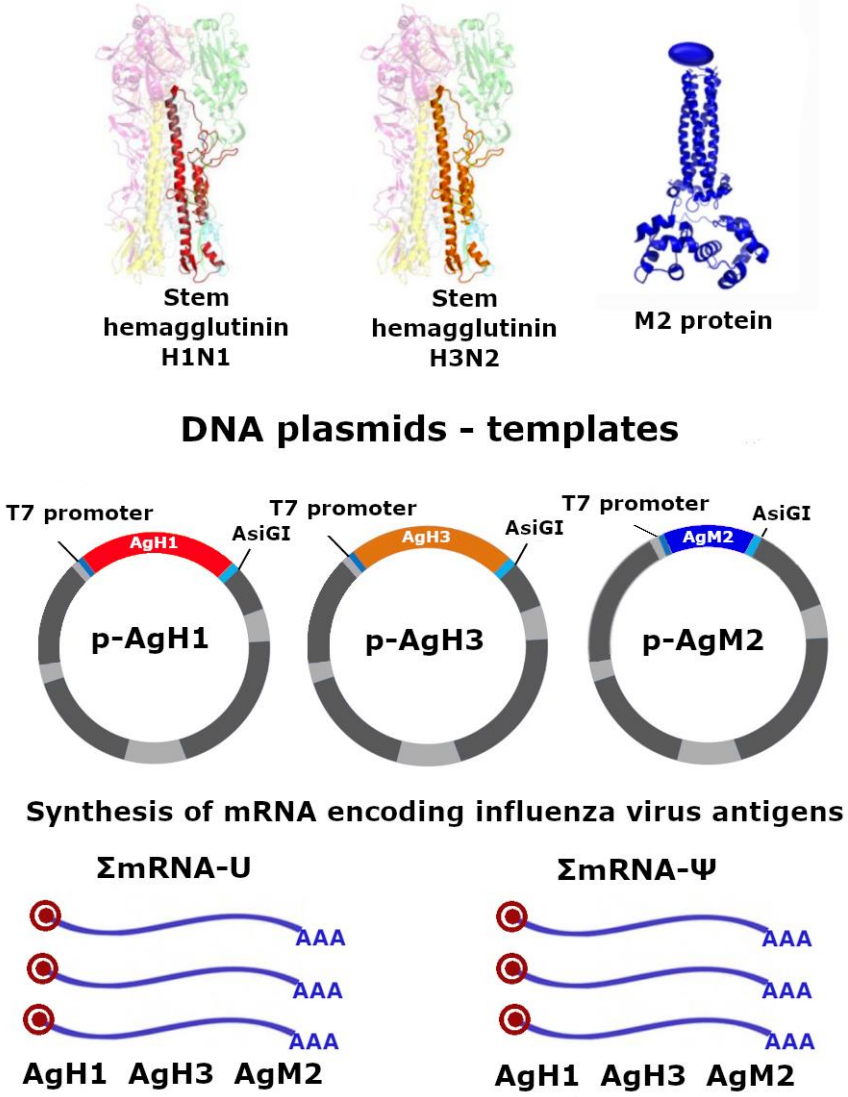
- Influenza viruses cause acute respiratory tract infections that occur periodically in the form of epidemics and pandemics. Influenza remains a major unresolved public health problem worldwide. Annual influenza epidemics cause 3 to 5 million cases of severe respiratory diseases, up to 650,000 of which are fatal.



3d illustration showing influenza viruses with RNA.
Image Credit: Axel Kock / Shutterstock

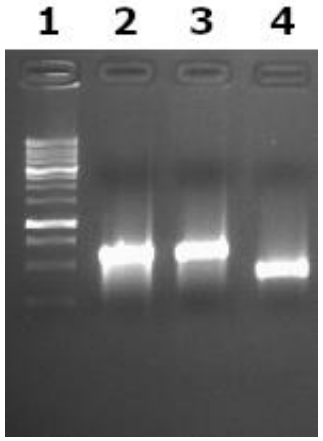
Design of influenza virus immunogens

- The study used an approach aimed at creating a universal influenza virus vaccine by encoding two variants of the influenza hemagglutinin stem and the conserved protein M2.
- In this study, we transformed those antigens from a DNA vaccine format to an mRNA vaccine format using different variants of nucleotide modifications.
- The impact of modified nucleotides on RNA stability and protein translation efficiency was assessed in the model mRNA encode GFP.

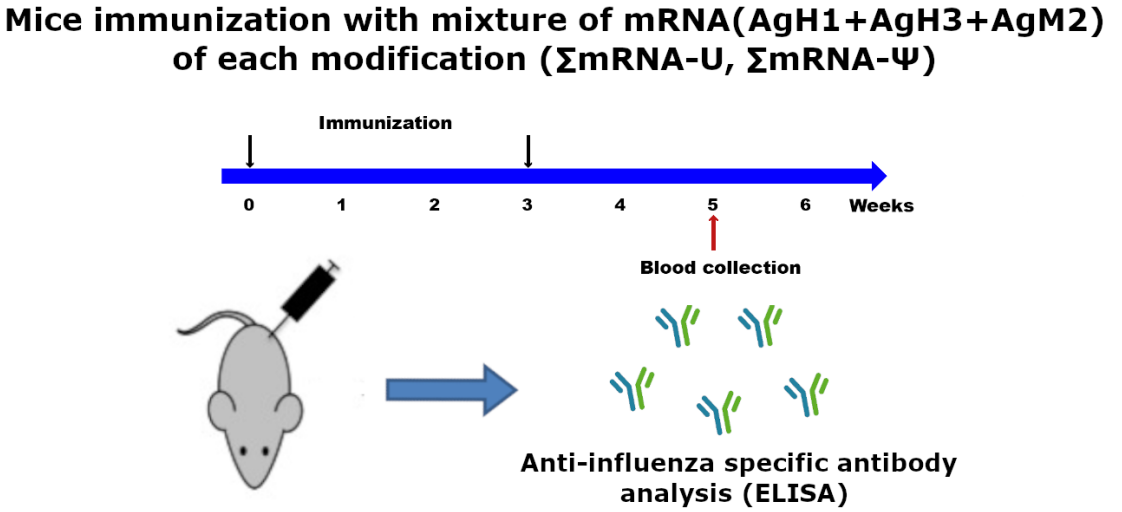


Materials and Methods

- mRNA synthesis that encoded AgH1, AgH3, AgM2 antigens and GFP was conducted using relevant DNA templates in two modifications: mRNA-U with uridine, mRNA-Ψ with pseudouridine.
- The mRNAs were synthesized using in vitro RNA synthesis kit T7 mScript™ Standard mRNA Production System (CellScript, Madison, USA) with NTP mixes containing different NTP (A,G,C and U or Ψ).
- Purified proteins of the influenza virus were used as a sorbent for ELISA.

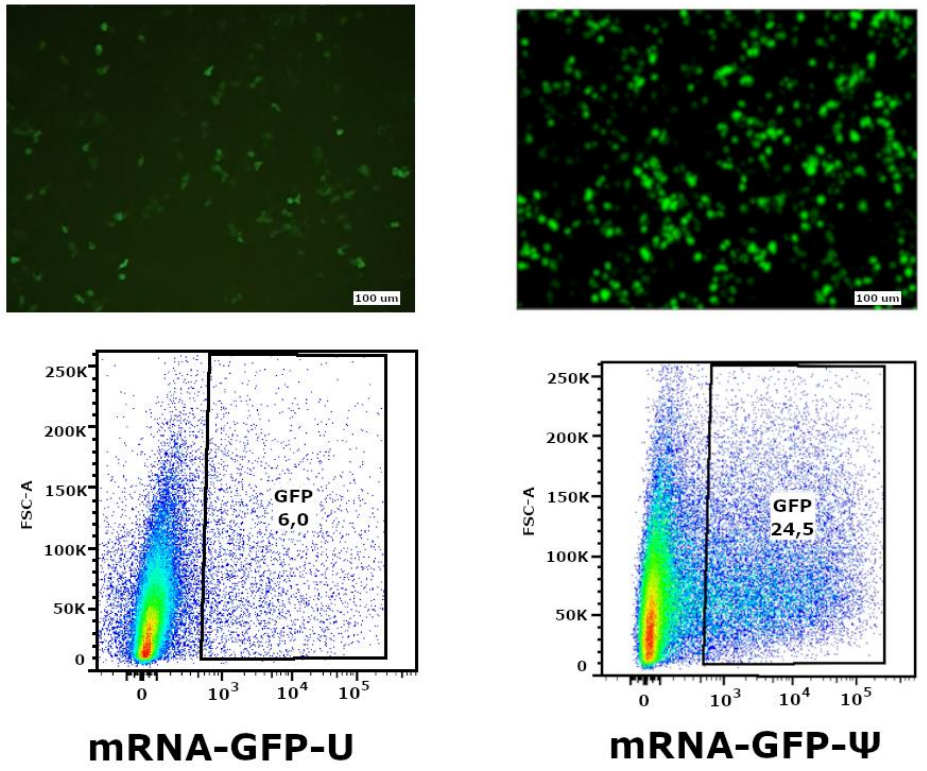


Control of mRNA synthesis.
1 - Marker M12 (SibEnzyme),
2 - mRNA-AgH1,
3 - mRNA-AgH3,
4 - mRNA-AgM2,



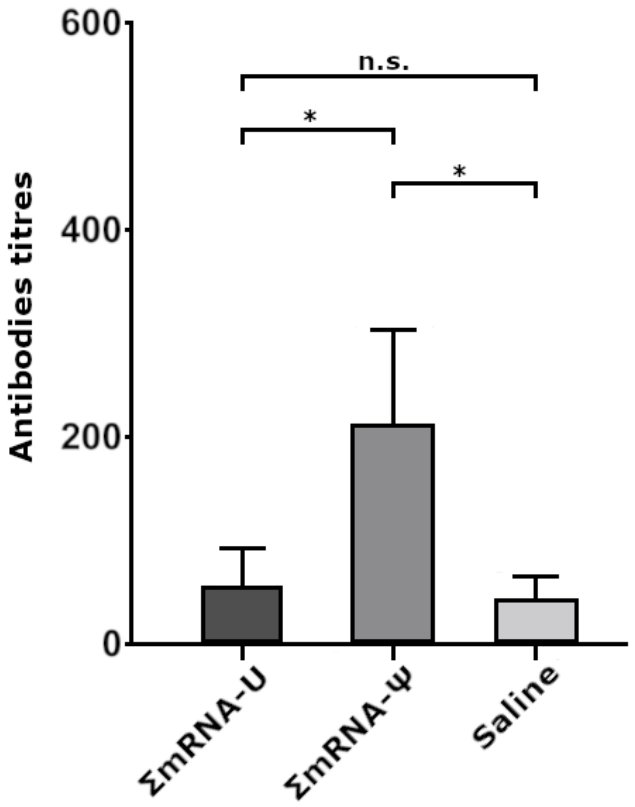
Assessment of nucleotide modifications on the GFP mRNA model.

- Transfection efficiency of HEK293FT cells, obtained via mRNA, was assessed through GFP fluorescence signal intensity, which was registered using microscopy and flow cytometry.
- The data obtained enabled us to conclude that the most efficient protein synthesis was observed in human cells when there was 100% substitution of U with Ψ.



Analysis of Immunogenicity mRNA-vaccine

- The combinations ΣmRNA-U, ΣmRNA-Ψ (15 µg of each immunogen per mouse) were used to immunize BALB/c mice.
- Data obtained using ELISA showed that 2 weeks after the second immunization, titers of specific antibodies in mice immunized with the mRNA vaccine constructs increased. Statistically significant levels of antibodies were registered in group immunized with ΣmRNA-Ψ.



ΣmRNA = mRNA-AgH1+mRNA-AgH3 + mRNA-M2

Conclusions

- Modified naked mRNA-vaccines encoding artificial antigens influenza viruses can induce a specific antibody response in mice.
- The immunogenicity of mRNA vaccines in the form of naked RNA molecules, despite the use of modified nucleotides, was insufficiently high. This may have been due to their degradation by RNases and weak effectiveness of delivery in antigen-presenting cells.
- We believe that obtained mRNA vaccine constructs will demonstrate protection against viral when using efficient delivery methods (i.e., electroporation, cationic polymers, dendrimers, and lipid nanoparticles). This will be the subject of our further studies.

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