

Novel vaccine technology and the future of vaccine science

Shan Lu

University of Massachusetts Medical School; Worcester, MA USA

A phone call from Dr. Harriet Robinson forever changed my career. It was 1990, and I was about to graduate from the Immunology PhD program at the University of Massachusetts Medical School (UMMS). It had been five years since I came to the US to pursue my research training after I completed my medicine residency training in China.

At that time I was interviewing for postdoctoral fellow positions. Previously, I had a brief discussion with Dr. Robinson about working in her lab as she just moved from a local research foundation to UMMS and I heard she is a leader in retrovirology. She was interested in me but she did not have funding available for a new postdoc.

In this unexpected phone call, Harriet told me that there would be an opening in her lab due to an unexpected departure of a technician. I was quite happy to take her offer. Not having to leave the UMMS was important to me since my wife was also studying at UMMS as a PhD student at that time.

Going to Harriet's lab provided a tremendous training opportunity for me. My PhD thesis was in the area of antigen processing and presentation. At that time, the crystal structures of major histocompatibility complex (MHC) molecules were just being solved. I was studying peptide binding to MHC molecules. Technically, I was running peptide synthesis, HPLC purification, amino acid analysis, and many 2D gels (up to 24 a day) to analyze interactions among peptide, class II MHC and its associated invariant chain. In contrast, Harriet's lab mainly worked in the field of virology with a molecular biology approach. I thought a postdoc fellow

training in molecular virology would complement my PhD work related to protein chemistry in the field of immunology.

Two unexpected things happened rather quickly. First, the size of Harriet's lab continued to shrink over the course of my first two years in the lab. She told me that she had lost her interest in continuing her work on the Avian Leukemia Virus (ALV) as future work will be on ALV protein structure, which is beyond her expertise. She wanted to do more HIV-1 research. The unfortunate result of this transition was no new immediate grant funding, I felt that I should try to help and submitted a fellowship application to the Howard Hughes Medical Institute without realizing how competitive it was.

The second event was some unexpected data that Harriet told me about one day: she suspected that an antigen gene delivered by a viral vector can stimulate immune responses by direct gene expression *in vivo*, not through viral vector expression, and the resulting protein can serve as the immunogen.

I was quite intrigued. Harriet mentioned this idea to several people in the lab hoping someone may be interested in taking this project. But with the uncertain future of the lab at that time, no one seemed interested except for me. I volunteered to do additional mouse experiments testing various vector designs while continuing my main project.

With data generated by my own work, I became more convinced that we were onto something significant. However, the funding situation continued to get worse before we could convince grant reviewers. Before long, the lab was left with just Harriet and me. Luckily, I got the news that my Howard Hughes Fellowship application

Correspondence to: Shan Lu;
Email: shan.lu@umassmed.edu

Submitted: 03/13/2014

Accepted: 03/13/2014

Published online: 05/23/2014

<http://dx.doi.org/10.4161/hv.29042>

was selected for funding! Then the visionary chairman of our department provided \$100,000 of special funding to Harriet so that we could survive until someone realized the value of our work. This money supported the hiring of a new technician and another postdoc fellow to expand our work on DNA vaccines.

At the same time, DNA vaccines started to gain more and more attention. Several research groups, in addition to ours, presented DNA vaccine data at a Cold Spring Harbor vaccine conference without prior knowledge of each other's work. Then three leading papers in this evolving new technology were published to which we were invited as reviewers. Within our lab, DNA vaccine studies expanded from influenza to HIV-1. While I was conducting all of the flu DNA vaccine experiments in the lab, I was asked to give that project to the new postdoc and started the HIV-1 project.

That decision sealed the direction of the next 20 y' of my research in HIV vaccine development. Once I started to study DNA vaccines using HIV-1 antigens, I quickly realized that the immunogenicity of HIV-1 antigens was very different from influenza antigens although both were tested by the same DNA vaccine vectors. This led me to think that the nature of an antigen may play a key role in determining the immunogenicity of a DNA vaccine, and that a process of "antigen engineering" as I called it, is needed to optimize the immunogenicity of a protein antigen delivered by gene-based vaccines. I started to make modifications on the HIV-1 Env gene inserts and immediately saw that the gp120 form of the Env insert was more immunogenic in eliciting antibody responses than the gp140 or gp160 forms. While the optimal design of Env to elicit neutralizing antibodies is still controversial, it was very clear from my data on binding antibodies that a secreted Env was significantly more immunogenic in eliciting overall antibody responses.

The other benefit of DNA vaccines is the possibility of mixing different DNA vaccine plasmids to produce a "polyvalent" formulation. Using this approach, different antigens or different subtypes of the same antigen can be delivered quite easily without the worry of interactions



About Dr. Lu

Dr. Shan Lu obtained his MD from Nanjing Medical University (Nanjing, Jiangsu, China) in 1982, and completed his medicine residency at the First Affiliated Hospital of Nanjing Medical University in 1985. He moved to the United States to earn his PhD in Immunology at the University of Massachusetts Medical Center (Worcester, MA) in 1990 and his MBA focusing on Health Administration at Clark University (Worcester) in 1991. Dr. Lu has remained affiliated with the University of Massachusetts Medical School (UMMS), first as a Howard Hughes Research Fellow, and later as a faculty member. Since 1999 and 2006, respectively, he has served as Attending Physician in the Division of General Medicine and Primary Care, and Professor in the Department of Medicine at UMMS.

Dr. Lu's research interests include the immunogenicity of protein antigens and is a pioneer in DNA vaccines. He has been a leader in AIDS vaccine research, with a special focus on using DNA vaccines to prime the host immune system to elicit high-quality, antigen-specific B cell and antibody responses, which can be greatly amplified through boosting with other vaccine forms, including recombinant proteins, inactivated vaccines, and live-attenuated vaccines. The heterologous prime-boost theory advocated by Dr. Lu has been shown highly effective in recent human studies with HIV-1 and influenza vaccines.

His research has resulted in over 150 publications in international peer-reviewed scientific journals, as well as over a dozen of chapters in books related to vaccines and immunology. He is regularly invited to speak at national and international vaccine conferences, and he has played key role in organizing the Annual Global Congress of the International Society for Vaccines (ISV), for which he served as President up to the end of last year. Dr. Lu is an active member of several professional societies and serves on the editorial board of various journals, including *Journal of Virology*, *Vaccine*, and *Emerging Microbes and Infections* for which he is Deputy Editor-in-Chief. Among many other honors and awards, Dr. Lu was named Fellow of American College of Physicians (FACP) and Fellow of the International Society for Vaccines (FISV) in 2013.

among different vaccine components included in a traditional polyvalent formulation. Polyvalent formulations have been required for vaccines against pathogens with multiple subtypes, such as seasonal influenza or pneumococcus. By using a DNA vaccine-based polyvalent formulation, Harriet and I designed a SIV DNA vaccination study in rhesus monkeys and achieved remarkable partial protection against an intravenous challenge of uncloned SIV251 stock. The level of protection achieved in this study set a bar in the field that has been difficult to surpass, even until today.

While encouraged by the progress of my work in Harriet's lab, I soon had to make a decision about the next career step once my HHMI fellowship ended. I wanted to continue my work in DNA vaccines since I believed in the great potential of this new technology but at the same time, I saw the need to develop a unique translational program that would allow me to use my previous clinical experience alongside the knowledge I learned from research to benefit the human population, especially in the field of vaccine science.

I took and passed the US Medical License Exams and was accepted to do a

medicine residency at UMMS. The Chair of Medicine supported me to continue my research work while completing my clinical training. I joined a special clinical scientist pathway (75% time in clinical work and 25% time in lab) authorized by the American Board of Internal Medicine. I was given a small lab space and the title of research-track Assistant Professor, without a start-up package. I learned how to write a grant quickly as I only had limited time to be away from my busy clinical work. A total of 12 grant applications, regardless of monetary value, were submitted by the end of my three years of residency training. I was fortunate enough to have received funding from eight of them.

Most importantly, my research continued to move forward even while maintaining a busy 80-h resident clinical work schedule, in addition to grant writing. It was in this period that I identified the real value of DNA vaccines: their potential to elicit high-quality antibody responses for vaccine development. The Chair of my department was very excited and convinced me to stay on as an Attending in the Division of General Medicine at the end of 3 y, then promoted me to Associate Professor with a tenure-track appointment.

Facing the challenges of eliciting neutralizing antibodies (Nab) against HIV-1, I noticed that immunizing animals first with an Env-expressing DNA vaccine leads to much improved NAb when the animals were later boosted with the recombinant Env protein. When I combined this prime-boost approach with the polyvalent Env vaccine formulation, the breadth of NAb that was elicited was quite impressive.

Based on this finding and with additional funding from the NIH, my research group moved from small animal studies into studies with non-human primates and even a phase I clinical study. The short summary of this remarkable progress is that DNA priming is effective in all of the species we tested, including humans. The DP6-001 formulation developed by my lab and tested in humans included five HIV-1 gp120 immunogens (representing various subtypes) delivered in the form of a DNA prime-protein boost, plus a Gag-expressing DNA vaccine during the priming phase, all by simple needle injection

without adjuvant. High-level Env-specific T-cell immune responses and antibody responses were detected in 100% of volunteers. Cross-subtype Nab were also detected in all volunteers although the titers were relatively low against the most difficult to neutralize viruses.

Our subsequent analysis of antibody profiles in both animal and human immune sera showed that priming with an Env DNA vaccine was effective in eliciting antibodies against conformational epitopes, such as the critical CD4 binding site, while recombinant Env protein alone elicited mostly antibodies against linear epitopes. This difference in the quality of antibody responses may be responsible for the difference in neutralizing antibody activities we observed with the DNA prime-protein boost approach. DNA priming was also able to increase the avidity of polyclonal antibody responses indicating a role for better antibody affinity maturation. We then discovered that DNA priming can greatly enhance the development of antigen-specific B cells, which can be shown by improved germinal center B cell development using enhanced help from T follicular cells.

Therefore, our understanding on DNA vaccines has gone full circle. Two decades ago, DNA vaccines were touted as a novel approach to elicit T-cell immunity. Now our results demonstrate that DNA vaccines have superb potential to elicit high-quality antibody responses as the result of eliciting strong helper T-cell responses.

This finding has major ramifications for the vaccine field. Immunogenicity of protein-based vaccines, including inactivated vaccines and recombinant protein vaccines, is limited unless a strong adjuvant is included in the formulation. Even so, multiple boost immunizations are needed but are not always effective in establishing high-level and long-lasting antibody responses. While in a way the DNA vaccine is a type of subunit vaccine, delivery of the antigen gene, but not the same antigen protein, demonstrated unexpected potential in priming antigen-specific B cells to produce high quality antibody responses.

In this way, we may finally find a role for DNA vaccines. Recent work from the NIH Vaccine Research Center also

demonstrated that human volunteers who received a one-time priming with influenza HA DNA vaccine elicited better protective antibody responses following a second immunization with an inactivated flu vaccine when compared with the control group, which received two immunizations with the same inactivated flu vaccine.

The story of DNA vaccines is still evolving. To develop a more practical way of using DNA vaccines, it is feasible that a mixture of a DNA vaccine and a conventional vaccine that share the same immunogen, can achieve better immune responses than each component alone. Preliminary data from my group supports this idea in both HIV-1 and HBV model systems. I named this function of DNA vaccines “antigen-specific adjuvant effects.”

Furthermore, it would be interesting to determine if it were possible for people to receive only a priming immunization to achieve protection. This notion is based on an observation I made in Harriet’s lab more than two decades ago: mice immunized via needle injection with HA DNA vaccine showed limited detectable antibody responses (no B cell measurement at that time) but they were protected from subsequent influenza challenge, and interestingly, they developed higher antibody responses than control naïve mice. This finding may be useful for the development of pre-pandemic influenza vaccination, in which the general population can be primed once with a mixture of DNA vaccines covering multiple projected pandemic influenza subtypes. Later in the event of a real pandemic outbreak, those previously primed with a DNA vaccine will have reduced symptoms or a decreased mortality even without a boost vaccination, which can take time to produce given the limited capacity to produce enough doses to immunize the world’s population.

DNA vaccines may also be useful for biodefense and emerging infection applications since these vaccines would only be needed for special targeted human populations. Conventional vaccines take a longer time to develop and produce, especially for stockpile purposes. We have shown good immunogenicity of DNA vaccines

against anthrax, plague, smallpox, SARS, cholera, EV71, and *Clostridium difficile*. DNA vaccines can also serve as a tool to screen and optimize candidate immunogens, as we have done for HCMV and various subtypes of the influenza virus. DNA vaccines also hold promise as therapeutic vaccines, and we have used this approach to establish the principle of therapeutic HBV vaccines. DNA immunization is also highly effective in producing high affinity monoclonal antibodies because of its role in stimulating B cells to produce conformation antibodies. We have produced rabbit and human monoclonal antibodies using this approach against certain difficult epitopes.

However, the more progress we achieve with DNA vaccines, the more I realize

the limitations associated with the current model of vaccine development. Major pharmaceutical companies continue to play decisive roles in vaccine product development yet they are facing severe financial restraint in making such commitments, especially when the market value of new vaccines and their covered population are not high. Furthermore, it is difficult to test and apply new technologies, such as DNA vaccines.

In 2008, I joined the Executive Board of the International Society for Vaccines (ISV) (<https://www.isv-online.org>) and served as its President between 2011 to 2013. My goal was to push for more interaction among worldwide vaccine scientists. I co-chaired the 2nd to 6th Annual Global Congress jointly organized by ISV to

establish a completely novel non-commercial conference as a platform to exchange progress in novel vaccine technologies. I managed the fast growth of ISV, including the establishment of the first website for professional vaccinologists, organization of a bi-annual open election of ISV leadership, and many other activities to establish a strong and continuous interaction among individual vaccinologists and various groups interested in vaccines.

Vaccine technologies, such as the DNA vaccine, are growing very rapidly but their benefits have not been yet demonstrated in licensed products for humans. Public and private sectors of both developing and developed countries need to work together to reform our current system to create the future of vaccine science.