A Multicistronic DNA Vaccine Induces Significant Protection against Tuberculosis in Mice and Offers Flexibility in the Expressed Antigen Repertoire.

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The Development of Vaccines

1\textsuperscript{st} generation vaccines
Whole-organism vaccines
Live attenuated
ex: BCG, measles

Inactivated (killed)
ex: polio, hepatitis A

2\textsuperscript{nd} generation vaccines
Subunit vaccines
Contain only fragments
(subunits) of the pathogen
ex: tetanus, diphtheria toxoid

3\textsuperscript{rd} generation vaccines
DNA vaccines
genetically engineered
vector coding antigen gene
ex: HIV DNA vaccine
Whole-organism Vaccines

- **Live attenuated vaccines:**
  - uses pathogens that are living but have **reduced virulence**.
  - contain replicating microbes that can stimulate a strong immune response.
  - Risk of reversion to pathogenicity

- **Inactivated (killed) vaccines:**
  - safer than live vaccines-
    - they cannot replicate or mutate to a virulent form
  - Multiple dose typically required
  - Adjuvants normally needed
Subunit Vaccines

- Protein-based vaccines
  - nucleic acid of the pathogen has been removed, only protein subunits remain.
  - the subunits have less risk of causing adverse reactions.
  - need to be administered with a potent adjuvant.
DNA vaccines use episomal vectors expressing antigens under eukaryotic promoters in the vaccinated host.

- can elicit potent **humoral and cellular immune responses** comprising both CD4+ and CD8+ T cells.

(Adapted from Wikipedia)
DNA vaccines generate antigen inside the cell

Humoral response

T cell response

Free Ag

MHC1

antigenic peptides

The Efficacy of DNA Vaccines

Review- Genetic vaccine and Therapy (2003)

Strategies to improve the efficacy of DNA vaccination:

- **vector backbone DNA sequence**
  - strong promoter
  - ex: CMV promoter, SV40 promoter

- **transgene sequence**
  - antigen

- **co-expression of stimulatory sequences**
  - ex: adjuvants

- **delivery system** used for the vector
### Table 1: Comparison of promoters used in DNA expression studies in vitro and in vivo

<table>
<thead>
<tr>
<th>Expressed antigen</th>
<th>Promoters/enhancers compared</th>
<th>In vitro/in vivo comparison</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hepatitis B surface antigen (HBsAg)</td>
<td>CMV, desmin</td>
<td>The promoters performed equally well in vitro, and CTL and Th1 serum antibody responses against HbsAg in mice were of similar magnitude.</td>
</tr>
<tr>
<td>Hepatitis B envelope proteins</td>
<td>CMV, desmin</td>
<td>Greater in vitro expression of antigen was attributed to the desmin promoter. However, comparable humoral and cytotoxic immune responses were stimulated following i.m. injection of mice.</td>
</tr>
<tr>
<td>Rabies virus G protein</td>
<td>CMV, SV40</td>
<td>Comparable G antigen-specific antibody titres were stimulated in mice. Slightly higher T cell responses were observed from the CMV construct.</td>
</tr>
<tr>
<td>Influenza virus H5 hemagglutinin (HA)</td>
<td>CMV, β-actin</td>
<td>Constructs containing the CMV or β-actin promoters provided comparable protection against influenza in chickens.</td>
</tr>
<tr>
<td>Influenza virus H5 hemagglutinin (HA)</td>
<td>CMV, β-actin, RSV, SV40</td>
<td>Similar in vitro expression of HA. The greatest HA-specific antibody and protection against influenza in chickens was provided with the CMV construct.</td>
</tr>
<tr>
<td>Bovine herpesvirus glycoprotein D (gD)</td>
<td>RSV, CMV/IA</td>
<td>CMV/IA construct produced higher neutralising antibody titres against gD in i.d. injected cattle.</td>
</tr>
<tr>
<td>HIV-1 gag/env</td>
<td>CMV, AKV murine leukemia viral long terminal repeat</td>
<td>CMV showed 10–20 fold greater activity than AKV in vitro. Immunised macaques developed high humoral responses with the CMV construct only.</td>
</tr>
<tr>
<td>SV40 large tumour antigen</td>
<td>CMV, SV40</td>
<td>The CMV construct induced higher levels of antibody and protection in the murine experimental metastasis model than the SV40 construct.</td>
</tr>
<tr>
<td>M. tuberculosis apa + proteins</td>
<td>CMV, UbC</td>
<td>The CMV promoter was the most efficient tested.</td>
</tr>
<tr>
<td>Adenovirus E4 ORF3</td>
<td>CMV, RSV, SV40, UbC, EF-1α</td>
<td>Following i.n. dosing to mice, constructs containing the UbC and EF-1α promoters stimulated the most stable expression of antigen.</td>
</tr>
</tbody>
</table>
Delivery methods of DNA Vaccines

- **Injection**
  - Aqueous solution in saline
    - intramuscularly (i.m.)

- **Gene gun**
  - DNA-coated gold beads
    - intradermally (i.d.)

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DNA vaccine technology is showing increasing promise in the treatment of disease in humans.

Numerous animal models are under investigation for the use of DNA vaccines in humans:
- Malaria
- AIDS
- Herpes
- Rotavirus (childhood diarrhea)
- Tuberculosis
Tuberculosis (TB) is a disease mainly caused by Mycobacterium tuberculosis.
spread through the air
form granulomas in the lung
Chronic infection
They developed *Mycobacterium bovis* bacillus Calmette-Guérin (BCG) in 1921.
Bacillus Calmette-Guérin (BCG) Vaccine

- live attenuated *Mycobacterium bovis* bacillus

- BCG provides some useful level of protection against childhood TB.
- BCG provides little or no protection against adult pulmonary TB.
The safety and efficacy of BCG emphasize the need for alternative TB vaccines.

1. Live vaccines
   - Attenuated *Mycobacteria tuberculosis*
   - Recombinant BCG

2. Subunit vaccines
   - secreted antigen proteins
   - cell-bound antigen proteins
   - surface exposed antigens
Current Subunit Vaccines against TB

- Secreted antigens:
  - CFP-10, ESAT-6, Ag85B, Ag85A
  
- Dormancy antigens:
  - HspX, Hsp65, Hsp70

- Resuscitation antigens:
  - Rv3407

- Polyprotein: fusion of *M. tuberculosis* antigens
  - ESAT6 + Ag85B
  - Mtb72F: Mtb39A + Mtb32C

Skeiky, Y. A. et al. (2004)
Olsen, A. W. et al. (2004)
Roupie, V. et al. (2007)
Stages of *M. tuberculosis* infection

- Invasion, colonization
- Transmission
- Disease Reactivation
- Dissemination
- Continuum or distinct stages?
- Latency/Dormancy/persistence
- Resuscitation
- Secreted antigens
- Dormancy antigens
- Granuloma
- Resuscitation antigens
Multicistronic DNA Vaccine

- Multicistronic DNA vaccine
- DNA plasmid coding multiple antigens
Multicistronic Strategies

- Fusion proteins
- Bidirectional promoters
- Internal ribosomal entry site (IRES)
- 2A peptide sequence

Picornaviruses, such as poliovirus and foot-and-mouth disease virus (FMDV), encode all of their proteins within a single open reading frame (ORF).

In the case of the FMDV, the 18 aa. 2A region has a major role in polyprotein processing.
Co-translational, intraribosomal cleavage of polypeptides by FMDV 2A peptide

- The FMDV 2A sequence functions during co-translational translocation.
- Protein sequences following 2A are excluded from the ER lumen.

(de Felipe et al. (2003) JBC 278:13  p.11441-8.)
Vector Construction

Cytomegalovirus (CMV)
Transfection of HEK293T cells

Transfection reagent
lipofectamine

DNA
V-2A

pCMV.tPA

DNA-lipofectamine complex

transfection

HEK293T cells
72 hour

Collect the cells

ELISA
Protein coexpression by V-2A

multicistronic vector

Control vector

pCMV-tPA

monocistronic vector

pCMV-tPA-Rv3407

Western blotting analysis

Expression of Rv3407

1. Positive control (R.Protein)
2. Transfection control
3. pCMV.tPA- transfected
4. pCMV.tPA-Rv3407-transfected
5. pCMV.tPA-2A-transfected

Expression of Ag85-A

1. pCMv.tpA-2A-transfected
2. Transfection control
3. pCMV.tPA- transfected
4. Positive control (R.Protein)

Expression of HspX

1. Positive control (R.Protein)
2. Transfection control
3. pCMV.tPA- transfected
4. pCMV.tPA-2A-transfected
Coexpression of four proteins

**transfection**

48 h

HEK293T cells

**Fluorescent microscopy**

Expression of active Green fluorescence

**Control vector**

- pCMV-tPA

**monocistronic vector**

- pCMV-tPA
  - GFP

**multicistronic vector**

**Fluorescent microscopy**

Expression of active Green fluorescence

A

- pCMV-tPA
- pCMV-tPA-GFP
- V-2A-GFP
- Quantitative RT-PCR
  - SYBR green
  - dsDNA dye
  - GADPH
  - Internal control

No significant difference in Ct value between monocistronic (pCMV-tPA-Rv3407) and multicistronic (V-2A).
Cellular and Humoral Immune Response after DNA Vaccination

100 μg DNA

Humoral response

BALB/c mice

IFN-γ

TNF-α

IL-2

Th1 Response

IgG1

IgG2a
Cellular Immune Response induced by V-2A

Lymph node

- IFNγ+
- TNFα+
- IL-2+

Spleen

- IFNγ+
- TNFα+
- IL-2+

% of CD4 gated cells

- Naive
- Naked Vector
- V-2A

* $P < 0.05$
** $P < 0.01$
*** $P < 0.001$
Cellular Immune Response induced by V-2A

Lymph node

- IFNγ+
- TNFα+
- IL-2+

Spleen

- IFNγ+
- TNFα+
- IL-2+

Legend:
- Naive
- Naked Vector
- V-2A

Significance:
- * P < 0.05
- ** P < 0.01
- *** P < 0.001
Humoral Immune response induced by V-2A

Antibody titers were measured by ELISA

![Graph showing antibody titers at different days](image_url)

*P < 0.05
**P < 0.01
Protective efficacy after Aerosol Challenge

V-2A vaccination

Aerosol challenge

10^6 CFU *M. tuberculosis*

CFU counting of lung tissue

BCG vaccination

4 weeks

90 days

BALB/c mice

Day 30

Day 60

Day 90

* P < 0.05

** P < 0.01
Summary

V-2A-GFP

In vitro Western Fluorescence microscopy quantitative RT-PCR

In vivo
Cellular Immune response Humoral Immune response
Protective efficacy against TB

Multicistronic DNA Vaccine may be a potential treatment for TB

V-2A is an effective DNA vaccine

1st generation vaccines
Whole-organism vaccines

2nd generation vaccines
Subunit vaccines

3rd generation vaccines
DNA vaccines

A person may contract pulmonary tuberculosis from inhaling droplets from a cough or sneeze by an infected person

Granuloma in lung tissue

Why didn’t you say in the first place? We could have used this new all-in-one vaccination rather than the traditional multiple injections. Oh well! We’ll do it this way next year . . . How about that?

V-2A-GFP

Granuloma in lung tissue

Objective
Antigen

Quantitative RT-PCR

V-2A-C

pCMV
Rv3407

Ag85A

LNFDDLKLADGVESNPQ

Protein expression levels are equal

V-2A is an effective DNA vaccine
Thank You!