One Group's Historical Reflections on DNA Vaccine Development

Ellen F. Fynan
Worcester State College

Let us know how access to this document benefits you.

Follow this and additional works at: https://escholarship.umassmed.edu/oapubs

Part of the Amino Acids, Peptides, and Proteins Commons, Genetics and Genomics Commons, Immunoprophylaxis and Therapy Commons, and the Therapeutics Commons

Repository Citation

Creative Commons License
This work is licensed under a Creative Commons Attribution 4.0 License.
This material is brought to you by eScholarship@UMMS. It has been accepted for inclusion in Open Access Articles by an authorized administrator of eScholarship@UMMS. For more information, please contact Lisa.Palmer@umassmed.edu.
One Group’s Historical Reflections on DNA Vaccine Development

Ellen F. Fynan,1 Shan Lu,2 and Harriet L. Robinson3,*

1Department of Biology, Worcester State College, Worcester, Massachusetts; 2Department of Medicine, University of Massachusetts Medical School, Worcester, Massachusetts; and 3GeoVax, Inc., Smyrna, Georgia.

DNA vaccines were pioneered by several groups in the early 1990s. This article presents the reflections of one of these groups on their work with retroviral vectors in chickens that contributed to the discovery and early development of DNA vaccines. Although the findings were initially met with skepticism, the work presented here combined with that of others founded a new method of vaccination: the direct inoculation of purified DNA encoding the target antigen.

Keywords: DNA vaccines, antibody, cytotoxic T cells, heterologous prime-boost

In 1992, our laboratory was one of the pioneers on the use of in vivo DNA-expressed proteins to elicit protective immune responses. As with many novel concepts, this “radical” method of vaccination met with skepticism and doubt. Jenner self-published his use of variolation to protect against smallpox because the Royal Society considered that they might damage the Society’s reputation by publishing his findings in the Proceedings of the Royal Society.1 So too, the idea of using DNA as a vaccine was first considered questionable. However, convincing experimental evidence from our laboratory and others over the past 25 years has demonstrated the powerful potential of this method for immunization and contributed to the use of in vivo expression of DNA-encoded proteins for gene therapy, cancer immunotherapy, and monoclonal antibody production.2–4

The development of live vaccinia virus as an expression vector and its use as a vaccine in 1982 generated interest in the use of viral vectors for vaccination.5,6 Recognizing the potential of this method and possible extension to avian diseases, our group inserted the gene for avian influenza hemagglutinin into a replication-competent avian retrovirus vector.7 Transfection of the recombinant retroviral vector into chick embryo fibroblasts resulted in production of the vector and expression of the influenza hemagglutinin insert for >2 weeks. In experiments conducted in collaboration with Rob Webster of St. Jude’s Children’s Research Hospital (which had the appropriate BSL3 laboratory for testing avian influenza virus infections in chickens), the retroviral vector–based vaccine completely protected chickens against a lethal influenza virus challenge.7 In contrast, birds within the control group succumbed to influenza. Given this, we next tested an infectious, replication-defective pseudotype of the retroviral vector for the ability to provide protection. This replication-defective pseudotype, despite inoculating <1×10⁶ infectious units, also achieved 100% protection, demonstrating that even low titers of a replication-defective vector could achieve protective immunity.

Retroviruses have DNA and RNA forms of their genetic information: RNA in infectious virus and DNA in infected cells. Given the ability of relatively few infectious units of the infectious, replication defective pseudotype to achieve protection and a growing body of evidence for successful in vivo transfection,⁵,⁹ we tested the ability of the DNA forms of both the replication-competent and replication-defective vectors to achieve protection. We made as much DNA as we could and asked Rob to vaccinate chickens with 300 μg of vaccine DNA.

*Correspondence: Dr. Harriet L. Robinson, GeoVax, Inc., 1900 Lake Park Drive, Suite 380, Smyrna, GA 30080. E-mail: hrobinson@geovax.com

© Ellen F. Fynan et al. 2018; Published by Mary Ann Liebert, Inc. This Open Access article is distributed under the terms of the Creative Commons License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.
or empty vector DNA (the control). Each chicken received 300 μg of DNA at weeks 0 and 4 delivered by three routes (subcutaneous, intraperitoneal, and intravenous). A lethal influenza virus challenge was administered at week 5. We learned that our first DNA experiment had worked when Rob left the message “Send more vaccine.” We had achieved 100% protection in both groups of chickens, receiving either the replication-competent or the replication-defective vectors. We immediately set out to repeat the trial, telling nobody of the results until a patent had been filed. Once we had filed, we began to present the results, but these were met with disdain and skepticism. The first question at the summer 1992 American Society of Virology meeting was “You don’t think this will be useful, do you?” Our grants were triaged and our manuscripts returned (despite Nature sending the report to multiple reviewers). Fortunately, our department chair, Guido Majno, a pathologist with broad interests in the history of science and medicine and author of the bestselling book, The Healing Hand, recognized the potential of what we were doing and provided departmental funds to keep us going. We knew we were onto something, and we kept going.

The new technology first achieved public acceptance at the fall 1992 Cold Spring Harbor Vaccine meeting, “Modern Approaches to New Vaccines,” which was attended by a number of the early players in DNA vaccines. We presented our protective studies in chickens and mice. Margaret Liu, Jeff Ulmer, and John Donnelly of Merck showed that protective cytotoxic T cells could be elicited, David Weiner from the University of Pennsylvania described the generation of Ab responses for human immunodeficiency virus type 1 (HIV-1), and researchers from Vical presented their results on introducing DNA into muscle. The attendees clustered around the DNA posters. The field of DNA vaccines had been born! That fall, the Department of Agriculture awarded our first funding for DNA vaccines, and Shan Lu, a new postdoctoral fellow in the lab, received a Howard Hughes fellowship for studying DNA-based immunizations. It would, however, take another year and increasing sophistication in immunology on our part to “merit” National Institutes of Health funding.

Taken together, it was becoming clear that transfection could occur in vivo and that low numbers of cells expressing a plasmid were sufficient to stimulate an immune response. However, given the concern that an endogenous virus might render our replication-defective retroviral vectors infectious, we undertook in vivo antigen expression with a non-retroviral DNA vector, comprised of a mammalian expression plasmid with the gene for the influenza hemagglutinin antigen under the control of a strong eukaryotic promoter. These studies readily replicated the success achieved with the retroviral vectors.

With protection against disease shown in DNA-vaccinated chickens, we moved our studies into much more tractable mouse models. Influenza hemagglutinin expressing plasmid DNA successfully protected BALB/c mice following intramuscular and intravenous inoculations using a hypodermic needle and syringe; intranasal inoculations, using nose drops; and epidermal inoculations using a gene gun. A prototype gene gun (Accell15) was acquired from Agracetus (Middleton, WI) where it had been developed primarily to introduce DNA into plant cells and, later, live animals.10–12 In our experiments, we used the gene gun to blast gold particles coated with the plasmid DNA into the shaved abdominal skin of mice. In earlier biologic studies, Stephen Johnston had used a gene gun to deliver human growth hormone to the outer ears of mice and realized that he had not affected mouse growth but had elicited Ab to human growth hormone.13 The use of the Agracetus gun (the size of a refrigerator) generated a great deal of excitement (and noise) within the department, but did not allay suspicions about our laboratory’s endeavors.

Our initial experiments in mice were highly successful: 95% of the mice inoculated intramuscularly survived the lethal influenza virus challenge. Even more striking were the results of the gene gun inoculations. Mice were protected against an influenza challenge virus with 250–2,500 times less plasmid DNA than with the other routes of administration.14 These results—along with pioneering work by Jon Wolff of the University of Wisconsin and Phillip Felgner of Vical on intramuscular delivery of DNA,15 Margaret Liu, Jeff Ulmer, and John Donnelly at Merck, which had licensed the delivery of “naked DNA” to muscle from Vical;16 Stephen Johnston of the Southwestern Medical Center on ballistic delivery of DNA to elicit Ab;13,16 David Weiner of the University of Pennsylvania;17 Heather Davis and Bob Whalen of the Pasteur Institute;18,19 Hildegund Ertl of the Wistar Institute;20 and Britta Wahren of the Karolinska Institute19—gained acceptance for this new vaccination method and encouraged others to try this novel avenue of vaccine research.21
Table 1. Timeline for DNA vaccines

<table>
<thead>
<tr>
<th>Year</th>
<th>Event</th>
</tr>
</thead>
<tbody>
<tr>
<td>1992</td>
<td>Demonstration of the ability to elicit antibody*</td>
</tr>
<tr>
<td>1993</td>
<td>First protective studies in animals</td>
</tr>
<tr>
<td>1994</td>
<td>Naming of technology, WHO*</td>
</tr>
<tr>
<td>1995</td>
<td>First prophylactic Phase I human trial*</td>
</tr>
<tr>
<td>1996</td>
<td>FDA points to consider for DNA-based vaccines*</td>
</tr>
<tr>
<td>1998</td>
<td>HIV, malaria, influenza, herpes, and hepatitis B virus vaccines in clinical trials</td>
</tr>
</tbody>
</table>

*Demonstrated in a “gene therapy” experiment in which human growth hormone was being delivered to mice to enhance growth.

**Names under consideration included genetic immunization, polynucleotide immunization, gene vaccines, and DNA vaccines.

The elicitation of low-titer antibody responses was evident in our earliest experiments in chicken where large amounts of DNA (300 μg of DNA) raised essentially undetectable Ab responses. The “undetectable” Ab responses did undergo strong anamnestic expansions post challenge, sufficiently strong to protect against infections that could kill within a week of challenge. These strong anamnestic responses differed from anamnestic responses through increased efficiency of DNA delivery. Work on expression cassettes has identified promoters, enhancers, and introns that optimize immunogenicity of the Zika glycoprotein, and sets a precedent for DNA immunizations with other highly immunogenic proteins holding good promise for success. Following electroporation, Ebola, Marburg, and Middle East respiratory syndrome (MERS) vaccines have shown promise in nonhuman primates, and the MERS vaccine has been advanced to clinical trials (ClinicalTrials.gov NCT02670187).

As for the authors, we are still using DNA for vaccination. Two of us are using heterologous prime-boost regimens for the development of a HIV
vaccine. One of us (H.L.R.) is using the DNA as a prime for modified vaccinia Ankara boosts. In this case, the DNA facilitates the display of the native form of the HIV Env on virus like particles. Another (S.L.) is using DNA as a prime for gp120 protein subunit boosts as part of a polyvalent HIV vaccine strategy. The last (E.F.F.) is teaching the next generation of experimental biologists.

ACKNOWLEDGMENTS

The authors would like to acknowledge Joe Santoro for his technical support of the early DNA vaccine experiments in the Robinson laboratory. We are eternally indebted to Dr. Guido Majno for his provision of Departmental support for our early work on DNA vaccines and to the Howard Hughes Medical Institute for having supported postdoctoral work on DNA vaccines for S.L.

AUTHOR DISCLOSURE

H.L.R. currently works for GeoVax, Inc., which is advancing a DNA prime-MVA boost vaccine. She owns stock in GeoVax as well as being on patents. S.L. is on DNA vaccine-related patents. E.F.F. is on DNA vaccine-related patents.

REFERENCES


Received for publication March 30, 2018; accepted after revision June 30, 2018.
Published online: July 2, 2018.