New Technologies and Vaccine Development

Margaret A. Liu, M.D.
Courtesy of T Sharrar, Smithsonian Institution
## Need for New Vaccines

<table>
<thead>
<tr>
<th>Disease</th>
<th>Annual New Cases</th>
<th>Annual Deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diarrheal Diseases</td>
<td>1,300</td>
<td>2.5-4</td>
</tr>
<tr>
<td>Acute Respiratory Diseases</td>
<td>3.7</td>
<td>2-3</td>
</tr>
<tr>
<td>Tuberculosis</td>
<td>7-8</td>
<td>2-3</td>
</tr>
<tr>
<td>HIV</td>
<td>5.8</td>
<td>3</td>
</tr>
<tr>
<td>Malaria</td>
<td>500</td>
<td>1.5-3</td>
</tr>
</tbody>
</table>
Issues for Live Attenuated Virus Vaccines

- Natural infection may not induce immunity or optimal immune responses
- Some viruses cause deleterious immune responses
- Potential reversion to virulence
  - Concern for HIV
- Decreased efficacy due to pre-existing antibodies
  - Influenza
- Decoy antigens on the virus
Comparison of Vaccine Technologies

- **Live attenuated viruses**
  - Highly effective
  - Potential risk
  - Manufacturing challenge

- **Recombinant proteins**
  - Potent antibody response
  - Non-native forms
  - Not induce CTL

- **Viral vectors**
  - Risk
  - Resistance / pre-existing antibody
  - Inflammation

- **DNA vaccines**
  - Need for increased potency
  - Designer immune response
    - e.g., Type of $T_H$
  - Specificity: avoid deleterious or diversional antigens
  - Stability
  - Safety
  - Generic manufacturing
  - Cost
HIV Clade (Strain) Diversity

Heterogeneity of HIV Strains
Exogenous Protein Results in Generation of T Cell Help But Not CTL

Modified from McDonnell WB and Askari FK, NEJM 334:42 (1996)
DNA Vaccine

Gene Encoding Antigen

CMVintA
Promoter

Bacterial Selection Gene

PolyA (BGH)
Terminator
Generation of CTL by DNA Vaccines

Proteasome cleaves protein into short peptides

DNA Vaccine

mRNA

Nucleus

CD8 + Cytotoxic T cell (CTL)

CD8

T cell Receptor

Modified from McDonnell WB and Askari FK, NEJM 334:42 (1996)
1918 Flu Pandemic

20 Million Deaths

Courtesy of T Sharrar, Smithsonian Institution
Initial Demonstration of Efficacy of DNA Vaccines

- Generation of CTL by DNA vaccine
- Protection by DNA vaccine against infectious challenge
- Cross-strain protection

DNA Vaccine Protects Against Cross-Strain Influenza Challenge

Addition of Irrelevant Plasmid DNA Increases Antigen-Specific Immune Responses

Donnelly JJ... Liu MA, Ann Rev Imm 15:627 (1997)
Immune Responses of DNA Vaccines

Results from:

- Specific immunity against encoded antigen
- Non-specific immune effects of plasmid backbone

Modified from Krieg, AM, Current Op Imm 12: 35 (2000)
Plasmid Non-Specific Stimulation

Due to:

- PuPuCGPyPy sequences
  - "CpG motifs"
- Potential means to increase / decrease / or change nature of immunogenicity of DNA Vaccines

Klinman DM...Krieg AM, PNAS 93:2879 (1996)
Klinman DM...Ishijatubo Y, JI 158:3635 (1997)
HIV
Different Forms of HIV Envelope Used for Immunizations

- **Monomer gp120**
  - Recombinant protein

- **Soluble Oligomer gp140**
  - Recombinant protein

- **Membrane Bound gp160**
  - DNA vaccine
B cell

DNA Vaccine

Antibodies

Proteasome cleaves protein into short peptides

Cytosolic Antigen

MHC Class 1 Glycoprotein

T cell Receptor

mRNA

Nucleus

Golgi Apparatus

CD8 + Cytotoxic T cell (CTL)

Modified from Mc Donnell WB and Askari FK, NEJM 334:42 (1996)
Clinical Trials of DNA Vaccines

- HIV
  - Therapeutic and prophylactic
  - Multiple vaccines / multiple trials
- Influenza
- Malaria
  - Multiple vaccines / multiple trials
  - Antigen + cytokine genes
- Hepatitis B
- Cancer
- (Gene Therapy)
Second Generation DNA Vaccines

- Increased potency
- “Designer” immune response
- Oral delivery
Area of Mucosal Surfaces:
1½ Basketball Courts
Encapsulated DNA: Microparticles
DNA Vaccine Replicons Rapidly Produce More Protein Antigen
“Designer Gene Vaccines”

Replicon:
- Amplify antigen mRNA

Genes Encoding:
- Cytokines
- Co-stimulatory molecules
- Targeting molecules

↑ or ↓ CpG Content:
(immunostimulatory sequences)
Sequential Immunization with DNA then Protein Generates Optimal Antibody Responses

<table>
<thead>
<tr>
<th>Prime</th>
<th>Boost</th>
<th>Percent seroconversion</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA</td>
<td>—</td>
<td>90%</td>
</tr>
<tr>
<td>DNA</td>
<td>DNA</td>
<td>100%</td>
</tr>
<tr>
<td>DNA</td>
<td>PROTEIN</td>
<td>100%</td>
</tr>
<tr>
<td>PROTEIN</td>
<td>—</td>
<td>0%</td>
</tr>
<tr>
<td>PROTEIN</td>
<td>PROTEIN</td>
<td>50%</td>
</tr>
<tr>
<td>PROTEIN</td>
<td>DNA</td>
<td>90%</td>
</tr>
</tbody>
</table>

Anti-Gag Ab titers

DNA: DNA Immunization, PROTEIN: Protein Immunization.
Protection of BALB/c mice after immunization with plasmid DNA and/or recombinant MVA

<table>
<thead>
<tr>
<th>Immunization 1</th>
<th>Immunization 2</th>
<th>% Protection*</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA</td>
<td>DNA</td>
<td>0</td>
</tr>
<tr>
<td>MVA</td>
<td>MVA</td>
<td>20</td>
</tr>
<tr>
<td>DNA</td>
<td>MVA</td>
<td>100</td>
</tr>
<tr>
<td>MVA</td>
<td>DNA</td>
<td>0</td>
</tr>
</tbody>
</table>

*5 animals/group
Antigens used: PbCSP + PbTRAP
DNA Vaccines: Tool for Functional Genomics/Proteomics

- Genome
- Select Genes
- Ligate
- In Vitro Expression
- In Vivo Expression/Function or Immunogenicity

- Select
- Ligate
Characteristics of DNA Vaccines

- Able to generate CTL, antibodies, $T_H$
  - Cross-strain protective CTL
  - Advantages of antigen structure for antibodies
    - Transmembrane protein
    - Native glycosylation
  - $T_H$ intrinsically $T_H$ 1
    - Can co-deliver cytokines to augment or alter $T_H$ phenotypes
  - Mechanisms for CTL and $T_H$ generation elucidated
  - Ability to stimulate desired immune responses not induced by wild-type disease
  - Avoid certain limitations/concerns of viral vectors
Characteristics of DNA Vaccines

- Second generation DNA Vaccines
  - Increased potency
  - Oral/Mucosal delivery
  - Facile manipulation of immune responses

- Potential advantages for clinical usage
  - Ability to generate T cell immunity: critical for many unconquered diseases
  - Key characteristics relevant to globally-needed vaccines
    - Generic technology
    - Stability
    - Manufacturing ease
    - Cost
    - Potential duration of immune response
### Disease Models in Which DNA Vaccines Have Demonstrated Efficacy

**Infectious Diseases**

**Viruses**
- HIV
- Influenza
- Rabies
- Hepatitis B,C,D
- Ebola
- Herpes Simplex
- Papilloma
- CMV
- Rota
- Measles
- LCMV
- St. Louis Enceph

**Parasites/Protozoa**
- Malaria
- Mycoplasma
- Leishmania
- Schistosoma
- Taenia ovis
- Toxo. gondii

**Bacteria**
- B. Burgdorferi
- C. tetani
- M. Tb
- S. typhi

**Cancer**
- Breast (Her2/neu)
- Colon
- Prostate
- Myeloma
- Lymphoma
- E7-Induced
- Fibrosarcoma

**Allergy**
- House Dust Mite
- Peanut
- Experimental Airway Hyperresponsiveness

**Autoimmune Disease**
- Diabetes
- EAE (MS model)