The
Scientific
Future of
DNA for
Immunization
The Scientific Future of DNA for Immunization

Prepared by:

Harriet L. Robinson, Ph.D.
Harold S. Ginsberg, M.D.
Heather L. Davis, Ph.D.
Stephen A. Johnston, Ph.D.
Margaret A. Liu, M.D.

A report from
The American Academy of Microbiology
COLLOQUIUM STEERING COMMITTEE

Harold S. Ginsberg, M.D. (Co-Chair)
National Institutes of Health

Harriet L. Robinson, Ph.D. (Co-Chair)
University of Massachusetts Medical School

Heather L. Davis, Ph.D.
Loeb Medical Research Institute

Stephen A. Johnston, Ph.D.
University of Texas, Southwestern Medical Center

Margaret A. Liu, M.D.
Chiron Corporation

BOARD OF GOVERNORS

American Academy of Microbiology

Rita R. Colwell, Ph.D., Sc.D. (Chair)
University of Maryland Biotechnology Institute

Joseph M. Campos, Ph.D.
Children’s National Medical Center

R. John Collier, Ph.D.
Harvard Medical School

Julian E. Davies, Ph.D.
University of British Columbia

Harold S. Ginsberg, M.D.
National Institutes of Health

Martha M. Howe, Ph.D.
University of Tennessee, Memphis

Mary E. Lidstrom, Ph.D.
University of Washington

Eugene W. Nester, Ph.D.
University of Washington

Mary Jane Osborn, Ph.D.
University of Connecticut Health Center

Moselio Schaechter, Ph.D.
San Diego State University

Melvin I. Simon, Ph.D.
California Institute of Technology
COLLOQUIUM PARTICIPANTS

Abul K. Abbas, MBBS
Harvard Medical School
Boston, Massachusetts

M. Teresa Aguado, Ph.D.
World Health Organization
Geneva, Switzerland

Heather L. Davis, Ph.D.
Loeb Medical Research Institute
Ottawa, Canada

Hildegund C.J. Ertl, M.D.
Wistar Institute, University of Pennsylvania
Philadelphia, Pennsylvania

Harold S. Ginsberg, M.D.
National Institutes of Health
Rockville, Maryland

Joel R. Haynes, Ph.D.
Agracetus Inc.
Middleton, Wisconsin

Maurice R. Hilleman, Ph.D.
Merck Institute for Therapeutic Research
West Point, Pennsylvania

Vanessa Hirsch, D.Sc.
National Institutes of Health
Rockville, Maryland

Stephen L. Hoffman, M.D., D.T.M.H.
Naval Medical Research Institute
Rockville, Maryland

Stephen A. Johnston, Ph.D.
University of Texas, Southwestern Medical Center
Dallas, Texas

Dennis Klinman, M.D., Ph.D.
Food and Drug Administration
Rockville, Maryland

Myron M. Levine, M.D., D.T.M.H.
University of Maryland School of Medicine
Baltimore, Maryland

Margaret A. Liu, M.D.
Chiron Corporation
Emeryville, California

Pierre Meulien, Ph.D.
Pasteur Mérieux Serums and Vaccines
March L’Etoile, France

Bernard Moss, M.D., Ph.D.
National Institutes of Health
Bethesda, Maryland

Carol A. Nacy, Ph.D.
Rockville, Maryland

Terence A. Partridge, Ph.D.
Royal Postgraduate Medical School
London, England

David S. Pisetsky, M.D., Ph.D.
Duke University Medical Center
Durham, North Carolina

Ian Ramshaw, Ph.D.
Australian National University
Canberra, Australia

Harriet L. Robinson, Ph.D.
University of Massachusetts Medical School
Worcester, Massachusetts

Michael Sheppard, Ph.D.
Pfizer Central Research
Lincoln, Nebraska

Frederick R. Vogel, Ph.D.
National Institutes of Health
Bethesda, Maryland

Britta Wahren, Ph.D.
National Bacteriology Laboratory
Stockholm, Sweden

Robert G. Webster, Ph.D.
St. Jude’s Children’s Hospital
Memphis, Tennessee

David B. Weiner, Ph.D.
University of Pennsylvania
Philadelphia, Pennsylvania
A novel approach to the development of needed vaccines uses DNA for immunization. DNA represents the genetic blueprint for life. When DNA is used for immunization, the DNA in plasmid form provides the code for the vaccinating protein. The actual production of the immunizing protein takes place in the DNA-inoculated host, initiating both humoral and cellular immunity. DNA vaccines are administered in saline using hypodermic needles or by propelling DNA-coated gold beads into skin using gene guns. Recent results obtained in animal models indicate that this new technology may revolutionize the vaccination of humans. Protective immunity has been achieved for such major killers as diarrhea-causing viruses, tuberculosis-inducing bacteria, and malaria-inducing parasites. These new DNA vaccines also hold promise for being safer, less expensive, and easier to produce and administer than conventional vaccines. This report is based on a colloquium of experts convened to consider this new and extremely promising technology.
INTRODUCTION

Historical Importance of Vaccination and the Need for New, Improved Vaccines

The widespread use of vaccines has resulted in the global eradication of smallpox, the near elimination of poliomyelitis and measles from the United States, and dramatic reductions in cases of diphtheria, tetanus, whooping cough, mumps, and rubella (German measles) (see Table 1). No other medical procedure has had such profound and long-lasting effects on world health. No other medical procedure has resulted in the actual eradication of disease or rivaled the cost effectiveness of vaccines. Each development of a vaccine has proved a triumph of humankind against disease. Nonetheless, infectious agents remain major killers and debilitators (see Figure 1), despite worldwide improvements in sanitation and vaccination. Emerging infections, the reemergence of historical scourges, and microorganisms as yet uncontrolled continue to pose major risks to world health.

Emerging diseases. Increased population density, the soaring frequency of travel, and the juxtaposition of species in changing natural environments promote the emergence and dissemination of infectious agents (see Figure 2). HIV-1, the virus that induces AIDS, unrecognized in human populations before 1984, has become the eighth leading cause of death in the United States and now infects an estimated 24,000,000 adults worldwide. Horrifying viruses that cause hemorrhagic fevers, such as the Ebola virus of central Africa and the Hanta viruses of mice, have scrambled for brief, but unsuccessful, toeholds in human populations. Although multi-drug therapies are providing new hope for stemming the inexorable progression of HIV-1 infected humans to AIDS, relatively few nations can provide these expensive and regimented treatments. True control of AIDS awaits an effective vaccine. The ultimate promise for containment of such emergent agents as Ebola and Hanta also lies in vaccine development.

Reemerging diseases. Microorganisms not eliminated by drugs or vaccines pose continuing threats of reemergent pandemics. Dreaded, drug resistant forms of tuberculosis have emerged not only in humans but in cattle and deer. Tuberculosis is responsible for more deaths in adults than any other single infectious agent. The combination of increasing numbers of immunocompromised individuals with HIV infections and the emergence and spread of multi-drug resistant tubercule bacilli has led to a dramatic worsening of the impact of this disease. Malaria is a major killer of both

Table 1. Comparison of Maximum and Current Morbidity for Vaccine-Preventable Diseases

<table>
<thead>
<tr>
<th>Disease</th>
<th>Maximum Cases</th>
<th>Year</th>
<th>1992</th>
<th>Percentage Change</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diphtheria</td>
<td>206,939</td>
<td>1921</td>
<td>4</td>
<td>-99.99</td>
</tr>
<tr>
<td>Measles</td>
<td>894,134</td>
<td>1941</td>
<td>2,237</td>
<td>-99.75</td>
</tr>
<tr>
<td>Mumps(^2)</td>
<td>152,209</td>
<td>1968</td>
<td>2,572</td>
<td>-98.31</td>
</tr>
<tr>
<td>Pertussis</td>
<td>265,269</td>
<td>1934</td>
<td>4,083</td>
<td>-98.46</td>
</tr>
<tr>
<td>Polio (paralytic)</td>
<td>21,269</td>
<td>1952</td>
<td>4(^3)</td>
<td>-99.98</td>
</tr>
<tr>
<td>Rubella(^4)</td>
<td>57,686</td>
<td>1969</td>
<td>160</td>
<td>-99.72</td>
</tr>
<tr>
<td>CRS(^5)</td>
<td>20,000</td>
<td>1964-65</td>
<td>11</td>
<td>-97.12</td>
</tr>
<tr>
<td>Tetanus(^6)</td>
<td>1,560</td>
<td>1923</td>
<td>45</td>
<td>-97.12</td>
</tr>
<tr>
<td><em>Haemophilus</em>(^) influenzae* type b</td>
<td>20,000(^7)</td>
<td>1984</td>
<td>1,412</td>
<td>-92.94</td>
</tr>
<tr>
<td>Hepatitis B</td>
<td>26,611</td>
<td>1985</td>
<td>16,126</td>
<td>-39.40</td>
</tr>
</tbody>
</table>

\(^1\)Adapted from (11).
\(^2\)First reportable in 1968.
\(^3\)Subject to change due to retrospective case evaluation of late reporting.
\(^4\)First reportable in 1966.
\(^5\)Congenital rubella syndrome estimated for the 1964–65 pandemic.
\(^6\)Cases first reportable in 1947. Maximum based on number of deaths.
Figure 1. Major Causes of Death in the World

A. All ages, 1995 estimates

- hook worm
- leishmaniasis
- pertussis (whooping cough)
- tetanus (neonatal)
- AIDS
- measles
- hepatitis B virus
- malaria
- tuberculosis
- diarrhea
- acute respiratory infections

B. Children under 5 years of age, 1992 estimates

- dengue
- meningitis
- tuberculosis (children)
- acute respiratory infections (viral)
- pertussis
- neonatal tetanus
- viral diarrhea
- malaria
- measles
- bacterial diarrhea
- acute respiratory infections (bacteria)

Diseases in order of increasing importance

Millions of deaths per year

 Millions of deaths per year

Figure 2. New Infectious Diseases in Humans and Animals Since 1976.

Countries where cases first appeared or were identified

*Animal cases only.*

children and adults. Emergence of resistance of the parasites to antimalarial drugs and of the mosquito vectors to insecticides, the deterioration of the health care systems of countries in turmoil, and the refugee crisis resulting in the movement of non-immune individuals to malarious areas have resulted in malaria reemerging where it was once under control and worsening in areas where it has always been a serious problem. Viruses, such as the influenza viruses, can emerge from animal reservoirs.8,9 Pandemic influenza viruses emerge after reassortment between human and avian influenza viruses in pigs and are transmitted via the pig to humans.

The spread of antibiotic resistance in bacteria represents an ecological disaster (see Table 2). Penicillin resistant pneumococci jeopardize the treatment of serious childhood diseases. Multi-drug resistant tuberculosis threatens to become the norm, and enterobacteria that cause critical diarrheal diseases often prove resistant to antibiotic treatment.10 These increases in resistance stem from excessive use of antibiotics in patient populations, as well as the use of agents with clinical applications in agriculture and aquaculture. In Denmark in 1993, human use of a new glycopeptide antibiotic (vancomycin) totaled 22kg, while animals consumed 19,000kg of a closely related compound (avoparcin).10

**Endemic infections.** Most microbial agents represent pathogens for which no effective vaccines exist (see Table 3). The annual financial cost of common infectious diseases in the United States, according to the National Science and Technology Council, totals $120 billion per year.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Diseases</th>
<th>Resistance To</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Enterobacteriaceae</strong></td>
<td>Bacteremia, pneumonia, urinary tract, surgical wound infections</td>
<td>Aminoglycosides, beta-lactams, trimethoprim, vancomycin, chloramphenicol</td>
</tr>
<tr>
<td><strong>Haemophilis influenzae</strong></td>
<td>Pneumonia, sinusitis, epiglottis, meningitis, ear infections</td>
<td>Beta-lactame, tetracycline, chloramphenicol, ampicillin, trimethoprim + sulphonamide</td>
</tr>
<tr>
<td><strong>Mycobacterium spp.</strong></td>
<td>Tuberculosis</td>
<td>Aminoglycosides, isoniazid, ethambutol, pyrazinamide, rifampin</td>
</tr>
<tr>
<td><strong>Neisseria gonorrhoeae</strong></td>
<td>Gonorhoea</td>
<td>Beta-lactams, penicillins, spectinomycin, tetracycline</td>
</tr>
<tr>
<td><strong>Shigella dysenteriae</strong></td>
<td>Severe diarrhoea</td>
<td>Ampicillin, chloramphenicol, trimethoprim + sulphonamide, tetracycline</td>
</tr>
<tr>
<td><strong>Pseudomonas aeruginosa</strong></td>
<td>Bacteremia, pneumonia, urinary tract infections</td>
<td>Aminoglycosides, beta-lactams, tetracycline, chloramphenicol, ciprofloxacin, sulphonamides</td>
</tr>
<tr>
<td><strong>Staphylococcus pneumoniae</strong></td>
<td>Bacteremia, pneumonia, surgical wound infections</td>
<td>Chloramphenicol, rifampin, ciprofloxacin, clindamycin, erythromycin, beta-lactams, tetracycline, trimethoprim</td>
</tr>
<tr>
<td><strong>Streptococcus pneumoniae</strong></td>
<td>Meningitis, pneumonia</td>
<td>Chloramphenicol, penicillins, erythromycin</td>
</tr>
<tr>
<td><strong>Bacteroides spp.</strong></td>
<td>Anaerobic infections, septicaemia</td>
<td>Penicillins, clindamycin</td>
</tr>
<tr>
<td><strong>Enterococcus spp.</strong></td>
<td>Catheter infections, blood poisoning</td>
<td>Penicillins, aminoglycosides, vancomycin, erythromycin, tetracycline</td>
</tr>
</tbody>
</table>

Vaccines and Vaccination

Nature of a vaccine. Vaccines mobilize the immune system (white blood cells and lymphoid tissues) to combat and control infectious diseases. Put simply, a vaccine is a nondisease causing mimic of an infectious agent. Vaccination is the administration of a vaccine and the elicitation by the vaccine of an immune response capable of controlling a specific infectious agent. Successful vaccination protects both individuals and populations. Individuals are protected against the development of disease; populations are protected against the spread of the disease-causing agent.

Antigens and the expansion of the immune response. Immunization with a vaccine primes the immune system to recognize and contain an invading infection. White blood cells (lymphocytes) that reside in tissues, or patrol the body in the blood and lymph, recognize and respond to infectious agents as foreign. These blood cells recognize specific structural shapes (epitopes)
within molecules (antigens) of the infectious agent. Each microbe has unique structures, and thus unique epitopes. The priming of an immune response expands and activates “naive” white cells (those that have not previously seen an immunogen) to become “effector” cells that actively combat the infection. Each naive cell has the potential for seeing one—and only one—epitope, a recognition system often likened to a key fitting into a lock. Only those cells that recognize their cognate epitope become effector cells.

Antibody-producing B cells. Recognition of specific immunological epitopes can expand antibody producing white blood cells, called B-cells. Antibodies are proteins that bind to foreign antigens. Each B-cell makes one and only one antibody. This antibody has the potential to bind to one conformational or linear epitope within an antigen. Each antibody binds to its specific epitope. Lymphocytes that produce antibody are called B-cells because scientists first recognized them in the bursa of Fabricius, a lymphoid organ of chickens.

T-helper cells and cytolytic T-cells. Recognition of specific antigens also expands lymphocytes called T-cells (or thymus dependent cells). These T-cells can be “helper” or “cytolytic” cells. The helper cells secrete growth factors for lymphoid cells (called lymphokines) that stimulate the activation and function of B- and T-cells. The cytolytic T-cells recognize and either directly, or indirectly, kill cells infected by a microorganism. Like B-cells, each T-cell has receptors specific for one and only one epitope. However, unlike B-cells, whose immunoglobulin receptors recognize determinants in native forms of protein, T-cell receptors recognize fragments of proteins that are displayed on the cell surface by major histocompatibility complexes (MHC).

Major histocompatibility (MHC) antigens. A central problem for the immune system is its deployment against invading organisms and not against one’s self. It accomplishes this by “restricting” its destructive activities to foreign antigens that the host cell MHC proteins present to T-cells. The co-discoverers of this phenomenon, Peter Doherty and Rolf Zinkernagel, received the 1996 Nobel Prize in Physiology and Medicine.

There are two different types of MHC proteins, Class I and Class II. Both of these present proteolytically degraded fragments of proteins to T-cells (see Figure 4). Class I molecules present fragments of proteins to cytolytic T-cells. Class II molecules present protein fragments to T-helper cells. In most instances, Class I presents foreign proteins synthesized in a cell. For presentation by Class II, the foreign protein either can be synthesized in the cell or taken up by the cell from the outside. If an antigen is synthesized in a cell and presented by both Class I and Class II molecules, both antibody-producing B-cells and cytolytic T-cells are raised. However, if an antigen originated outside of a cell and is expressed only by Class II, the specific immune response is largely limited to T-helper cells and antibody production.

Memory responses. As an infection is contained and the inducing antigen eliminated, the immune system creates “memory” B- and T-cells. Memory cells represent inactive yet rapid-response patrols on guard for the reappearance of their specific antigen. The power of vaccination lies in the memory response. Under the best
MHC I, Synthesis of immunogen in cell

- Synthesis of vaccinating immunogen in cell
- Proteasome digestion of the immunogen and loading of peptide fragments onto MHC I molecules in the endoplasmic reticulum
- Transport of peptide-MHC I complexes to cell surface
- Presentation of peptide-MHC I complexes to CTL

MHC II, Synthesis of immunogen in cells or internalization of immunogen from outside of cell

- Synthesis of vaccinating immunogen in cell
- Internalization of vaccinating immunogen into an endosome
- MHC II blocked by invariant chain
- Digestion of the immunogen and the invariant chain by lysosomal proteases
- Loading of peptide fragments of the immunogen onto MHC II and transport to cell surface
- Presentation of peptide-MHC II complexes to T-helper cells
case scenario, vaccines require only one administration to provide life-long protection. And, if given worldwide, vaccinations can achieve eradication, eliminating both the disease and the need for further vaccination.

The Development of Human Vaccines

The roots of vaccination trace back to early Western and Eastern civilizations. Roman scholars suggested that livers of mad dogs could protect against rabies, and 16th century Taoist healers used crusts from smallpox to protect against smallpox disease. An 18th century British physician, Edward Jenner, made the first scientific demonstration of vaccination. In 1796, he used cowpox (taken from the lesion on a milk maid’s hand) to inoculate an 8-year-old boy. The boy developed a localized infection that rapidly healed. Approximately two months later, Jenner inoculated (challenged) the boy with smallpox virus. The boy was completely resistant to the disease. The cowpox virus had sufficient antigens in common with the deadly smallpox to provide protective memory. After the British Royal Society rejected Jenner’s report “lest he damage his reputation,” Jenner privately published a paper. The news that one could prevent the then leading cause of adult death by inoculation with the cowpox led to the rapid acceptance of this revolutionary medical technique.

Despite this initial success, the development of further vaccines awaited another century, gaining momentum only as fundamental work by Louis Pasteur and Robert Koch demonstrated that microorganisms (not poisons) cause infectious diseases and that specific microorganisms cause specific diseases. The realization that specific microorganisms caused specific diseases provided the rationale for using a non-pathogenic form of a microbe for vaccination. Currently human vaccines utilize four different forms of non-pathogenic agents: live attenuated microorganisms, killed whole microorganisms, purified components from microorganisms (subunits), and genetically engineered components of microorganisms (see Table 4).

**Live attenuated vaccines.** Jenner’s cowpox vaccine represented the first use of a live, attenuated organism for vaccination. In Jenner’s case, attenuation resulted from using a non-human virus in a human. The cowpox virus (which may have come from a horse) grows sufficiently in the human to induce immune responses. However, it does not grow sufficiently to cause disfigurement beyond the site of inoculation; only in rare instances did it cause death.

The use of live attenuated organisms has since proved effective in preventing diseases such as rabies, yellow fever, poliomyelitis, measles, mumps, rubella, and most recently chicken pox (see Table 4, column 1). Since World War II, viruses for vaccines have been attenuated by serial passage of the human virus in cell culture of nonhuman origin—for example, chicken embryo or monkey kidney cells. During a successful attenuation (and not all efforts at attenuations are successful), the virus loses virulence while retaining the antigens that stimulate protective immunity. The current “Jeryl Lynn” mumps vaccine represents one of several attempts at attenuation, with success occurring only after a 16-passage attenuation of a fresh isolate cultured from a case of clinical mumps in the 6-year-old daughter of the developer.

**Killed whole vaccines.** A second type of vaccine, killed whole organisms, emerged in the late 19th century. At first, developers used heat to inactivate (kill) the *Salmonella typhi* and *Vibrio cholera* bacteria that cause typhoid fever and cholera respectively (see Table 4). Initially tested in British troops in India and the Boer War, these heat-inactivated vaccines became mandatory for virtually all soldiers by the first World War. Since World War II, chemicals have replaced heat to inactivate viruses used in killed whole vaccines. The poliovirus vaccine that Jonas Salk developed offers one example of a chemically (formaldehyde) inactivated vaccine.

**Purified component vaccines.** A third type of vaccine comprising purified components from pathogenic organisms was developed in the early 20th century to prevent tetanus and diphtheria.
Table 4. The Development of Human Vaccines

<table>
<thead>
<tr>
<th>Time Period</th>
<th>Vaccines</th>
</tr>
</thead>
<tbody>
<tr>
<td>18th Century</td>
<td>Smallpox 1798</td>
</tr>
<tr>
<td>19th Century</td>
<td>Rabies 1885</td>
</tr>
<tr>
<td></td>
<td>Typhoid 1896</td>
</tr>
<tr>
<td></td>
<td>Cholera 1896</td>
</tr>
<tr>
<td></td>
<td>Plague 1897</td>
</tr>
<tr>
<td>Early 20th Century</td>
<td>Bacille Calmette-Guerin 1927 (tuberculosis)</td>
</tr>
<tr>
<td></td>
<td>Yellow Fever 1935</td>
</tr>
<tr>
<td></td>
<td>Pertussis 1926 (whole cell)</td>
</tr>
<tr>
<td></td>
<td>Influenza 1936</td>
</tr>
<tr>
<td></td>
<td>Rickettsia 1938</td>
</tr>
<tr>
<td></td>
<td>Diptheria 1923</td>
</tr>
<tr>
<td></td>
<td>Tetanus 1927</td>
</tr>
<tr>
<td>Post-World War II</td>
<td>Polio (oral)</td>
</tr>
<tr>
<td>(cell culture)</td>
<td>Measles</td>
</tr>
<tr>
<td></td>
<td>Mumps</td>
</tr>
<tr>
<td></td>
<td>Rubella</td>
</tr>
<tr>
<td></td>
<td>Adenovirus (Type 4)</td>
</tr>
<tr>
<td></td>
<td>Typhoid (salmonella Ty21a)</td>
</tr>
<tr>
<td></td>
<td>Varicella</td>
</tr>
<tr>
<td></td>
<td>Polio (injected)</td>
</tr>
<tr>
<td></td>
<td>Rabies (new)</td>
</tr>
<tr>
<td></td>
<td>Japanese B encephalitis</td>
</tr>
<tr>
<td></td>
<td>Hepatitis A</td>
</tr>
<tr>
<td></td>
<td>Tickborne encephalitis</td>
</tr>
<tr>
<td></td>
<td>Pneumococcus</td>
</tr>
<tr>
<td></td>
<td>Meningococcus</td>
</tr>
<tr>
<td></td>
<td>Haemophilus influenzae (PRP)*</td>
</tr>
<tr>
<td></td>
<td>Hepatitis B (plasma-derived)</td>
</tr>
<tr>
<td></td>
<td>H. influenzae PRP*-protein (conjugate)</td>
</tr>
<tr>
<td></td>
<td>Typhoid (V)</td>
</tr>
<tr>
<td></td>
<td>Acellular pertussis</td>
</tr>
</tbody>
</table>

*PRP: capsular polysaccharide (polyribosylribitol phosphate)

(Table 4, column 3). The bacteria that cause tetanus and diphtheria secrete disease-causing toxins. Inactivated forms of these toxins, called toxoids, can raise “neutralizing” (inactivating) antibodies against the toxin. With improvements in fermentation (growth of bacteria) and in the purification of macromolecules, inactivated toxins could be produced in bacterial cultures and used as vaccines. Following the introduction of the diphtheria toxoid vaccine, cases of diphtheria declined from more than 200,000 per year in the United States in 1921 to 2 per year in 1984. The recent reappearance of diphtheria in the former Soviet Union, where social and economic upheavals led to a lapse in adequate immunization, emphasizes the continuing importance of this vaccination.

Since World War II, purified polysaccharides from bacterial pathogens have served as highly effective vaccines. A combination of polysaccharides from 23 strains of pneumonia-inducing pneumococcal bacteria vaccinates against invasive pneumococcal infections. A vaccine composed of capsular polysaccharides from six strains of the bacterium Hemophilus influenzae b coupled to carrier proteins (to increase immunogenicity) prevents hemophilus b-induced pneumonia and meningitis. The carrier-coupled vaccine is effective in infants as well as adults. Prior to the introduction of the Hemophilus influenzae b vaccine, meningitis caused by this bacterium ranked as the leading cause of acquired learning disabilities in the United States.

**Genetically engineered vaccines.** Genetically engineered vaccines, which use proteins produced by recombinant DNA technology, represent a late 20th century approach to vaccine development. Recombinant DNA technology
allowed the production of proteins for agents that would not grow in culture. The recombinant hepatitis B virus vaccine, whose immunizing protein is produced by DNA introduced into yeast or mammalian cell cultures,\(^2\),\(^17\) represents an example of this type of vaccine. Prior to the genetically engineered production of the hepatitis B virus surface antigen, this protein had been purified from the blood of infected carriers.

The advent of recombinant DNA technology also enabled the development of live recombinant and live attenuated vaccines. In live recombinant vaccines, one or more genes encoding critical determinants for immunity are introduced into a benign, yet live vector. Live recombinant vaccines now use a number of different viruses or bacteria as vectors.\(^18\) These recombinant virus vectors possess many advantages of live attenuated vaccines, while expressing only selected genes of a pathogen. Recombinant DNA technology also allows construction of live-attenuated vaccines by directly mutating virulence genes within the genome of a microorganism. No live genetically engineered vaccine has yet won approval for human use. Some, however, are in human testing and several are licensed for veterinary uses.

A New Technology, DNA Vaccines

Recently, a new approach to vaccination opened up with the demonstration that direct inoculation with DNA that encodes a foreign antigen can initiate protective immune responses. DNA represents the genetic information of all cellular life. It provides the code (blueprint) for the synthesis of the macromolecules that make up living organisms. DNA vaccines containing the genes of foreign proteins provide cells in the vaccinated host with the code for the synthesis of the immunizing antigens (see Figure 5).

Construction of a DNA vaccine. Vaccine DNAs consist of bacterial plasmids (small circular DNAs that can replicate in bacteria). Each vaccine consists of a plasmid bearing an insert (see Figure 5). The insert comprises sequences for the vaccinating protein as well as control elements (termed promoter and poly A in Figure 5) that allow expression of the vaccinating protein in eukaryotic cells. The construction of bacterial plasmids with vaccine inserts is accomplished using recombinant DNA technology. Once constructed, the vaccine plasmid is introduced (transformed) into bacteria. There the growth of the bacteria produces many plasmid copies. The plasmid DNA is purified from the bacteria, using relatively simple techniques for separating small circular plasmid DNAs from the much larger bacterial DNA and other bacterial impurities. The purified DNA (a stable molecule) is the vaccine.

Administration of DNA vaccines. DNA vaccines can be injected in saline solutions into muscle or skin using a syringe and needle.\(^19\),\(^20\) DNA vaccines can also be given by coating the DNA onto microscopic gold beads and then using a “gene gun” to fire the beads into cells.\(^21\) The saline injections deliver DNA into extracellular spaces, whereas gene guns bombard DNA-
Table 5. Immunogenicity and Efficacy of DNA Vaccines Successfully Used in Animal Models

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Protein(s)</th>
<th>Antibodies</th>
<th>CTL</th>
<th>Protection</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>VIRUSES</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>cytomegalovirus</td>
<td>pp89</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>22</td>
</tr>
<tr>
<td>encephalitis virus</td>
<td>prM/E</td>
<td>+</td>
<td>ND</td>
<td>+</td>
<td>23</td>
</tr>
<tr>
<td>hepatitis B virus</td>
<td>HBsAg, HBcAg</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>24–27</td>
</tr>
<tr>
<td>herpes virus, bovine</td>
<td>gD</td>
<td>+</td>
<td>ND</td>
<td>+</td>
<td>28</td>
</tr>
<tr>
<td>herpes simplex virus 1,2</td>
<td>gB, gD</td>
<td>+</td>
<td>equivocal</td>
<td>+</td>
<td>24–33</td>
</tr>
<tr>
<td>simian immunodeficiency viruses and simian/ human immunodeficiency virus chimeras</td>
<td>Gag, Pol, Env, Vif, Vpr, Vpu, Rev, Tat, Nef</td>
<td>+</td>
<td>+</td>
<td>+/-</td>
<td>34–37 (for review see 38)</td>
</tr>
<tr>
<td>influenza</td>
<td>HA, NP</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>39–43</td>
</tr>
<tr>
<td>lymphocytic choriomeningitis virus</td>
<td>NP</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>44–46</td>
</tr>
<tr>
<td>measles</td>
<td>HA, F, NP</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>47,48</td>
</tr>
<tr>
<td>papilloma</td>
<td>L1</td>
<td>+</td>
<td>ND</td>
<td>+</td>
<td>49</td>
</tr>
<tr>
<td>rabies</td>
<td>G</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>50</td>
</tr>
<tr>
<td>rotavirus</td>
<td>VP6</td>
<td>+</td>
<td>ND</td>
<td>+</td>
<td>51,52</td>
</tr>
<tr>
<td><strong>BACTERIA</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mycoplasma pulmonis</td>
<td>library</td>
<td>+</td>
<td>ND</td>
<td>+</td>
<td>53</td>
</tr>
<tr>
<td>mycobacterium tuberculosis</td>
<td>Ag85, hsp65</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>54,55</td>
</tr>
<tr>
<td><strong>PARASITES</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>malaria</td>
<td>CSP, HEP17</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>56,57</td>
</tr>
<tr>
<td>ND: not done</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

ND: not done

coated gold beads directly into cells. The immune responses raised by injection and bombardment require different amounts of DNA and can raise different types of T-cell help (see below).

**Animal trials with DNA vaccines.** Animal trials of DNA vaccines have revealed the ability of this new technique to raise protective immunity against a number of agents for which we need new or improved vaccines (see Table 5). Preclinical studies also have revealed that fairly similar doses of DNA are effective in essentially all animal species (mice, chickens, cows, and monkeys). Thus, this novel method of immunization offers high promise for the realization of much needed vaccines.

Choosing the name “DNA vaccines.” The World Health Organization, among the first to realize that something new was happening in vaccinology, convened a meeting in May of 1994 to hear its pioneers present their results. On the second day of the meeting, a vote was taken on a name for the new technique from a list of candidates: genetic immunization, polynucleotide vaccines, gene vaccines, and nucleic acid vaccines. Voters split; however, the majority chose nucleic acid vaccines, with subterms DNA vaccines or RNA vaccines. The rationale for choosing the name nucleic acid vaccines focused on public perception. To gain wide acceptance, the new technology’s name needed to convey its purpose as a protective vaccine without suggesting that it modified the genetic information of the recipient.
Advantages of DNA Vaccines Over More Classical Vaccines

Introduction. DNA vaccines have a number of advantages over more classical vaccines (see Figure 6). In regard to viruses, DNA vaccines mimic live attenuated vaccines and live recombinant vectors by producing the immunizing material in the host. However, DNA vaccines are unlike live viral vaccines in that they do not cause infections. DNA vaccines are subunit vaccines in that they express only one, or a subset of proteins, from a pathogen. Classical subunit vaccines are produced in fermenters or cell cultures and the desired antigen(s) purified. Then the purified components are inoculated into the vaccinee. Recombinant virus vectors revolutionized this process by producing the subunit vaccine in the host. DNA vaccines take this one step further by eliminating the need for a virus vector. The production of subunit vaccines in hosts by DNA has a number of important consequences for the success of vaccination. It also greatly simplifies vaccine development and production.

Antibody against the natural forms of viral proteins. The ability of DNA to produce the immunizing protein(s) in host cells yields a vaccinating protein in its “native” form. Many viral proteins have folded structures that the purification process can easily disrupt. Most antibodies recognize folded structures. If the preparation of a subunit vaccine has resulted in a misshapened protein, the antibody response will recognize the disrupted structure, rather than the normal protein present on the microorganism. Antibodies that fail to recognize the native form of a protein frequently prove ineffective at containing an invading microorganism.

Induction of cytolytic T-cells. A distinguishing feature of DNA vaccines, as opposed to more classical subunit vaccines, is the production of the immunogen in host cells. This supports processing and presentation by both Class I and Class II MHC molecules (see Figure 4). By contrast, killed-whole or protein subunit vaccines undergo presentation by Class II MHC molecules. These differences in presentation result in DNA vaccines raising both cytolytic T-cells and antibody, whereas more classical subunit vaccines raise antibody mostly. The importance of raising cytolytic T-cells lies in their ability to directly kill pathogen-containing cells. The use of DNA, a nonliving agent, to raise cytolytic T-cells represents a milestone in vaccinology.

Long-lived responses. As early vaccine development progressed from live-attenuated to killed-whole organism vaccines, workers rapidly came to appreciate that raising and maintaining immunity requires multiple inoculations of inactivated vaccines. This stands in contrast to live-attenuated vaccines, where a single inoculation can result in long-term protection. In the
In the United States, the DPT vaccine (inactivated diphtheria, pertussis, and tetanus) is administered five times between birth and 7 years of age. In preclinical studies, DNA vaccines appear similar to live vaccines, in that they have the potential to raise long-lived immune responses. For researchers who pioneered DNA vaccines, the two most exciting results are the achievement of protection and the realization that the protection could last a long time.

**Facilitation of combination vaccines.** In the United States, the full course of childhood immunizations currently requires 18 visits to a physician or clinic. Giving several vaccines at once could reduce this number of visits, but differences in the formulations of the different vaccines limits their use in combination. DNA vaccines, by having the same formulation, might eliminate this problem.

**Generic production and validation.** All DNA vaccines can be produced using similar fermentation, purification, and validation techniques. This ability to use generic production and verification techniques vastly simplifies vaccine development and production.

**Stability.** In contrast to many conventional vaccines, such as live viruses and protein subunits, DNA vaccines remain stable at both high (below boiling) and low temperatures. DNA vaccines can be stored either dry or in an aqueous solution. The good stability of DNA vaccines should facilitate distribution and administration and eliminate the need for “the cold chain”—the series of refrigerators required to maintain the viability of a vaccine during its distribution. Currently, maintaining the cold chain represents 80% of the cost of vaccinating individuals in developing nations.

**Recovery of candidate vaccines from diseased tissue.** Of the complex molecules that make up living organisms, DNA is the easiest with which to work. A technique called polymerase chain reaction (PCR) supports the recovery and amplification of specific DNA sequences—even those present at very low levels—from biological materials. The ability to recover microbial DNA sequences using PCR revolutionized vaccine development. For known organisms for which we can design PCR reagents, candidate vaccines can be directly recovered from infected tissue, eliminating the weeks to months required to culture a microbe from a diseased tissue. The ability to make a vaccine directly from the tissue of an infected animal also allows construction of vaccines for microorganisms that fail to grow in cultures. Examples of such pathogens include such major killers as the hepatitis B and C viruses, diarrhea-causing viruses, and papilloma viruses.

**Library screening.** The ability to use DNA as a vaccine allows researchers to screen genes from an organism for their ability to raise protective responses. Even academic laboratories can construct and test multiple candidate vaccines, a feat once undreamt of by even the largest industrial giants using conventional vaccine development approaches. In cases where the immunologically relevant genes remain unknown, DNA “libraries,” or collections of DNA sequences, can be used to screen the entire genome of a microorganism for sequences that raise protective immune responses. This technique is called “expression library immunization.” As protective libraries are identified, these are subdivided and further tested to identify which gene or genes within the library provide protection.

**Manipulation of the vaccine response.** DNA vaccines can be used to bias an immune response towards one of two different types of T-cell help (see Figure 7). These two types of T-cells, called T-helper 1 and T-helper 2, affect the types of antibody raised and the nature of the inflammatory cells mobilized to fight an infection. The type of T-help and its associated inflammatory responses are important in determining how effectively the body clears an infection. The ability of DNA immunizations to bias the type of T-cell help may also support the use of this new technology for the control of autoimmune disease (an inappropriate T-helper 1 response) and allergy (an inappropriate T-helper 2 response).
**Limitations of DNA vaccines over more classical methods of vaccination**

**Limited to protein antigens.** DNA can be used to raise immune responses against the protein components of pathogens. Proteins are the major building blocks of all life. However, certain microbes have outer capsular structures that are made of polymerized sugars (polysaccharides). This limits the extent of DNA vaccines’ usage because they cannot substitute for polysaccharide-based subunit vaccines (e.g., pneumococcus).

**Mucosal delivery.** DNA vaccines have yet to be developed for intranasal and oral delivery. Most infectious agents enter humans through the respiratory, intestinal, or genital tract. Vaccination for microorganisms that cross mucosal surfaces is most effective when induced at the entry surface because memory cells patrol the surfaces where they first encountered an antigen. For example, the Sabin oral poliovirus vaccine initiates vaccination in the gut, the site of poliovirus infection. DNA vaccines administered to skin or muscle against influenza virus (a respiratory tract infection) and to skin against rotavirus (an intestinal infection) successfully prime for protective mucosal responses. Whether delivery of DNA vaccines to mucosal surfaces will improve this protection remains to be determined.

**Special consideration of DNA vaccines for the control of viruses, parasites, and bacteria**

**Unique patterns of protein modifications for bacteria and parasites.** Whereas viruses use the machinery of animal cells to synthesize proteins, parasites and bacteria provide their own machinery for protein synthesis. As proteins are synthesized they undergo modifications, the most frequent of which is the addition of sugar groups (glycosylation). Animal cells, bacteria, and parasites have distinctive patterns of protein modification. A bacterial protein produced by an animal cell will not have the same modifications and structure as the protein produced by bacterial cells. Thus, a unique advantage of DNA vaccines for immunization against viruses—production of the native form of the viral protein—does not necessarily hold for vaccinations against bacterial or parasitic proteins that carry modifications distinct from those conferred by animal cells.

Despite this limitation, early work with DNA vaccines has provided impressive results against bacterial and parasitic agents. Development of vaccines for these more complex organisms has relied on both DNA-expressed proteins that represent known targets of immune responses and expression library immunizations to identify as yet unidentified protective genes.

**Importance of multi-valent DNA vaccines for parasites and bacteria.** For complex microorganisms, such as bacteria and parasites, the ability to use multiple DNAs for immunization represents a particular strength of the DNA approach. For example, parasites go through different stages of infection in humans, such as the blood, liver, and reproductive stages of a malarial infection. Each of these stages involves different antigens. The ability to use multiple DNAs for immunization allows the use of immunogens against each stage. This ensures that those parasites that make it past stage one (a transit time accomplished in minutes) again will be embattled at stages 2 and 3. The use of multiple DNAs will also be important for controlling *Mycobacterium tuberculosis*, the cause of tuberculosis. In this case, multiple vaccine targets should act much as multidrug therapy, limiting low-level survival and the outgrowth of vaccine-resistant variants.
IMPLEMENTATION OF A NEW TECHNOLOGY FOR VACCINATION

Implementing a new technology requires basic research, applied research, and product development (see Figure 8). Competitive government basic research grants were seminal to the realization and demonstration that DNA could be used to raise immune responses. Applied research is now translating this basic finding into vaccines. With the identification of candidate vaccines, product development—including scale-up for manufacture, safety testing, and marketing—ensues. Government supports most basic research through grants to academic institutions or its support of government laboratories, such as those at the National Institutes of Health. Applied research receives both government and industrial support. The final step, product development, falls largely to private industry, with government resources provided only up to that point where a fairly certain (and cost justifiable) path to an actual vaccine emerges.

Need for basic research on DNA vaccines

The use of DNA for immunizations opens a very new area of research. We currently understand very little about how immune responses are raised and what factors modulate the T-helper types of these immune responses. Fortunately, developing an effective vaccine does not require understanding how the vaccine works. However, gaining knowledge of how DNA-based immunizations function should provide springboards for more effective application of this exciting technology and also support insights into the cellular and molecular biology of immune responses. Therefore, there is a cogent practical, as well as intellectual, promise for basic research on DNA vaccines.

Inoculation with more classical forms of subunit vaccines requires microgram amounts of protein to raise immune responses. By contrast, DNA immunizations appear to need much less protein (on the order of 1,000 times less).\textsuperscript{21,60} How such low levels of DNA-expressed proteins raise such effective immune responses remains a profound and unsolved puzzle.

Target site and lymphoid tissue. Determining the relative roles of the target site (muscle or skin delivery site) and lymphoid tissues in DNA-raised responses represents a high priority for basic research.\textsuperscript{61} Is this novel immunization method obeying classical rules for provoking immune responses or are DNA-transfected muscle cells or skin cells, and not lymphoid cells, presenting antigen? If lymphoid cells present antigen, how do they obtain antigen? Does DNA directly transfect these cells or do they acquire protein from transfected skin or muscle cells?

T-helper biases. A second priority seeks a better understanding of the T-helper preference raised by DNA immunizations and what determines these different biases. In human warfare, appro-
Appropriate circumstances exist for deployment of the army or the navy; for the immune system there are appropriate circumstances for the deployment of T-helper 1, as opposed to T-helper-2 responses. The realization that the immune system has different branches to its defense mechanisms came relatively recently\textsuperscript{62,63} (see Figure 7). Each branch has T-helper cells with distinctive patterns of lymphokine production. In murine models, where most of the work has been done, these distinctive patterns of lymphokines determine the type of antibody produced and the types of inflammatory cells mobilized and activated during the memory response.

The method of DNA delivery,\textsuperscript{58,59} the form of DNA-expressed antigen,\textsuperscript{47,49,64} and in some instances, co-transfected lymphokines,\textsuperscript{65,66} can affect the type of T-help that DNA-based immunizations raise. Quite impressively (but in agreement with how memory responses work), the T-helper bias established by a DNA immunization is maintained during the amplification of its memory response.\textsuperscript{39} Thus, one can use DNA inoculation, or a DNA-expressed antigen, to bias the inflammatory response that a challenge infection will mobilize. For example, vaccines for those bacterial infections best controlled by phagocytosis can be specifically designed to establish T-helper 1 memory that mobilizes phagocytic defenses preferentially (see Figure 7). And, vaccines for infections that mast cells control best, such as worms, could preferentially be designed to elicit T-helper 2 responses.

Identification of antigens that raise protective responses. A third important area for research on DNA-based immunizations involves identifying the antigens, combinations of antigens, and forms of antigens most effective at inducing protective immunity against microbial infections. We have already discussed the ability to screen different genes from an organism rapidly and the ability to use multiple genes for immunization. In addition to these two advantages, the use of recombinant DNA for immunization allows one to test different forms of antigens or antigens constructed to favor presentation by MHCI or MHC II complexes.\textsuperscript{53}

Immunization of neonates. From the point of view of logistics and disease prevention, the day of birth offers the most favorable time for immunization. However, the presence of maternal antibody and the immaturity of the infant immune system pose major problems to vaccinating neonates. At present, relatively little is understood about either of these phenomena and how they might interact with DNA-based immunizations. Maternal antibodies neutralize the infecting virus in live attenuated vaccines and, therefore, block immunization.\textsuperscript{11} DNA would not be subject to such neutralization. The immune system undergoes rapid development immediately after birth. Will the temporal expression of DNA (generally initiated within 12 to 24 hours of inoculation) or the longevity of DNA expression (presumed to last for at least several days) support successful immunization? If responses occurred, how would these differ from those in the adult? Could infant vaccines also deliver lymphokines or co-stimulatory molecules, known to participate in the elicitation of immune responses, to provide the necessary machinery for the neonate to establish protective vaccine responses?

Vaccine vectors and delivery methods. At present, vaccine vectors are modeled on the vectors used to achieve high-level production of therapeutic proteins, such as tissue plasminogen activator. Researchers need to evaluate a host of approaches to improve vaccine vectors. These include the improvement of antigen expression and the evaluation of the effect of immunostimulatory “CpG” DNA motifs on immunizations.\textsuperscript{67,68,69} Immunostimulatory CpG motifs can stimulate the production of lymphokines—IFNγ, IFNβ, IL-12—which in turn can affect the efficiency and, potentially, the T-helper type of an immune response.\textsuperscript{67}

Many different delivery systems are being evaluated for the administration of DNA vaccines, especially to mucosal surfaces. These include the use of intracellular bacteria as carriers for vaccine DNAs,\textsuperscript{70} the use of DNA containing microspheres,\textsuperscript{71} and the use of lipid-based DNA delivery.\textsuperscript{72} More effective systems for delivering DNA to the nucleus also need evaluation.
Evaluation of therapeutic immunizations for chronic infections, autoimmunity, allergy, and cancer. Chronic infections occur when a host cannot clear an infection. DNA immunizations may play a therapeutic role in such situations by augmenting an existing immune response or broadening a response to include additional antigens. In instances where an infection fails to elicit cytotoxic T-cells, DNA vaccinations may generate such cells, which in turn may destroy infected cells.

Researchers also need to evaluate DNA immunizations for their ability to provide therapy for autoimmune and allergic diseases. Autoimmune disease occurs when the host mounts an immune response against one of its own components. For example, in multiple sclerosis, immune cells target myelin basic protein in the brain. Inflammatory responses supported by T-helper 1 cells mediate these debilitating attacks on one’s own body. DNA-based immunizations may selectively destroy the immune cells supporting the autoimmune attack. In contrast to autoimmunity, which is associated with complement-binding IgG and T-helper 1-biased responses against one’s own proteins, allergy stems from IgE and T-helper 2-based responses against foreign proteins. Most allergy-causing proteins are ones to which humans undergo regular exposure, such as house mites, cat fur, and pollen. If the antibodies against these allergens can be changed from IgE to IgG, and the response changed from T-helper 2 to T-helper 1, then allergic symptoms will be relieved. Indeed in mice, IgE-initiated allergic responses have been modulated towards IgG and T-helper 1 using intramuscular saline injections of allergen encoding DNA.74,75

Finally, DNA vaccines to control cancer merit further development. We could prevent a remarkably large number of cancers by vaccinating for the infections that cause the cancer—a process already started for hepatitis B virus (see Figure 3). For those cancers unrelated to microbial infection, DNA vaccines may raise immune responses for proteins specific to the cancer and thus kill cancer cells. Indeed, some success has been obtained using DNAs to raise antibodies specific to certain B-cell lymphomas.76 Success may also come from using DNAs to express lymphokines or co-stimulatory molecules in cancer cells to attract and activate immune system cells to kill the cancer.77

Special considerations for applied research on DNA vaccines

Safety issues. Regulatory agencies considering the application of DNA vaccines for human use have identified several key areas of concern (Figure 9). Each of these is briefly discussed below.

Integration. Integration is the insertion of a DNA sequence into the chromosomal DNA of a host organism. Each insertion represents a mutagenic event. A fraction of these mutations have the potential to cause cancer by perturbing the structure or expression of genes that control cell growth and differentiation. Integrations can take place between identical DNA sequences in a plasmid and a host cell or between differing sequences, in which case the integration event is referred to as an illegitimate recombination. Efforts to find integrations of vaccine plasmids into mouse DNA have failed to detect insertions of the injected plasmid. These studies could have detected one integration event for each 150,000 nuclei, a mutation rate estimated at 1,000 times less than the spontaneous mutation rate of DNA.

Tolerance. In some experimental systems, repeated injection of small quantities of antigen leads to the development of immunologic unresponsiveness or tolerance. Since DNA immunization produces a small amount of antigen, and the expression of antigen after DNA immunization appears to persist, the

Figure 9. Safety Issues for DNA Vaccines

The potential integration of the plasmid DNA into the genome of transfected cells.
The potential induction of immune tolerance to the vaccine antigen.
The potential induction of autoimmunity.
The potential induction of antibodies to the injected plasmid DNA.
possibility exists that unresponsiveness, rather than protective immunity, might result. This needs further investigation in both infants and adults.

**Autoimmunity.** Autoimmunity is the attack of the immune system on one's own cells. Autoimmune responses might occur as a result of the immune-mediated destruction of cells expressing a DNA vaccine, or by virtue of the DNA converting a normally nonantigen presenting cell into an antigen presenting cell. However, both the destruction of one's own cells and the expression of foreign antigens occur in the course of viral and bacterial infections. Thus, DNA vaccines may pose no greater risk of inducing autoimmunity than natural infections.

**Anti-DNA antibodies.** The induction of anti-DNA antibodies by plasmid DNA—for example, the immune system would see the vaccine DNA as foreign—poses another safety consideration. Antibodies to DNA can cause disease and are associated with systemic lupus erythematosus. To date, studies have not associated DNA vaccines with the induction of anti-DNA antibodies. This appears to reflect several factors. First, purified double stranded DNA does not readily induce anti-DNA antibodies. Second, nonpathogenic anti-DNA antibodies are found in most humans. These antibodies are specific for the DNA of particular bacterial species and do not cross-react with mammalian DNA, which suggests that these antibodies were generated during bacterial infections. Third, vaccination of lupus-prone mice with purified plasmid DNA has little effect on the levels of anti-DNA antibodies and no detrimental autoimmune effect in this animal model. Finally, vaccination of normal animals with DNA vaccines has induced few or no anti-DNA antibodies, as measured by ELISA, immunoblot, or radioimmunoassay. Therefore, while it remains unknown whether DNA vaccines will induce anti-DNA antibodies in humans, animal studies suggest this appears unlikely.80

**Pre-clinical and Clinical Trials**

**Pre-clinical evaluation.** The issues for pre-clinical efficacy of DNA vaccines are the same as those for traditional vaccines; they must demonstrate immunogenicity and protective or therapeutic efficacy in animal models. Concerns about the relevance of results in animal models to human applications exist for all vaccines. These concerns arise from potential differences in immune responses, as well as possible differences in disease development. Nevertheless, the basic issue of the relevance of pre-clinical immunogenicity and efficacy of DNA vaccines and traditional vaccines remains the same. And this necessitates the usual consideration of the type of immune response, the relevance of a disease model to clinical disease, and the significance of observations in inbred laboratory animals for the human population.

**Clinical trials.** As for any biomedical agent, the critical issue is the risk-benefit ratio for a disease. For prophylactic vaccination, this means that the safety and protective efficacy of the vaccine are very important. While safety ranks as the most important component for prophylactic DNA vaccines, the particular disease and population under consideration would affect the risk-benefit. To this end, it is important to evaluate carefully the safety of DNA vaccines in pre-clinical and then clinical studies, while at the same time considering the benefit of a protective vaccination. New technologies to evaluate safety have greatly facilitated the ability to address the safety concerns described above.

**Unique Aspects for Product Development**

**Generic production.** One advantage of DNA vaccines lies in the likelihood that their manufacture will require only generic technology, since vaccines for different diseases will utilize similar plasmid backbones, differing mainly by the vaccine gene inserted (see Figure 5). Thus, unlike other vaccine technologies that necessitate
unique growth and attenuation processes for different vaccines, or specific purification or chemical processes for inactivated or subunit vaccines, all DNA vaccines will rely largely on the same growth and purification process.

Large-scale fermentation conditions and processes already exist for various recombinant DNA technologies. Fermentation for manufacture of DNA vaccines would differ from these previous efforts. In DNA vaccines, the plasmid itself—DNA—rather than a produced protein is the desired product. Both the state of the art for large-scale bacterial fermentation and the generic nature of DNA vaccines offer significant advantages for manufacturing DNA vaccines cost effectively.

**Purification.** Purifying DNA plasmid from the bacterial cellular components and any contaminants introduced during manufacture (fermentation) and purification should be relatively simple. Acceptable limits on the presence of other components in the final product and their effect upon the potency or tolerability of DNA vaccines are only being determined now. For example, bacterial endotoxin, present in host bacteria and known to affect both immune responses and reactogenicity, requires purification to an acceptable extent. Preliminary work indicates that the technology to purify DNA vaccines could be scaled up, but the ultimate cost per dose will depend upon current unknowns. Those include human potency and the number of constructs needed for a given vaccine.

**Characterization.** One can characterize a vaccine plasmid at various levels, including the actual DNA sequence of the vaccine, the form of the DNA (linear, open-circular, or super-coiled), and the presence of other materials such as inert substances of the manufacturing process. Although various plasmid forms may be biologically active, a licensed DNA vaccine must meet specific criteria to ensure the stability and potency of its different manufactured lots.

Recently, the U.S. Food and Drug Administration’s Center for Biologics Evaluation and Research released a “Points to Consider” document. The process of licensing DNA vaccines would closely follow that for traditional vaccines in requiring a demonstration of the safety and efficacy of the vaccine commensurate with the risk-benefit ratios of the targeted disease.

**Potential for Utilization in Developing Countries**

The crucial criteria for utilizing DNA vaccines in developing countries are the same as those for developed countries: safety and efficacy for relevant diseases. The feasibility of DNA vaccines for developing countries will depend upon the success of large-scale manufacturing processes to produce vaccines that remain stable under local conditions, presumably without a cold chain. Currently, it appears that DNA vaccines will prove both practical and affordable for developing nations.
SOCIETAL ISSUES

Responsibilities of Organizations and Societies

Scientific community. The primary responsibility of the scientific community in the development of DNA vaccines is to conduct the necessary research as quickly and efficiently as possible. This will require that individual scientists disseminate their findings through scientific presentations and publications to minimize the duplication of efforts and provide cross-fertilization of ideas. This is particularly important for DNA-based immunization. This novel technology’s simplicity makes it accessible to many levels and types of scientists, even those in countries with less developed research programs and less sophisticated facilities.

Industry. The new technology of DNA vaccines has piqued the interest of established pharmaceutical giants as well as small biotechnology companies. Major pharmaceutical companies have established in-house research and development programs, provided external funding to academic scientists, and supported scientific meetings on DNA vaccines. Ideally, such support will continue and industry will openly share information and reagents to encourage continued rapid development of DNA vaccines. Many biotechnology companies were established for research and development on gene therapy, an expertise easily transferred to DNA vaccines.

Government. Governments have and will continue to play an important role in the development of DNA vaccines. Governments can expedite the development of DNA vaccine technology by providing funding for basic and applied research on DNA vaccines. The National Institutes of Health (NIH) provided the competitive research grants that fostered the inception and initial discovery of DNA vaccines. Funding for sequencing the genomes of microbial pathogens should be encouraged, as the knowledge of DNA sequences is basic for the development of DNA vaccines. Governmental agencies might also establish targeted research programs on DNA vaccines. Currently, the NIH has initiatives, such as funded production facilities for gene therapy and national centers for antigen-based vaccine trials, that could provide needed reagents and infrastructure for the clinical testing of DNA vaccines.

Another important role for governmental agencies involves the regulatory issues associated with the approval of clinical trials and the licensing of new products for use in humans and animals. DNA vaccines should be treated as any other new product. For some applications the potential benefits clearly outweigh the risks, and expeditious approval of clinical trials will provide important safety information for other applications where the potential risks initially appear of greater importance. As trials are undertaken, careful surveillance should be undertaken.

International organizations. The World Health Organization (WHO) has shown the greatest interest in DNA vaccines of any international body. At a very early stage, WHO saw the potential benefits for application of this technology to the poorer and less developed areas of the world. WHO has played a significant role in facilitating the development of DNA vaccines through direct support of academic research as well as the organization and support of international symposia.

WHO also has established a system to ensure that vaccines, including DNA vaccines, reach citizens of poor as well as wealthy countries. The world’s nations have been divided into tiers based on population and gross national production (see Figure 10). Actual vaccine purchases
for the poorest countries would be made by the United Nations International Children’s Education Fund (UNICEF). Such a system would make vaccines available to those nations that could not afford to purchase them under other circumstances.

Other Societal Issues

**Intellectual property and patents.** The awarding of patents for DNA vaccines is just beginning. It is essential that those who hold key patents foster the cross-licensing agreements that will allow the rapid development and potentially broad use of DNA vaccines.

**Environmental issues.** DNA vaccines appear to pose no environmental risk. Furthermore, DNA vaccines should reduce existing environmental risks, by reducing endemic infections.

**Communication and the role of the media.** The media will play a large and important role in the implementation of DNA vaccines. While the potential benefits of DNA vaccines are enormous, until the true benefits are known, the media need to inform the public with cautious optimism. Unrealistic optimism could generate a backlash if hopes fail to become reality in a timely fashion. Thus, it is important that the media play an active, yet cautious role during the development of this novel technology that holds such high promise for world health.

Source: ref. (81).
References and Suggested Reading


38. Robinson HL. DNA vaccines for immunodeficiency viruses. AIDS suppl. (in press).


The Scientific Future of DNA for Immunization

American Academy of Microbiology
This report is based on an American Academy of Microbiology colloquium held May 31–June 2, 1996.

The colloquium was supported by the following sponsors:

Agracetus, Inc., Middleton, Wisconsin
Amgen, Inc., Thousand Oaks, California
Astra Arcus, Sodertalje, Sweden
Bayer AG GB-Pharma, Wupertal, Germany
Bayer AG, Animal Health Group, Leverkusen, Germany
Bio-Rad Laboratories, Hercules, California
Chiron Corporation, Emeryville, California
Connaught Laboratories, North York, Ontario, Canada
IRIS, Research Institute of Biocine Sclavo, Siena, Italy
Lederle-Praxis Biologicals, Wayne, New Jersey
Merck Research Laboratories, West Point, Pennsylvania
Pasteur Mérieux Connaught, La Coquette, France
Pfizer Central Research, Lincoln, Nebraska
QIAGEN GMBH, Hilden, Germany
Univax Biologics Inc., Rockville, Maryland
Vida Labs, Dallas, Texas