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LLNL-TR-666002

Nanolipoprotein Particles (NLPs) as Versatile Vaccine Platforms for Co-delivery of Multiple Adjuvants with Subunit Antigens from Burkholderia spp. and F. tularensis - Technical Report

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Disclaimer

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CBM QUARTERLY PROGRESS REPORT	PROPOSAL / CONTRACT NUMBER CBCALL12-PLAT2-2-0010	
	PERIOD COVERED BY THIS REPORT	
PROGRAM DIRECTOR / PRINCIPAL INVESTIGATOR Nicholas O. Fischer, Ph.D.	FROM July 1, 2014	THROUGH September 30, 2014

APPLICANT ORGANIZATION — CURRENT TRL

Lawrence Livermore National Laboratory

TITLE OF PROJECT

Nanolipoprotein particles (NLPs) as versatile vaccine platforms for co-delivery o

DUAL USE RESEARCH OF CONCERN

Select Agent/Toxin: Please indicate the select agents or toxins studied in this project.

☐ Does not involve use of virulent select agent, toxin or genetic components

☐	*Bacillus anthracis*	☐	Ebola virus
☒	*Francisella tularensis*	☐	Marburg virus
☐	*Yersinia pestis*	☐	Variola major virus
☒	*Burkholderia mallei*	☐	Variola minor virus
☒	*Burkholderia pseudomallei*	☐	Rinderpest virus
☐	Botulinum neurotoxin	☐	Foot-and-mouth disease virus
☐	Toxin producing strains of *Clostridum botulinum*	☐	Avian Influenza virus (highly pathogenic)
		☐	Reconstructed 1918 Influenza virus

Experiments of Concern: The following categories of experiments will be used to determine dual use research of concern. Please indicate the type of research conducted from the list below.

☒ Research conducted does NOT involve any of the listed experiments

☐	Enhances the harmful consequences of the select agent or toxin
☐	Disrupts immunity or the effectiveness of an immunization against the select agent or toxin without clinical and/or agricultural justification
☐	Confers to the select agent or toxin resistance to clinically and/or agriculturally useful prophylactic or therapeutic interventions against that select agent or toxin or facilitate their ability to evade detection methodologies
☐	Increases the stability, transmissibility, or the ability to disseminate the select agent or toxin
☐	Alters the host range or tropism of the select agent or toxin
☐	Enhances the susceptibility of a host population to the select agent or toxin
☐	Generates or reconstitutes an eradicated or extinct biological agent or toxin from select agent or toxins listed above

ABSTRACT

The goal of this proposal is to demonstrate that colocalization of protein subunit antigens and adjuvants on nanolipoprotein particles (NLPs) can increase the protective efficacy of subunit antigens from *Burkholderia spp*. and *Francisella tularensis* against an aerosol challenge. In the second quarter of the third year, LLNL finalized all immunological assessments of NLP vaccine formulations in the F344 model. Battelle has immunized rats with three unique NLP formulations by either intramuscular or intranasal administration. All inoculations have been completed, and protective efficacy against an aerosolized challenge will begin at the end of October, 2014.

STUDIES AND RESULTS

Task 4.1: This quarter was focused on finalizing all immunological assessments on NLP on conducting experiments integral in laying the groundwork for aerosolized *F. tularensis* SCHU S4 challenges in the Fischer 344 rat model. LLNL conducted experiments aimed at establishing the immunological responses of NLP vaccine formulations in the F344 rats. The goal of these analyses was to identify the most promising NLP vaccine formulation in a timely manner. As such, it was decided by the LLNL, the DTRA PM, and Battelle not to test non-NLP formulations. While the majority of the immunological assays were completed in the previous quarter (e.g. IgG serum titers and cytokine quantification), a few additional analyses were conducted. IgA titers were assessed in both serum (for IM and IN groups) and bronchoalveolar lavage (BAL; IN groups only) 4 weeks after the third (and final) inoculation. As illustrated in Figure 1, serum IgA levels were highest for IglC, primarily when formulated on mCpG:NLPs, regardless of administration route. While antigen-specific titers were lower for DnaK and KatG, those formulated with mCpG:NLPs exhibited the highest IgA titers. In the BAL, the low number of animals (n=4) precluded any significant conclusions to be drawn, but important trends were apparent (Figure 2). Higher IgG titers were generally observed with CpG formulations (in contrast to MPLA). Interestingly, only mCpG:NLPs elicited measurable IgA titers in the IN inoculated animals.

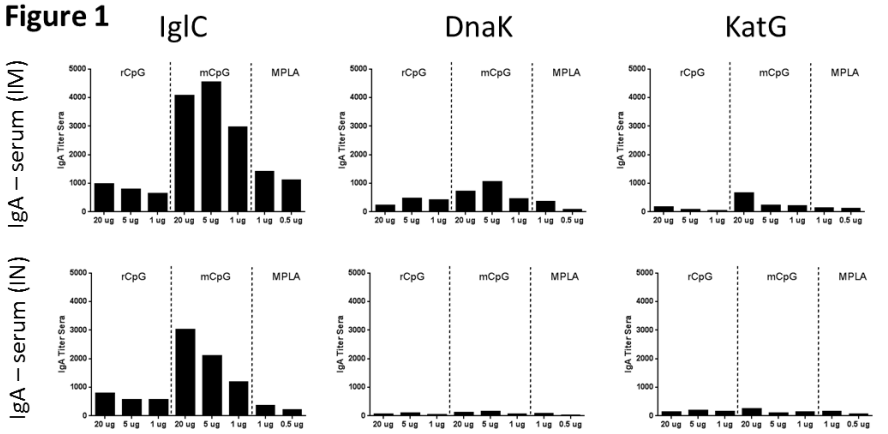

Figure 1: Quantification of antigen-specific serum IgA titers. Sera from animals was collected 12-weeks post prime (4-weeks after third inoculation), pooled, and analyzed for IgA titers. Data are grouped according to antigen (IglC, DnaK, KatG) and adjuvant formulation (mCpG [ODN1826], rCpG [ODN 2395], or MPLA) and dose (1, 5, 20 µg CpG or 0.5, 1 µg MPLA).

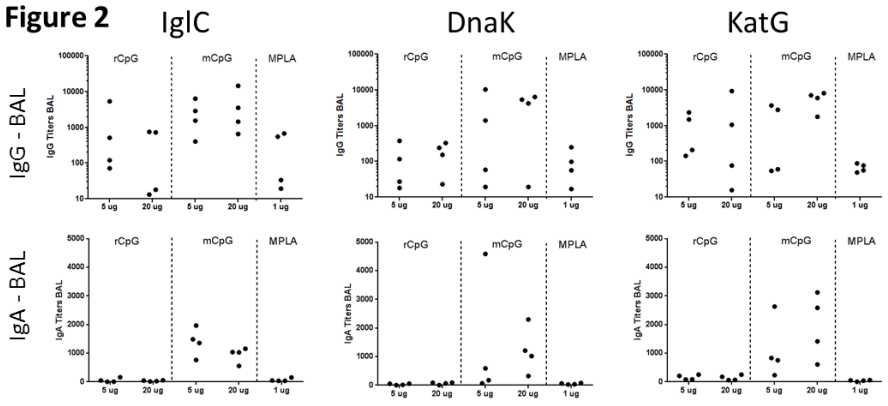

Figure 2: Quantification of IgG and IgA titers in BAL of animals (n=4) immunized via IN. Antigen-specific titers were determined for individual animals. Data are grouped according to antigen, adjuvant formulation, and dose (5, 20 µg CpG or 1 µg MPLA).

Task 4.3: Based on these recent data from Task 4.1, as well as the data accumulated in the previous Quarter, the mCpG (ODN 1826) was deemed to elicit the greatest humoral and T-cell responses in the animals. As both IM and IN elicited different yet strong responses, it was decided to utilize both routes (same formulations, but separate groups) for inoculating the animals that are to be challenged. Only mCpG formulations were chosen for this study, as these formulations elicited the greatest response. Table 1outlines the experimental design for the challenge experiment to be carried out at Battelle. NLP inoculation groups are formulated with all three antigens (IglC, DnaK, KatG) and mCpG:NLP. Formulations vary in total amount of CpG or total amount of each antigen (i.e. 5µg CpG/10µg each antigen; 5µg CpG/20µg each antigen; 20µg CpG/10µg each antigen). Inoculations were either IM or IN. This Task began its in vivo phase at Battelle on July 28, 2014. To verify consistency between vaccine formulation administration at Battelle and LLNL, sera collected 3-weeks post prime inoculation were collected and sent to LLNL for IgG titer analysis. As seen in Figure 3, overall titers were consistent between the two studies. An additional verification will occur after the final bleed (Day 77). These samples have been received at LLNL and are currently being processed. Animals will be challenged on October 20, 2014.

Table 1: Challenge Experimental Groups and Design

Group #	Vaccine Formulation	Vaccination Route	Number of F344 Rats		Challenge Dose (cfu)	Vaccination Points	Blood Collection Schedule (Study Day)	Post-Challenge Observations	Endpoint
			Male	Female					
Challenge with *F. tularensis* Strain Schu S4									
1	CpG:NLP (Adj5/Ag10)	I.M.	10	10	$10xLD_{50}$ (320 cfu)	I_1 = Day 0 I_2 = Day 28 I_3 = Day 56	21, 77	Twice Daily Clinical Observations Through Study Day 112	Survival, time-to-death
2	CpG:NLP (Adj5/Ag20)	I.M.	10	10					
3	CpG:NLP (Adj20/Ag10)	I.M.	10	10					
4	CpG:NLP (Adj5/Ag10)	I.N.	10	10					
5	CpG:NLP (Adj5/Ag20)	I.N.	10	10					
6	CpG:NLP (Adj20/Ag10)	I.N.	10	10					
7	PBS	I.M.	10	10					
8	LVS	I.M.	10	10		I_1 = Day 56	77		

I_1: First Immunization
I_2: Second Immunization
I_3: Third Immunization
Immunization Injection Volume: I.M. = 100 µL; I.N. = 50 µL (25 µL/nare)
Vaccination Site: Groups 1, 2, 3, 7, 8 = I.M.; Groups 4, 5, 6 = I.N.

Figure 3

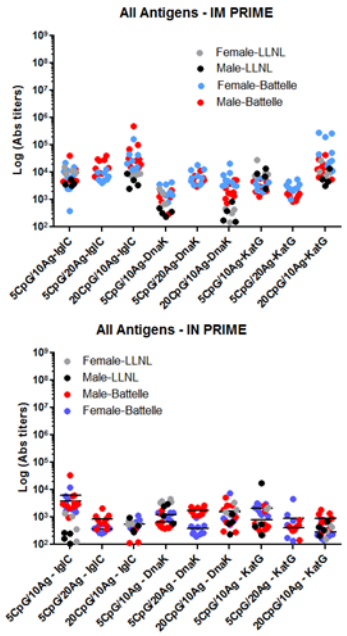

Figure 3: Comparison between titers elicited 3-weeks post prime at Battelle and LLNL. 11-week titers are currently being determined. Data are grouped according to antigen (IglC, DnaK, KatG) and mCpG:NLP formulation (5µg CpG/10µg each antigen ; 5µg CpG/20µg each antigen ; 20µg CpG/10µg each antigen)

ISSUES (TECHNICAL AND PROGRAMMATIC)

During the course of this study, it had been suggested by DTRA PMs and SMEs that blood be collected from the inoculated animals at Battelle to be analyzed at LLNL for antigen-specific serum titers. This is a very important metric for understanding the anticipated results from the upcoming challenge experiments. However, the costs for blood draws and training animals were not included in the original SOW or Battelle budget. To enable these activities, as well preparing LVS as a positive experimental control, we have increased the Battelle budget for this study, reciprocally reducing the budget for the final Battelle efficacy study. Modifications to the SOW were prepared and approved by the DTRA PM. No increase in budget was requested.

DISCUSSION AND SIGNIFICANCE

The second Quarter in Year 3 has been focused on identifying top NLP vaccine formulations and to begin inoculation regimen protective efficacy assessments against aerosol LD_{50} of *F. tularensis* SCHU S4 in the F344 model. The formulations and routes that elicited the greatest humoral and T-cell responses were prepared and shipped to Battelle. Analysis of IgG titers in animals vaccinated at Battelle (after prime inoculation) were assessed and compared to titer values previously obtained at LLNL: comparative levels were congruent, indicating that inoculations and immune responses are proceeding as expected. Challenge experiments are set to begin on October 20, 2014.

(Prepared by LLNL under Contract DE-AC52-07NA27344. LLNL-TR-666002).