

Immunogenicity and Efficacy of a Combination Anthrax/Plague DNA Vaccine in a Mouse Model

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Multiagent vaccines are a principal goal of next generation immunological therapeutics and likely to be DNA-based and/or consist of recombinant protein formulations. In this study we evaluate the ability of DNA and recombinant protein vaccines to mediate protection against the BW threat agents *Yersinia pestis* and *Bacillus anthracis* in a murine model. DNA vaccines, codon optimized for mammalian expression and encoding *B. anthracis* virulence factors Protective Antigen (PA) and Lethal Factor (LF) and *Y. pestis* virulence factors F1 and LcrV were engineered into the DNA vaccine vector pDNAVac to generate the DNA vaccines pDNAVac-hPA (human codon optimized), pDNAVac-LcrV-hLFn (hLFn = human codon optimized domain one of Lethal Factor fused downstream of *Y. pestis* V antigen), and pDNAVac-hLFn-F1 (human codon optimized domain one of Lethal Factor fused upstream of *Y. pestis* V antigen). A/J mice were immunized using the gene gun (PowderMed, UK) with DNA vaccine constructs, either individually or in groups, or with recombinant protein/fusion proteins rF1, rLcrV-LFn and rF1-LFn via the intramuscular route. The DNA vaccine encoded antigens induced isotypes dominated by IgG1 in the serum profiles. DNA vaccine-induced serum antibody levels for each specific antigen were not statistically different for mice immunized with a single DNA construct as compared to mice inoculated with multiple constructs with the exception of anti-LFn. The anti-LFn IgG1 antibodies were higher when given in the F1 fusion construct than those from the V antigen Lethal Factor fusion construct. Recombinant protein fused F1 antigen and Lethal Factor (rF1-LFn) induced approximately four-fold greater anti-LFn antibody levels than the recombinant V antigen and Lethal Factor fusion (rLcrV-LFn). Anti-F1 serum antibody levels in mice injected with rF1-LFn compared to mice injected with only rF1 were approximately two-fold higher. DNA and recombinant protein immunized mice were challenged by exposure to 10 median lethal doses (MLD) of aerosolized *Y. pestis* bacteria or *B. anthracis* spores. *Y. pestis* challenged mice previously inoculated with DNA constructs encoding *Y. pestis* virulence factors fused to Lethal Factor were partially protected. Co-immunization with these plasmids plus a second plasmid expressing Protective Antigen increased protection levels significantly. Mice inoculated with DNA constructs encoding *B. anthracis* virulence factors, either individually or co-administered in multi-construct formulations, were 100% protected against inhalational anthrax challenge. All mice immunized with recombinant protein/fusion proteins from *Y. pestis* (rF1, rLcrV-LFn or rF1-LFn) and *B. anthracis* (rPA, rLcrV-LFn or rF1-LFn) were completely protected against pathogen challenge. These data demonstrate the viability of utilizing DNA vaccines encoding

multiple antigens or recombinant heterologous fusion proteins to simultaneously confer significant protection against aerosolized *Y. pestis* and *B. anthracis*.

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***Gene gun study using plasmids encoding PA, LcrV-LFn and LFn-F1**

**Immunized by gene gun on day 0,14 and 28.
Protein immunization – 2 doses with alhydrogel**

**Aerosol challenged with *Y. pestis* GB at 1×10^3 - 1×10^4 cfu/mouse at day 56.
Aerosol challenged with *B.anthraxis* Sterne spores = 2.75×10^4 spores/mouse at day 56**

Protection study results:

Vaccine group	Survivors Plague	Survivors Anthrax
pDNAVac (empty control)	0/6 (0%)	1/5 (20%)
pDNAVac-TPA-hPA	0/6 (0%)	6/6 (100%)
pDNAVac-TPA-hLcrV-LFn	3/6 (50%)	6/6 (100%)
pDNAVac-TPA-hPA + pDNAVac-TPA-hLcrV-LFn	5/6 (83%)	6/6 (100%)
pDNAVac-TPA-hLFn-F1	0/6 (0 %)	5/5 (100%)
pDNAVac-TPA-hPA + pDNAVac -TPA-hLFn-F1	4/6 (67%)	6/6 (100%)
pDNAVac-TPA-hPA + pDNAVac-TPA-hLFn-F1 + pDNAVac-TPA-hLcrV-LFn	3/6 (50%)	6/6 (100%)
Protein data		
rPA	N.D.	6/6 (100%)
rF1	6/6(100%)	N.D.
rF1-LFn	N.D.	6/6 (100%)
rLcrV-LFn	6/6(100%)	6/6 (100%)

*Study completed in collaboration with DSLT Porton Down. BDRD completed cloning, protein expression and purification, DSTL completed animal gene gun study.