Molecular Therapy
Commentary

Preparing for Pandemics: RNA Vaccines at the Forefront

Jesse H. Erasmus[1] and Deborah Heydenburg Fuller[1]

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With coronavirus disease 2019 (COVID-19), it is now clear that the preparedness of the healthcare system for the levels of morbidity and mortality that could occur with a serious pandemic, whether due to influenza or COVID-19, is uncertain, but what is certain is the need for vaccine platforms that can be rapidly developed and scaled up to combat current and future pandemics. Prior to the emergence of severe acute respiratory syndrome (SARS)-coronavirus 2 (CoV-2), the virus that causes COVID-19, researchers were preparing for the next influenza pandemic. Influenza pandemics have occurred throughout history and continue to be a threat, but existing vaccines will be inadequate. Antibody responses induced by current influenza vaccines can protect against homologous viruses but are less effective against antigenic variants and provide little, if any, protection against genetically shifted viruses. In addition, the logistics and time frame for the manufacture and administration of conventional killed or live attenuated influenza virus vaccines require at least 6 months from the identification of a strain to vaccine distribution and then an additional 1–2 months for widespread delivery. Such a time frame will limit vaccine availability during a worldwide pandemic. A significant challenge, therefore, is to develop new vaccine strategies that have shortened production times. In addition, for influenza, and possibly SARS-CoV-2, the most effective pandemic vaccine will need to address genetic drift and shift by providing broad spectrum protection against divergent influenza strains. Such a universal influenza vaccine is believed to be possible if it can induce immune responses against conserved regions of influenza.

Nucleic acid vaccines, including RNA and DNA vaccines, offer the greatest potential to meet these needs because they can be quickly designed to encode any viral sequence and manufactured rapidly, requiring minimal to no process development for new antigenic variants. As mRNA vaccines do not require costly and time-consuming cell-based manufacturing, culture, or fermentation, they can be rapidly produced through simple synthesis methods. In addition, the formulated products demonstrate improved stability and, in multiple phase I human clinical trials, have been shown to be very safe. Importantly, both DNA and RNA vaccines can be designed to precisely focus the response on any given antigen of the virus, including more conserved antigen sequences that will need to be targeted by a universal influenza vaccine capable of inducing immunity against both seasonal drift and unknown future pandemics. With the likelihood that the continued spread of SARS-CoV-2 could be exacerbated during flu season,[1] the development of a universal influenza vaccine remains a high priority.

Even before mRNA vaccines caught the world's attention as the first COVID-19 vaccine approach to enter phase I human clinical trials,[2] they were making quick headway as an emerging front-runner for a universal influenza vaccine. The first mRNA vaccines were investigated in the early 1990s, but they were not initially pursued due to poor stability, limited capacity for scale up, and inefficient *in vivo* delivery. Since then, improvements in the delivery and stability of mRNA vaccines have placed them at the forefront of the pandemic response for COVID-19 and, even before that, in preparation for the next influenza pandemic. These include incorporation of RNA structural and sequence elements as well as purification methods to increase antigen expression and RNA stability and the development of lipid nanoparticles to enhance intracellular delivery of RNA into cells.[3–12]

In this issue of *Molecular Therapy*, Freyn et al.[13] describe the development of an intradermally delivered combination lipid nanoparticle (LNP) mRNA vaccine candidate and break down antibody and T cell responses to each antigen component as well as the efficacy associated with those responses. In terms of antigen selection, the authors decided on 3 structural gene-derived antigen candidates, including a previously described mini hemagglutinin (HA), comprising a structurally-optimized HA stem design, as well as neuraminidase (NA) and the matrix-2 (M2) ion channel and then a nonstructural gene-derived antigen based on the nucleoprotein (NP).

Using a nucleoside-modified co-transcriptional RNA production and capping process followed by purification using a dsRNA-removal process, they then demonstrated low, single-dose potency in mice following LNP-formulated intradermal vaccination. While the potency of this approach has been previously attributed to this particular preparation and delivery method, the authors have provided additional insight into the effect of combining multiple mRNA-encoded antigens into a single immunization on immunogenicity and efficacy compared to the individual components administered independently. While the combination of all 4 vaccines was able to completely protect against escalating challenge doses of H1N1 as well as diverse heterologous challenge viruses, including drifted H1N1 variants, H5N8, and a chimeric H6 virus, neuraminidase (NA) alone was only protective against H1N1 challenges, including the high-dose challenge (500 × 50% lethal dose [LD_{50}]). Additionally, while NP alone provided broad protection from mortality, albeit with varied levels of morbidity, this protection was not provided solely by antibody or T cells

[1]Department of Microbiology, University of Washington, Seattle, WA, USA

Correspondence: Deborah Fuller, PhD, Department of Microbiology, University of Washington, P.O. Box 358070, Seattle, WA 98109, USA.
E-mail: fullerdh@uw.edu

independently. In contrast, while broad protection with variable morbidity was observed following vaccination with either M2 or miniHA alone, with little to no protection at the high-dose challenge, this protection was largely mediated by antibodies alone. In all challenge cases, however, the combination of miniHA, M2, NA, and NP provided for complete protection from morbidity and mortality, even following a 500 × LD_{50} challenge.

The observation that a combination vaccine does not appear to induce any interference and that resulting immune responses are consistent with an additive effect in mice supports the continued development of this approach in larger animal models and eventually humans. However, the complexities and costs associated with releasing 4 separate drug products for a single clinical trial, in this case, may necessitate the testing of a single mRNA candidate encoding these 4 antigen genes or evaluating fewer combinations to determine the minimal composition of a universal flu vaccine. Additionally, minimizing the number of antigens in the combination while adding a booster dose may alleviate the need for multiple vaccine candidates in a single product, as the authors showed that booster doses improved antibody responses as well as efficacy, particularly for the miniHA candidate where antibody affinity maturation may contribute to the improved morbidity outcome.

With the emergence of COVID-19, the substantial progress made in the last 10 years in the development of new and better DNA and RNA vaccine technologies is suddenly coming of age as these technologies, long believed to have considerable potential for pandemic preparedness, are put to the test in the very real pandemic unfolding before us. The work reported here by Freyn et al.[13] highlights the feasibility and considerable promise of combination mRNA vaccines for developing a universal influenza vaccine as a strategy that could pre-empt a possible future flu pandemic. While the jury is still out on whether or not these types of vaccines will prove safe and efficacious in the human population, we are certainly very close to finding out, with first-in-human data anticipated this year.

REFERENCES

1. Singer, B.D. (2020). COVID-19 and the next influenza season. Sci. Adv., Published online May 29, 2020. https://doi.org/10.1126/sciadv.abd0086.
2. Thanh Le, T., Andreadakis, Z., Kumar, A., Gómez Román, R., Tollefsen, S., Saville, M., and Mayhew, S. (2020). The COVID-19 vaccine development landscape. Nat. Rev. Drug Discov. *19*, 305–306.
3. Cullis, P.R., and Hope, M.J. (2017). Lipid Nanoparticle Systems for Enabling Gene Therapies. Mol. Ther. *25*, 1467–1475.
4. Ball, R.L., Bajaj, P., and Whitehead, K.A. (2016). Achieving long-term stability of lipid nanoparticles: examining the effect of pH, temperature, and lyophilization. Int. J. Nanomedicine *12*, 305–315.
5. Kaczmarek, J.C., Kowalski, P.S., and Anderson, D.G. (2017). Advances in the delivery of RNA therapeutics: from concept to clinical reality. Genome Med. *9*, 60.
6. Sabnis, S., Kumarasinghe, E.S., Salerno, T., Mihai, C., Ketova, T., Senn, J.J., Lynn, A., Bulychev, A., McFadyen, I., Chan, J., et al. (2018). A novel amino lipid series for mRNA delivery: improved endosomal escape and sustained pharmacology and safety in non-human primates. Mol. Ther. *26*, 1509–1519.
7. Zhao, Y., and Huang, L. (2014). Lipid Nanoparticles for Gene Delivery. Adv. Genet. *88*, 13–36.
8. Li, B., Luo, X., and Dong, Y. (2016). Effects of Chemically Modified Messenger RNA on Protein Expression. Bioconjug. Chem. *27*, 849–853.
9. Karikó, K., Muramatsu, H., Welsh, F.A., Ludwig, J., Kato, H., Akira, S., and Weissman, D. (2008). Incorporation of pseudouridine into mRNA yields superior nonimmunogenic vector with increased translational capacity and biological stability. Mol. Ther. *16*, 1833–1840.
10. Karikó, K., Muramatsu, H., Ludwig, J., and Weissman, D. (2011). Generating the optimal mRNA for therapy: HPLC purification eliminates immune activation and improves translation of nucleoside-modified, protein-encoding mRNA. Nucleic Acids Res. *39*, e142.
11. Thess, A., Grund, S., Mui, B.L., Hope, M.J., Baumhof, P., Fotin-Mleczek, M., and Schlake, T. (2015). Sequence-engineered mRNA Without Chemical Nucleoside Modifications Enables an Effective Protein Therapy in Large Animals. Mol. Ther. *23*, 1456–1464.
12. Kauffman, K.J., Mir, F.F., Jhunjhunwala, S., Kaczmarek, J.C., Hurtado, J.E., Yang, J.H., Webber, M.J., Kowalski, P.S., Heartlein, M.W., DeRosa, F., and Anderson, D.G. (2016). Efficacy and immunogenicity of unmodified and pseudouridine-modified mRNA delivered systemically with lipid nanoparticles in vivo. Biomaterials *109*, 78–87.
13. Freyn, A.W., Ramos da Silva, J., Rosado, V.C., Bliss, C.M., Pine, M., Mui, B.L., Tam, Y.K., Madden, T.D., de Souza Ferreira, L.C., Weissman, D., et al. (2020). A Multi-Targeting, Nucleoside-Modified mRNA Influenza Virus Vaccine Provides Broad Protection in Mice. Mol Ther. *28*, this issue, 1569–1584.