Eukaryotic Gene Expression: Basics & Benefits

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Lecture 34

DNA vaccines
Eukaryotic protein expression systems-II (lecture 31)
Protein expression in mammalian cells (non viral vectors)
Cell-free protein expression systems

Eukaryotic protein expression systems-III (lecture 32)
Protein expression in mammalian cells (viral vectors)

Human gene therapy (lecture 33)

DNA vaccines (lecture 34)
- Viral vectors
  - Retroviral vectors
  - Adenoviral vectors
  - Adenovirus-associated viral vectors

- Non-viral vectors
  - Polymers (cationic)
  - Liposomes (cationic)
  - Calcium phosphate transfection

- Other methods
  - Electroporation
  - Microinjection
  - Particle bombardment (Gene gun)
Non viral vs viral gene delivery systems
efficacy vs cost

Naked DNA + Economics
WHAT IS NAKED DNA?

NAKED DNA is just an eukaryotic expression plasmid that is neither complexed with chemical formulations nor contains any viral components.

It had been assumed for a long time that such a plasmid cannot be taken up by cells efficiently.

In 1990, Wolff and his colleagues demonstrated that mouse skeletal muscle cells can be transfected with naked DNA.

This finding opened new possibilities for delivering genes in vivo without the use of chemical or viral components.

Wolff JA et al.
Direct gene transfer into mouse muscle in vivo.
Science 1990; 247: 1465-1468,

DNA VACCINES
Cationic lipids for *in vivo* gene transfer and gene therapy

**Felgner PL et al.** Lipofectin: a highly efficient lipid-mediated DNA transfection procedure. *Proc Natl Acad Sci USA* 1987; **84**: 7413-7417

**Felgner PL et al.** Improved cationic lipid formulations for *in vivo* gene therapy. *Ann NY Acad Sci* 1995; **772**: 126-139


**Nabel GJ et al.** Immune response in human melanoma after transfection of an allogenic class I major histocompatibility complex gene with DNA liposome. *Proc Natl Acad Sci USA* 1996; **93**: 15388-15399

**Gill DR et al.** A placebo controlled study of liposome-mediated gene transfer to the nasal epithelium of patients with cystic fibrosis. *Gene Therapy* 1997; **4**: 199-209
Cationic lipid-mediated gene transfer

- Cationic lipids assemble and entrap plasmid DNA
  - Cationic headgroup to condense DNA
  - Lipid moiety for fusogenic cellular entry

Dioleoyltrimethylammonium propane (DOTAP)
While assessing the efficacy of various cationic lipid formulations for transfection of plasmid DNA into mouse skeletal muscle, naked DNA alone was used as a control.

Surprisingly, it was observed that transfection of mouse skeletal muscle was more efficient when naked DNA was used alone than when complexed with cationic lipids.

Wolff JA et al.
Direct gene transfer into mouse muscle in vivo.
Science 1990; 247: 1465-1468,
RNA and DNA expression vectors containing genes for chloramphenicol acetyltransferase, luciferase, and beta-galactosidase were separately injected into mouse skeletal muscle in vivo.

Protein expression was readily detected in all cases, and no special delivery system was required for these effects.

In situ cytochemical staining for beta-galactosidase activity was localized to muscle cells following injection of the beta-galactosidase DNA vector.

After injection of the DNA luciferase expression vector, luciferase activity was present in the muscle for at least 2 months.

Expression of β-galactosidase in mouse skeletal muscle following direct injection of β-gal expression plasmid
Can naked DNA transfection be used for Gene therapy of Haemophilia?

• Hemophilia is an X-linked disorder affecting 1 in 10,000 males
• The disease is due to a defect in the gene encoding the blood clotting factor, FACTOR VIII
  • The normal circulating levels of Factor VIII is 200 ng/ml (1 unit)
  • Maintainence of 0.1-0.2 units (20-40 ng/ml) is accepted as clinically significant

INTRAMUSCULAR INJECTION OF PLASMID DNA ENCODING FACTOR VIII GENE DID NOT RESULT IN THE PRODUCTION OF CLINICALLY SIGNIFICANT LEVELS OF FACTOR VIII
When the human dystrophin expression plasmids are injected intramuscularly into dystrophin-deficient mdx mice, the human dystrophin proteins are present in the cytoplasm and sarcolemma of ~1% of the myofibres.

Myofibres expressing human dystrophin contain an increased proportion of peripheral nuclei.

The results indicate that transfer of the dystrophin gene into the myofibres of DMD patients could be beneficial, but a larger number of genetically modified myofibres will be necessary for clinical efficacy.

Human dystrophin expression in mdx mice after intramuscular injection of DNA constructs.
Acsadi et al., Nature 352, 815 - 818 (29 August 1991)
Direct gene transfer into nonhuman primate myofibers in vivo.

However, the efficiency of gene transfer into skeletal muscle and these other tissues by direct injection is relatively low and variable, especially in larger animals such as nonhuman primates.
Heterologous protection against influenza by injection of DNA encoding a viral protein.


Immunization of mice by intramuscular injection of plasmid DNA encoding an influenza viral protein generated specific cytolytic T cells and protected the mice against subsequent challenge with live influenza virus

Naked DNA points way to vaccines.
Mice injected with plasmid DNA encoding influenza nucleoprotein are protected from viral challenge

First report of naked DNA vaccine.

Ulmer et al., SCIENCE (1993) 259, 1745
The strain of challenge virus was different from the strain from which the vaccine was made.

H1N1 (1934)  H3N2 (1968)
Viruses escape immune responses by mutating the regions against which an immune response is generated.

Antigens which are expressed on viral surface are often the targets of generation of neutralizing antibodies and when these surface antigens are mutated, antibodies can no longer neutralize such virus strains.

Viral proteins which are internal are often ineffective in inducing antibody responses but they can still induce cytolytic T cell responses. However, in order for these internal antigens to induce a CTL response, they need to be synthesized inside the host cell and be presented on the infected cell surface by class I MHC. These internal proteins are more conserved than the surface antigens and thus immune responses targeted to these conserved proteins can lead to cross-strain protection.

Thus, in the case of influenza, DNA vaccination lead to protection against a strain of influenza virus that was not only a different subtype from the strain from which the gene for the DNA vaccine was derived but also had arisen 34 years later.
1993

The beginning of DNA vaccine research
ADVANTAGES OF DNA VACCINES

Production : Simpler and cheap

Stability     : Does not require cold chain

Infectivity  : No risk of infection

Versatility  : Activates both cellular and humoral immunity, long lasting immunity

Flexibility  : Fusion of multiple epitopes
Compared with other types of vaccine technologies, DNA vaccines potentially have more widespread applications.

Once a manufacturing and purification process for the plasmid DNA has been established, a similar process can be applied to a different DNA vaccine, since only the inserted gene will be different.

In contrast, each attenuated, inactivated, or recombinant protein vaccine requires a unique manufacturing and purification process.
Instead of injecting plasmid DNA into skeletal muscle, immune response can also induced by coating plasmid DNA onto gold beads and propel the beads into the skin by using a gene gun.
Genetic immunization is a simple method for eliciting an immune response.

To produce an immune reaction against a foreign protein usually requires purification of that protein, which is then injected into an animal.

The isolation and purification of protein is time-consuming and sometimes difficult. Immune response can be elicited by introducing the gene encoding a protein directly into the skin of mice using a hand-held form of the biolistic system which can propel DNA-coated gold microprojectiles directly into cells of an animal.

Genetic immunization may be time- and labour-saving in producing antibodies and may offer a unique method for vaccination.
The Helios GENE GUN from BIORAD, USA
**Mechanism of DNA vaccination**

**How does injection of plasmid expressing a foreign antigen into skeletal muscle induce an immune response?**

In order to elicit an immune response, antigenic peptides need to be expressed on host cell surface in conjunction with Class I or Class II MHC.

Class I molecules are expressed by almost all nucleated cells. However, their expression is very high in antigen presenting cells such as dendritic cells, langerhans cells etc. Cells such as fibroblasts, liver cells, muscle cells and neural cells express very low levels.

Thus, only antigen-presenting cells can efficiently prime cytolytic T cells.

Thus, if a non–antigen-presenting cell such as muscle cell takes up the DNA vaccine and produces the protein antigen, it must deliver the antigen in some form to a professional antigen-presenting cell by a process called *cross-priming*, in order for cytolytic T cells to be induced.
Mechanism of DNA vaccination

Antigen secreted from DNA inoculated cell is taken up by Antigen presenting cells (APCs) and presented in association with CLASS II MHC

Plasmid DNA is taken up by APC, antigen is made and presented in association with CLASS I MHC
Delivery of DNA vaccines into skin

The Langerhans cell population along with monocytes, granulocytes (basophiles, eosinophiles, neutrophiles), and natural killer cells comprise the innate arm of the immune response that are either recruited to the skin or recirculated between the skin and the blood system in response to infection and trauma.

In the presence of an infection or skin vaccination, the innate immune cells interact with the adaptive arm of the immune system, the B and T cells that also recirculate, to generate the specific immune response to foreign antigen.

The Gene Gun delivery system is specifically designed to deliver DNA encoding antigens of pathogenic organisms to the immune-competent Langerhans cells.
Disease targets for which DNA vaccines have been tested in animals

Viruses

Parasites
Cryptosporidium parvum, Leishmania, Plasmodium falciparum (malaria), Schistosoma.

Cancer-associated antigens
Carcinoembryonic antigen (CEA), melanoma-associated antigen, the MHC molecule HLA-B7.

Bacteria
Borrelia burgdorferi (Lyme disease), Moraxella bovis, Mycobacterium tuberculosis, Mycoplasma, Rickettsia, Salmonella, tetanus toxin.

There have been more than 42 Phase I/II clinical trials involving DNA Vaccines.
http://www.dnavaccine.com
Are DNA Vaccines Safe?

Plasmid DNA malaria vaccine: tissue distribution and safety studies in mice and rabbits.

To evaluate the safety of a plasmid DNA vaccine, tissue distribution studies in mice and safety studies in mice and rabbits were conducted with VCL-2510, a plasmid DNA encoding the gene for the malaria circumsporozoite protein from Plasmodium falciparum (PfCSP). After intramuscular administration, VCL-2510 plasmid DNA was detected initially in all of the highly vascularized tissues, but at later time points was found primarily in the muscle at the site of injection, where it persisted for up to 8 weeks. After intravenous administration, plasmid DNA initially distributed at a relatively low frequency to all the tissues examined except the gonads and brain. However, plasmid DNA rapidly cleared, and by 4 weeks postadministration could be detected only in the lung of one of six animals evaluated. In a safety study in mice, eight repeated intramuscular injections of VCL-2510 at plasmid DNA doses of 1, 10, and 100 microg had no adverse effects on clinical chemistry or hematology, and did not result in any organ pathology or systemic toxicity. In a safety study in rabbits, six repeated intramuscular injections of VCL-2510 at plasmid DNA doses of 0.15 and 0.45 mg had no discernible effects on clinical chemistry, hematology, or histopathology. No evidence of autoimmune-mediated pathology, anti-nuclear antibodies (ANA), or antibodies to dsDNA were observed in the mouse or rabbit studies.
Are DNA Vaccines Safe?

Plasmid DNA malaria vaccine: the potential for genomic integration after intramuscular injection.

It was found that the amount of plasmid DNA persisting in muscle tissue varied but averaged about 10 fg per microgram of genomic DNA (in the range of 1500 copies per 150,000 genomes). In two of four separate experimental injections of mouse muscle, PCR assays of genomic DNA fractions indicated that agarose gel purification removed plasmid DNA down to a level of ≤3 copies per 150,000 mouse genomes. In the two other experimental samples, 3-30 copies of plasmid DNA remained associated with purified genomic DNA. The time following injection (i.e., 30 or 60 days) was not a factor in the number of copies of plasmid associating with genomic DNA and it was not possible to conclude if such sequences were covalently linked to genomic DNA or simply adventitiously associated with the genomic DNA. However, if an assumption is made that the highest level plasmid DNA found associated with genomic DNA (i.e., 30 copies) represented covalently integrated plasmid inserts and that each insert resulted in a mutational event, the calculated rate of mutation would be 3000 times less than the spontaneous mutation rate for mammalian genomes. This level of integration, if it should occur, was not considered to pose a significant safety concern.
Points to Consider:
Plasmid DNA vaccines for Preventative Infectious Disease Indications

DECEMBER 1996
Guidance for Industry

Considerations for Plasmid DNA Vaccines for Infectious Disease Indications

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Biologics Evaluation and Research
November 2007
Companies which undertake custom synthesis of GMP grade plasmid DNA for use in Clinical Trials

pAlliance — The new force in contract manufacturing of therapeutic plasmid DNA

- Standard-setting plasmid purity — challenging the most stringent purity specifications for cGMP-grade plasmid DNA currently available
- Batch scales of up to 100 g cGMP-grade plasmid DNA — using 10-3500 liter fermenters, optimized fermentation protocols, state-of-the-art equipment, and proprietary process technologies
- Complete service — including strain selection, cell banking, formulation, and final filing of ready-to-use cGMP-grade plasmid DNA
- Dedicated expertise from plasmid purification specialists — encompassing cGMP contract production, upstream and downstream process development, and international regulatory affairs
DNA Vaccines – Manufacture

• Process development and QC issues
• Cell origin, genotype & phenotype
• Genetic stability (WCB)
• Source of process components
• Process contaminants in final product
• Adventitious agents (e.g., bacteriophage) in MCB & WCB
• Genetic characterization
• Verify DNA sequence of entire vaccine (vector plus insert) present in MCB
DNA Vaccines -- Safety

Local reactogenicity & systemic toxicity

Nature of the immune response

Tissue localization, persistence & integration

Challenge/protection studies (demonstrate rationale for vaccine use)

Prime/boost studies (support dose, schedule, route of each component)

Cytokine expression (immunomodulation)
DNA Vaccines - Integration

• Potential Consequences of:

• Genome instability

• Inactivation of specific genes (tumor suppressors)

• Activation of dominant oncogenes by insertion of promoters/enhancers

• Germline alteration

• Biodistribution - if no signal (plasmid <30,000 copies per μg host DNA) is detected at study termination (typically Day 60), an integration study is not required

• iodistribution studies might be waived for DNA vaccines:

• When a novel, but related, gene is inserted into a plasmid vector previously documented to have an acceptable biodistribution/integration profile. If minor changes are made to the vector
Rapid antibody production using genetic immunization

Conventional technologies usually either generate antibodies against purified proteins, or against synthetic peptides based on amino acid sequences derived from DNA sequence data.

Genetic immunization involves introducing the gene in the form of a cDNA directly into an animal which translates this cDNA into protein thus stimulating an immune response against the foreign protein.

Following conventional methods, proteins are either extracted directly from tissues or in a recombinant form after expression of the cDNA in bacteria, yeast or eukaryotic cells.

In all of these cases, the methods are both time-consuming and costly.

Protein purification is not necessary for the genetic immunization approach, which can save between four to six months in time over recombinant protein generation for developing monoclonal antibodies.

http://www.genovac.com/
http://www.genovac.com/

GENOVAC

Antibody production by genetic immunization
—the direct way from gene to antibody
High affinity antibodies

Antibodies generated by genetic immunization at GENOVAC have been shown to have binding affinities to the protein in the sub-nanomolar range, which are approximately 100x higher than conventionally developed antibodies.

GENOVAC’s results confirm published data for much higher avidity of sera generated by genetic immunization as compared with that gained by immunization with a corresponding recombinant protein.

This stronger binding is again an important characteristic of the type of antibody needed for diagnosis or therapy, especially where the proteins to be detected are only present at low concentrations.

Therefore, antibodies generated by genetic immunization are ideally suited for diagnostic and therapeutic applications.

http://www.genovac.com/
Tailor-made antibodies

Another property of antibodies generated by genetic immunization is that it is easy to manipulate DNA, thus it is possible to focus antibodies to specific regions of a protein, such as a functional domain in order to stimulate or inhibit a particular protein function, e.g., receptor binding sites.

This could have important implications for drug development.

Furthermore, DNA sequences can easily be mutated, so that different protein isoforms, i.e., variants of one protein, can be made.

http://www.genovac.com/
Genetic immunization for antibody generation in research animals by intravenous delivery of plasmid DNA

Mary Kay Bates¹, Guofeng Zhang², Magdolna G. Sebestyén¹, Zane C. Neal¹, Jon A. Wolff¹,², and Hans Herweijer¹

BioTechniques 40: 199-208 (February 2006)
Oral DNA Vaccines

Oral gene delivery with Chitosan-DNA nanoparticles generates immunologic protection in a murine model of peanut allergy
Nature Medicine April 1999

Protective immunity induced by oral immunization with a Rotavirus DNA vaccine encapsulated in microparticles.
DNA vaccines get under the skin

NATURE BIOTECHNOLOGY  SEPTEMBER 1999

- DNA encoding a hepatitis B antigen can be applied directly to the skin of mice and still induce immune responses similar to those produced by intramuscular injection of a commercially available recombinant hepatitis B polypeptide vaccine.

- Hepatitis B antigen DNA, at a certain threshold concentration, appears to be taken up by hair follicles rather than through the skin epidermis. What's more, vaccination failed to work in mice that lacked normal hair follicles.
THERAPEUTIC DNA VACCINES

Therapy of Tuberculosis in mice by DNA vaccination

Lowrie et al., Nature 15 July 1999

- DNA vaccination as an adjunct to Chemotherapy

- In mice infected with M. tb H37Rv, DNA Vaccination can switch the immune response from one that is relatively inefficient and bacteriostatic to one that is bacteriocidal.

- Clearance of bacteria from spleen and lungs was demonstrated by injection of plasmid encoding Hsp65 but not by that of blank vector or BCG vaccination.

- This clearance is due to a switch from type-2 to a type-1 immune response.

- The clearance of bacteria was also observed in the M. tb CLS52, a clinical isolate resistant to isoniazid, one of the main chemotherapeutic drugs.
Expression Library Immunization (ELI)

Stephen Johnston

University of Texas Southwestern Medical Centre, Dallas

• Clone the DNA of a pathogen into groups of about 1000 plasmids.

• Inject mice with each group and challenge.

• Select the group that conferred protection, break it into groups of 100 and re-examine.

• Repeat this process to the level of single plasmid.

• From the library, one can thus identify a few plasmids that can confer protection
Immunostimulatory effects of CpG motifs


- CpG containing oligos augment antigen-specific serum antibody levels up to ten fold and IFNg production up to six fold. *Vaccine* 1999; 17: 19-25

Unmethylated CpG in particular sequence contexts, are recognized as foreign by the innate immune system.

They activate macrophages, induce B cell proliferation and stimulate strong B cell responses.

They are as effective as Freund’s complete adjuvant.

They can act synergistically with other adjuvants such as alum.
DNA VACCINE FOR JAPANESE ENCEPHALITIS


DNA VACCINE FOR RABIES


Since 1993, DNA vaccines have gone from a scientific curiosity to one of the most dynamic fields of research.

Atleast two DNA vaccines have been licensed for veterinary use

• To protect horses from west nile virus

• To protect salmon fish from infectious hematopoietic necrosis virus
Equine Health Solutions

West Nile - Innovator® DNA

WORLD EQUINE LEADERS

http://fortdodgelivestock.com/equine/equine-westniledna.htm
Apex-IHN®
Time lines of the registration process

- Apex-IHN was the first commercial DNA vaccine licensed in Canada in 2005.
  - 2002 (Dec) – Dossier Submitted
  - 2003 (Oct) – Conditional License (310 days)
  - 2004 (Sept) – Field Trial Permit Issued
  - 2005 (July) – Full Product License Issued

- Total Review Time = 2.5 years

Apex®-IHN is the first effective vaccine to prevent Infectious Haematopoietic Necrosis (IHN) in farm-raised Atlantic Salmon.
DNA Plasmid and Inactivated Tissue Culture Rabies Vaccine Containing ≥ 2.5 I.U per ml adjuvanated with Aluminium hydroxide gel Store between 2°C and 8°C For Intramuscular use only Shake well before use

NOT FOR HUMAN USE FOR ANIMAL TREATMENT ONLY

Manufactured by

INDIAN IMMUNOLOGICALS LIMITED
A Wholly Owned Subsidiary of the National Dairy Development Board
Rakshapuram, Gachibowli
Hyderabad - 500 019.

Batch No. : 1/2000
Mfd. Date : NOV 2003
Exp. Date : OCT 2006
**Inovio**

**PENNVAX™-family of vaccines:**
Human Immunodeficiency Virus (HIV)

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- **Prostate Cancer**: PSMA
- **Hepatitis C Virus**: NS3/4A
- **Breast/Lung/Prostate**: V934: hTERT

http://www.inovio.com/products/HIV_PENNVAX_TM.htm
Can we vaccinate ourselves using mini-gene guns and disposable DNA vaccine cartridges?

POWDERJECT, United Kingdom
DNA Vaccine Reviews


The Mechanism of Naked DNA Uptake and Expression
Jon A. Wolff and Vladimir Budker Advances in Genetics (2005) 54: 1-20

Donnelly JJ, Ulmer JB, Shiver JW, Liu MA. DNA vaccines
DNA Vaccine Reviews


The antiprogestin-dependent GeneSwitch system for regulated gene therapy.

Regulated production of proteins from muscle using gene transfer: potential therapeutic applications.

DNA-based influenza vaccines: Evaluating their potential to provide universal protection.

DNA vaccines for cancer therapy.
Horton HM, Parker SE, Wloch MK, Norman JA.