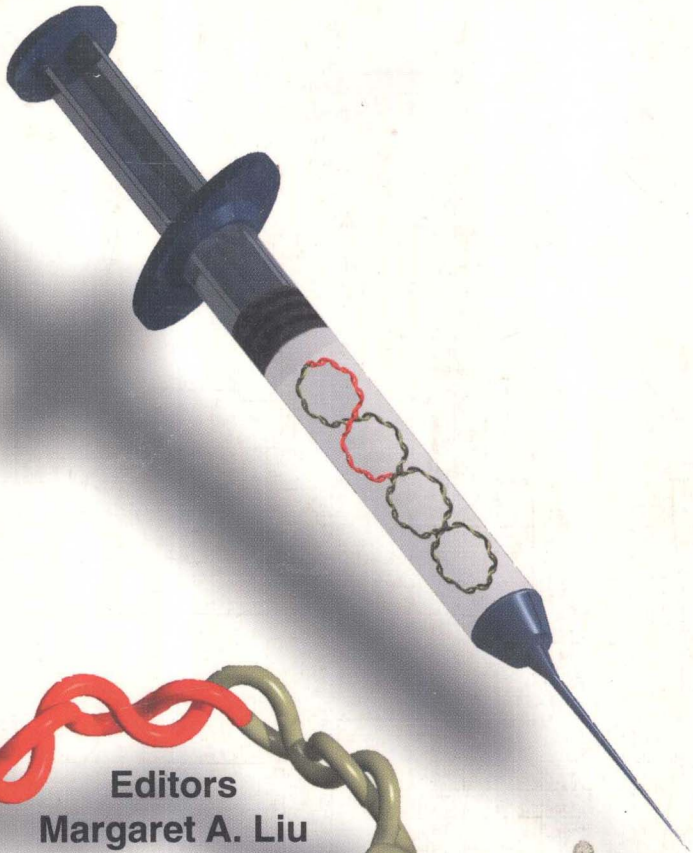
DNA VACCINES

A New Era in Vaccinology



Editors
Margaret A. Liu
Maurice R. Hilleman
Reinhard Kurth

ANNALS OF
THE NEW YORK
ACADEMY OF SCIENCES

VOLUME 772

ANNALS OF THE NEW YORK ACADEMY OF SCIENCES

Volume 772

The New York Academy of Sciences
2 East 63rd Street
New York, New York 10021

Cover: *The simplicity of DNA vaccines is illustrated by the syringe containing supercoiled plasmid DNA. (The coding region is shown in red.) Host cells synthesize the antigen encoded by the plasmid DNA. Protective cellular and antibody responses have been demonstrated in a number of preclinical disease models. (Illustration by Jeff Campbell.)*

Library of Congress Cataloging-in-Publication Data

DNA vaccines : a new era in vaccinology / edited by Margaret A. Liu, Maurice R. Hilleman, and Reinhard Kurth.
p. cm. — (Annals of the New York Academy of Sciences, ISSN 0077-8923 ; v. 772)
"Papers from a conference . . . held by the New York Academy of Sciences in Arlington, Virginia on April 6–9, 1995"—P. 7.
Includes bibliographical references and index.
ISBN 0-89766-997-5 (cloth : alk. paper). — ISBN 0-89766-998-3 (paper : alk. paper)
1. DNA vaccines—Congresses. I. Liu, Margaret A. II. Hilleman, Maurice R., 1942– . III. Kurth, Reinhard, 1942– . IV. Series.
[DNLM: 1. Vaccines, Synthetic—congresses. 2. DNA, Recombinant—therapeutic use—congresses. 3. Gene Therapy—congresses. 4. Immunotherapy—congresses. W1 AN626YL v.772 1995 / QW 805 D629 1995]
Q11.N5 vol. 772
[QR189.5.D53]
500 s—dc20
[615'.372]
DNLM/DLC
for Library of Congress 95-36708
CIP

Bi-Comp/PCP
Printed in the United States of America
ISBN 0-89766-997-5 (cloth)
ISBN 0-89766-998-3 (paper)
ISSN 0077-8923

DNA VACCINES

A NEW ERA IN VACCINOLOGY

[ANNALS OF THE NEW YORK ACADEMY OF SCIENCES]
Volume 772

DNA VACCINES

A NEW ERA IN VACCINOLOGY

Edited by Margaret A. Liu,
Maurice R. Hilleman, and Reinhard Kurth

The New York Academy of Sciences
New York, New York
1995

Financial assistance was received from:

Major Funder
- MERCK & CO., INC.—RESEARCH LABORATORIES AND VACCINE DIVISION

Supporters
- DIVISION OF AIDS—NATIONAL INSTITUTES OF HEALTH
- PASTEUR MÉRIEUX SÉRUMS ET VACCINS

Contributors
- MILES INC.
- SMITHKLINE BEECHAM PHARMACEUTICALS
- VICAL INC.
- WYETH-AYERST RESEARCH
- AVIRON

Preface

This symposium was devoted to the science and art of the new field of vaccination with recombinant DNA vectors encoding antigens. This approach has been termed DNA vaccination or nucleic acid vaccination. If the realization of DNA vaccines equals the promise shown to date, a whole new era of simplified vaccinology may be born. The papers presented during this meeting demonstrated how much progress has been made in various disease models since the publication of the first demonstration of *in vivo* protective efficacy in 1993.[1] Yet in order to bring DNA vaccines into full clinical evaluation, many issues beyond immunological mechanisms and preclinical proof of principle need to be addressed. Thus during this symposium, considerable emphasis was placed on many of the preclinical safety and efficacy issues from both the experimental and regulatory perspectives. Much useful exchange occurred between scientists of different disciplines.

Because DNA vaccines represent a new technology, although there clearly is a precedent for them in live virus vaccines, it is worthwhile examining how each of the different stages of vaccine development may differ for this technology vs more traditional approaches. For basic research, DNA vaccines encounter the same challenges as traditional technologies of demonstrating proof of principle (*i.e.*, that the technology can induce the desired and efficacious immune responses), and preclinical efficacy (*i.e.*, that in the appropriate animal models, protection and adequate duration of protection and immune correlates are seen). But in addition, given the current scientific climate and the novelty of DNA vaccines, increased emphasis has been placed upon determining the mechanisms of antigen presentation and elucidating the immune responses and correlates responsible for protection.

The generation of both antibody and cell-mediated immune responses has been shown in response to various antigens expressed from different vectors administered by different routes and protocols. For example, neutralizing antibodies have been generated by immunization with constructs encoding the viral glycoproteins: influenza hemagglutinin, rabies glycoprotein, bovine herpesvirus gIV, and HIV envelope, as well as L1, the nuclearly-directed major capsid protein of cottontail rabbit papillomavirus. In another arena, the generation of therapeutic antibodies in cancer is about to be tested in clinical trials for B cell lymphoma utilizing vectors encoding the idiotype of the tumor's antibody as the immunizing agent.

$CD8^+$ cytotoxic T lymphocytes (CTL) have been demonstrated following immunization with vectors encoding influenza nucleoprotein, malaria circumsporozoite protein, and SV40 large T antigen.

In a number of systems a surprisingly consistent demonstration of the generation of T helper cell responses with a T_h1-like cytokine phenotype was seen in splenocytes or peripheral blood (from animals immunized with a vector encoding a viral protein) upon reexposure of the lymphocytes to antigen *in vitro*. The duration of immune responses has been shown to be for months (*e.g.*, 6 months to date for helper responses, and out to two years for antibody and CTL) in some cases, although a more rapid decline of antibody or protection has been observed in other systems. It is not clear whether the persistence or difference in persistence of immune response relates to persistent expression of antigen or whether different antigens and routes of inoculation are more important features.

Different investigators have explored the use of facilitators or delivery systems to augment the uptake of the injected DNA. These include agents such as 25% sucrose or bupivacaine, a membrane-active anesthetic. In some laboratories these improved gene expression or immune responses, although in other systems there was no augmentation when co- or preinjection with these agents were utilized. These differences may reflect upon differences in the inherent activity of different vectors (*i.e.*, augmentation seen with vectors which express less protein). Work is ongoing to develop cationic lipids which complex with DNA, then facilitate its uptake. Further refinements include vector systems amenable to regulatable expression by administration of an exogenous agent such as tetracycline.

One potential advantage of DNA vaccines that was clearly demonstrated by the wide variety of systems studied is the generic nature of the technology. Utilizing identical or similar vector backbones and plasmid preparation and purification techniques, a wide range of infectious disease and cancer targets can be addressed. This is in contrast to recombinant protein technology where exploration of various host cells (*E. coli,* yeast, baculovirus, mammalian cells) is required to find the system where a protein can be properly and optimally expressed. This is then followed by development of process technologies which are unique for each recombinant protein, rather than generic for plasmid DNA regardless of the gene inserted.

Another attribute of DNA vaccines that was not emphasized at this meeting is its use as a laboratory tool to generate reagents: polyclonal and monoclonal antibodies.[2] In situations where a protein antigen is not available in sufficient quantities for vaccination purposes due to difficulties in purification or lack of availability of the gene product, a DNA plasmid encoding the desired protein may be a means to generate the desired antibody. Moreover, this provides an alternate means of assessing which gene products of a pathogen or transformed cell might generate desired immune responses (vs the older technique of purifying proteins from pathogens or tumor cells for immunization).

In the area of development, it seems somewhat obvious to note in words borrowed form Dr. Hilleman's opening lecture, that "DNA is the Thing!" Yet this is a fact of not small significance, in that DNA represents a novel entity for scale-up for vaccine and pharmaceutical manufacturers. In addition to needing to develop novel processes for production and purification, there is a need to develop new analytical technologies for characterizing the vaccines product. It is worth mentioning that while the lay press has quoted scientists remarking that DNA vaccines will be stable at room temperature, until the stability of a DNA vaccine is formally tested, it may be premature to equate the ability of DNA to sufficiently survive at room temperature to adequately transfect bacteria for subsequent production of plasmid with the ability of plasmid DNA to retain its potency as a vaccine. Finally, while most vaccines have been considered to be biological entities, it is possible that DNA vaccines might rather be considered chemicals. The distinction may be significant because of the different characterizations and measures of potency which may be used, *e.g.*, concentration vs biological activity.

During this meeting a significant amount of attention was devoted to the potential safety considerations of this technology. The major potential issues include: does integration of the plasmid with ensuing insertional mutagenesis occur? are pathogenic anti-DNA antibodies or anti-self immune responses generated? and does immunologic tolerance occur as a result of possible persistent expression of antigen? Regarding the question of insertional mutagenesis, it is worth remembering the precedents of DNA vaccines in the use of live DNA virus vaccines with the administration of the smallpox vaccine to millions of individuals and the successful eradication of smallpox without any known associated oncogenesis.

Additionally precedents exist in the use of viruses and retroviruses as vectors, although clearly in more restricted clinical settings to date. The theoretical risks of integration as well as the actual studies done to date give some reassurance concerning the safety of DNA vaccines in this regard. Yet because of the difficulty of detecting a very rare event, despite the extreme sensitivity of the polymerase chain reaction (PCR) technique that has been used for the laboratory studies reported at the meeting, further studies need to be done to address this important issue.

Regarding the issue of whether pathogenic anti-DNA antibodies are generated, double-stranded DNA has been considered to be poorly immunogenic. The generation of anti-DNA antibodies in experimental models has generally required the use of single-stranded DNA complexed to protein immunized in complete Freund's adjuvant. Yet this is likewise an area which needs further evaluation. Additionally, certain properties of DNA, such as particular sequences, may be responsible for specific immune activities, such as mitogenicity. Although such properties have not yet been confirmed for DNA vaccines, it is possible that they might be considered a positive attribute by conferring adjuvant-like activity upon the immunogen (the encoded protein).

In general, most of the data reported by various groups using DNA vaccines encoding a variety of proteins has demonstrated that antibody responses can be boosted substantially by repeat immunization. In a limited number of cases, the immune response is refractory to further boosting, but this has occurred following repeated immunization via multiple routes of vaccination. There is no evidence for the induction of tolerance by a more conventional protocol of vaccination involving a limited number of immunizations by a given route. Indeed, it is not known in general for DNA vaccines whether the DNA and antigen production persist for any length of time. It is worth noting that efforts are being made to improve conventional vaccines by developing formulations which result in sustained release of antigen, a process which may be mimicked by DNA vaccines.

In sum, although to date, no "mechanism-based" or unexpected safety limitations have been demonstrated, considerable attention is being directed towards these potential issues. This is entirely appropriate, because the critical initial regulatory consideration is that most of the vaccines under development are for prophylactic rather than therapeutic indications. While the technology may be very useful for therapy, and indeed, the initial clinical trials are devoted to evaluating the efficacy as immunotherapy of B cell lymphoma and HIV, the demonstration of safety must be more stringent before the technology could have any widespread use for prophylactic vaccines.

Just as was the case for the first vaccine made by recombinant protein technology, this technology offers the opportunity for partnership between the scientists and regulators at various regulatory agencies and the researchers and vaccine manufacturers evaluating and developing new vaccines. The National Institutes of Health in the U.S., specifically the Division of AIDS, and the World Health Organization were also proactive by providing for dialogue between the groups early on (February 3, 1994, and May 15–16, 1994).[3] As scientific advances have resulted in the eradication or control of certain infectious diseases, the expectations of the public and biomedical scientists alike have increased regarding efficacy, safety, and even convenience. The regulatory environment for vaccines necessarily reflects these expectations while also seeking to encourage the expeditious development of new technologies to improve human health.

This meeting highlighted the remarkable breadth of preclinical efficacy of DNA vaccines, and the equally impressive rapidity of the development of the field.

Three key issues will determine whether DNA vaccines will be a significant new vaccine technology for the future: their full safety profile, human efficacy, and process/stability features. One hopes that the technology will indeed provide a powerful new tool for the prevention and treatment of infectious diseases and cancer. Concomitantly, vaccinologists may be able to exploit the simplicity, versatility and power of this technology to further our understanding of the immune system and host defenses.

Margaret A. Liu
Maurice R. Hilleman
Reinhard Kurth

REFERENCES

1. ULMER, J. B., J. J. DONNELLY, S. E. PARKER, G. H. RHODES, P. L. FELGNER, V. J. DWARKI, S. H. GROMKOWSKI, R. R. DECK, C. M. DEWITT, A. FRIEDMAN, L. A. HAWE, K. R. LEANDER, D. MARTINEZ, H. C. PERRY, J. W. SHIVER, D. L. MONTGOMERY & M. A. LIU. 1993. Heterologous protective immunity to influenza A by intramuscular injection of DNA encoding a conserved viral protein. Science **259:** 1745–1749.
2. BARRY, M. A., M. E. BARRY & S. A. JOHNSTON. 1994. Production of monoclonal antibodies by genetic immunization. BioTechniques **16:** 616.
3. Vaccine **12:** Issue 16 devoted entirely to meeting proceedings (1994).

ANNALS OF THE NEW YORK ACADEMY OF SCIENCES

Volume 772
November 27, 1995

DNA VACCINES

A NEW ERA IN VACCINOLOGY[a]

Editors and Conference Chairs
MARGARET A. LIU, MAURICE R. HILLEMAN, AND REINHARD KURTH

CONTENTS

[a] This volume contains the papers from a conference entitled *DNA Vaccines: a New Era in Vaccinology*, which was held by the New York Academy of Sciences in Arlington, Virginia on April 6–9, 1995.

Part VII. Related Applications: Cancer and Gene Therapy

Poster Papers

DNA Vectors

Precedents and Safety

MAURICE R. HILLEMAN

Merck Institute for Therapeutic Research
Merck Research Laboratories (WP 53C-350)
West Point, Pennsylvania 19486

DNA Is the Thing!

When studying organic chemistry in the late 1930s (TABLE 1), students learned that nucleic acids are very interesting sticky substances that occur in the nuclei of animal and plant cells. Single strands of nucleic acid from yeast were found to be polymers of phosphoric acids, sugars and pyrimidines and purines hooked together much as they are known today. The next several years brought substantive increase in knowledge of the chemistry of nucleic acids, but their purpose and function were only speculative.

The landmark breakthrough which established DNA as the carrier of heritable genetic information was published in 1944 by Avery, MacLeod, and McCarty.[1] These workers showed that purified DNA extracted from encapsulated Type III pneumococci brought about heritable change in unencapsulated rough (R) variants of Type II pneumococci. The DNA transformed the rough variants into encapsulated Type III pneumococci and established DNA as the seat of genetic inheritance.

This work opened the way to other examples of genetic transfection and transformation in microbial species, but the next seminal discovery was that of Watson and Crick who defined the double helical structure of DNA and its role in encoding genetic information.[2,3] This basic advance was soon followed by discovery of the restriction nucleases (see Ref. 4), the elucidation of the genetic code and, finally, the evolvement of the DNA cloning techniques that opened the door to expression vectors for production of specific proteins, to genetic modification and hybridization of cells *in vitro,* and finally to engineered gene therapy and vaccines employing viral vectors. The recent accomplishment of effective antigen expression *in vivo* through use of recombinant plasmids consisting of DNA alone is the subject of the present symposium on DNA vaccines.[5] It is to be expected that there will be sharing of new knowledge essential to building the data base which is needed to arrive at the consensus required eventually to bring DNA vectorology to practical application. The present paper is an abbreviated overview and referencing will be to reviews, whenever possible.

Toward Application

The major advances (TABLE 2) that have been made in understanding and altering the genetic content and function of human somatic cells, *in vitro,* came from humble hopes that such knowledge might be applied to therapy of human genetic disease and to prophylactic control of infectious diseases. What seemed technically doable in the genetic and molecular sense in these applications was necessarily restricted by the regulatory control agencies whose responsibilities

TABLE 1. Highlights in Evolvement of DNA and Genetic Information

Nucleic acid was a mystique in the late 1930s and early 1940s.
1944. Avery *et al.* showed that DNA is the seat of inheritance.
1953. Watson and Crick established DNA structure and its genetic implications.
Restriction nucleases, genetic code, and cloning techniques followed.
Door was opened to expression vectors, genetic modification and hybridization, and viral vectors for gene therapy and vaccines.
A new era was initiated.

are to rule on what is acceptable in clinical research and what is acceptable for licensure for general use. The regulatory task is of tremendous magnitude and involves weighing the mass of current information, posing questions still to be answered, and exercising judgment as to what is acceptable and what is not. The buck stops at the desks of both the regulator and the sponsor.

Creation of the new field of gene therapy came as a radical departure from the old-time pursuit of descriptive human genetics to genetic modification of somatic cells of human beings by addition, deletion, substitution, or replacement of genes or in altering their function. Present application in the human species is centered on the somatic cell. Modification of the human germ line itself opens a vista of technical possibilities and ethical considerations that preclude clinical application for the foreseeable future.

Viral vectorology, in contrast to gene therapy, has a far longer history as an experiment of nature that extends, perhaps, to the time of evolution of the first cells. We think especially of the retroviruses, so well adapted to movement from host to host while picking up and transporting genetic baggage in the course of their travels. Such transfer of genetic information between individuals might have been essential and contributory to the evolution of mammalian species themselves.

Attenuated live virus vaccines, which transport their own genetic information, have been in use since the time of Jenner in the last part of the 19th century. From such early beginnings came live viral vaccines that have been used routinely for many decades. These vaccines were developed long before knowledge of gene enhancers, promoters, reporters, and terminators, and their safety and utility were established through use in hundreds of millions of persons throughout the world with huge savings in lives, morbidity and disabilities.

It may be a short step from attenuated live viral vaccines to recombinant viral vectors[6] for delivery of new genetic information in gene therapy. Though viral vectors may be in a favored position through long-time use as vaccines, they share the same concerns that are expressed in the regulation of all human gene therapy.

TABLE 2. Practical Application in Genetics and Vaccinology

Driven by hopes for gene therapy and prophylaxis of infectious diseases. Subject to control by regulatory agencies.
Human gene therapy is centered on modification of somatic cells. Germ line modification is off limits.
Viral vectorology is backed by a long history of observations of natural infections and live viral vaccines.
Viral vector vaccines and human gene therapy share common concerns and can learn from each other.

Whatever the course of progress, it seems certain that the sharing of knowledge between the fields of gene therapy and vaccinology will be useful for both.

Viral and DNA Vectorology

The routine application of attenuated live viral vaccines can be either to induce immunity against the virus itself or to deliver added genetic information if it is applied as a recombinant vector. Concerns for safety of prophylactic vaccines are of special importance since vaccines are given to normal healthy people to prevent an infection to which they may never be exposed and a disease that they may never develop. The call may be very different for use of vectored recombinant viruses in gene therapy. Their use will be governed by an added scientific and value judgment in which risk is weighed against benefit.

The importance of safety of viral vaccines has created general concepts of the relative safety of possible viral vaccine approaches shown in FIGURE 1 that may apply also to vectors for gene therapy. These represent but a single scenario based on a logic that might not be acceptable to everyone.

In general, *unmodified retroviruses* would not be acceptable and *virulent ordinary viruses* would not be pursued for vaccines except in very special circumstances as, *e.g.*, lack of virulence when given by an unnatural route of administration.

Replication-defective amphotropic retroviral vectors[6–8] have been a principal delivery system used in human gene therapy even though mandatory integration into the cell genome is essential for their function. Though generally considered to be of a high order of probable safety, concerns have been raised recently by experiments that showed production of replication-competent retrovirus on cultivation in cell cultures and demonstrated that amphotropic replication-compe-

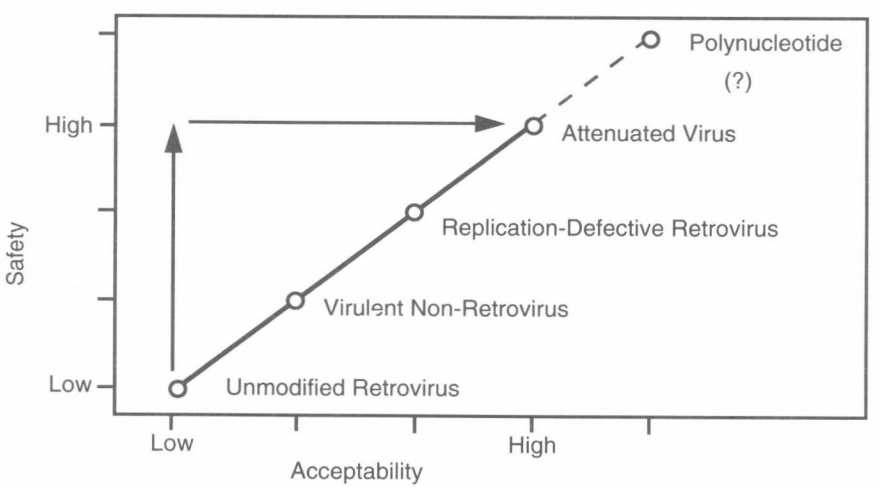


FIGURE 1. Perceptions for safety and acceptability of vaccine vectors within the realm of possibility.