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A comparison of *Yersinia Pestis* subunit vaccine platforms: Chimeric recombinant F1V versus Nanolipoprotein F1 and V particle formulation

Jennifer L. Dankmeyer, Nathaniel O. Rill, Michael L. Davies, Christopher P. Klimko, Jennifer L. Shoe, Melissa Hunter, Yuli Talyansky, Ju Qiu, Amy Rasley, Nicholas O. Fischer, Christopher K. Cote, and Sergei S. Biryukov

Bacteriology Division, United States Army Medical Research Institute of Infectious Diseases, Fort Detrick, Maryland, USA

Introduction

Yersinia pestis, a facultative gram-negative coccobacillus, is the etiological agent of plague that has been responsible for three pandemics and remains a current concern as demonstrated by recent outbreaks in Madagascar. *Y. pestis* infection results in a severe and rapidly progressing illness that can only be treated with antibiotics. Unfortunately, natural acquisition of antibiotic resistance has been reported. There are currently no FDA-approved vaccines. Previous vaccine attempts utilizing live attenuated strains or whole-cell inactivated formulations, with varying levels of reactogenicity, conferred short-lived protection against bubonic plague but failed to provide protection against pneumonic plague. Utilization of a subunit vaccine formulation may circumvent some of these whole cell vaccine-associated shortfalls while enhancing the efficacy and longevity of protection. **Here we compare the immune response of two different subunit vaccine platforms directed against *Y. pestis*.** The immunogenicity generated by the most advanced subunit vaccine formulation (rF1V), a chimeric fusion protein of Fraction 1 (F1) capsular antigen and a type III secretion system-associated protein LcrV antigen (V), was compared against a nanolipoprotein particle (NiNLP)-based vaccine, see **Figure 1**. The NLP vaccine, a mimetic of naturally occurring high-density lipoproteins, is a lipid bilayer disc stabilized by amphipathic "scaffold" apolipoproteins and has been shown to be an effective vaccine platform for subunit antigens targeting multiple disparate pathogens.

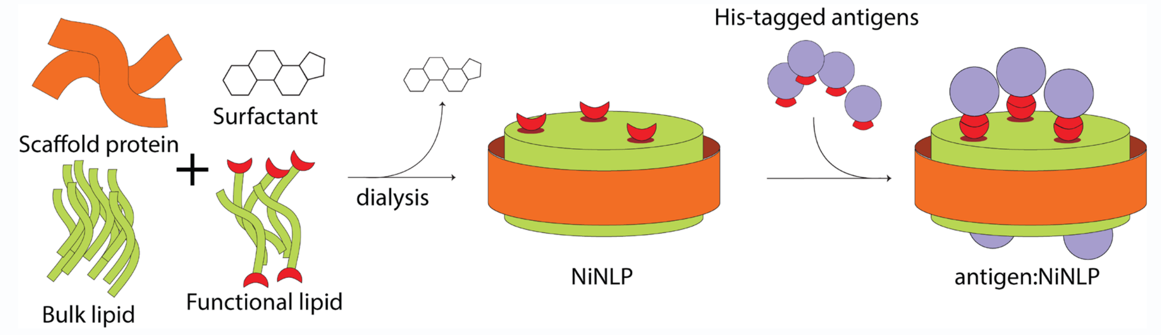


Figure 1. NiNLPs are prepared by self-assembly, initiated by dialyzing a solution of apolipoprotein and detergent solubilized lipid. NiNLPs are purified, sterilized, and lyophilized. Vaccine formulations are prepared by adding his-tagged antigens and adjuvant prior to vaccination.

Methods

Animal Vaccination: Female, 6- to 8-week-old, BALB/c, mice were obtained from Charles River (Frederick, MD). The mice (16 mice per group) were vaccinated twice, subcutaneously, four weeks apart, see **Figure 2**. Approximately 1 month after the last vaccine dose, the mice were exposed to aerosolized suspensions, created by a three-jet Collison nebulizer, of approximately 8 LD_{50}s of *Y. pestis* CO92 (1 LD_{50} = 6.8 x 10^4 CFU). Blood, spleen, and lung samples were collected from each vaccine and control group at various times before and after aerosol challenge, see **Figure 2**. Blood and tissue samples were examined for the presence of colony-forming-units (CFUs), cytokine/chemokine expression, and antibody titers. In addition, mice were observed for 21 days post challenge and early endpoint euthanasia was performed in accordance with approved euthanasia criteria.

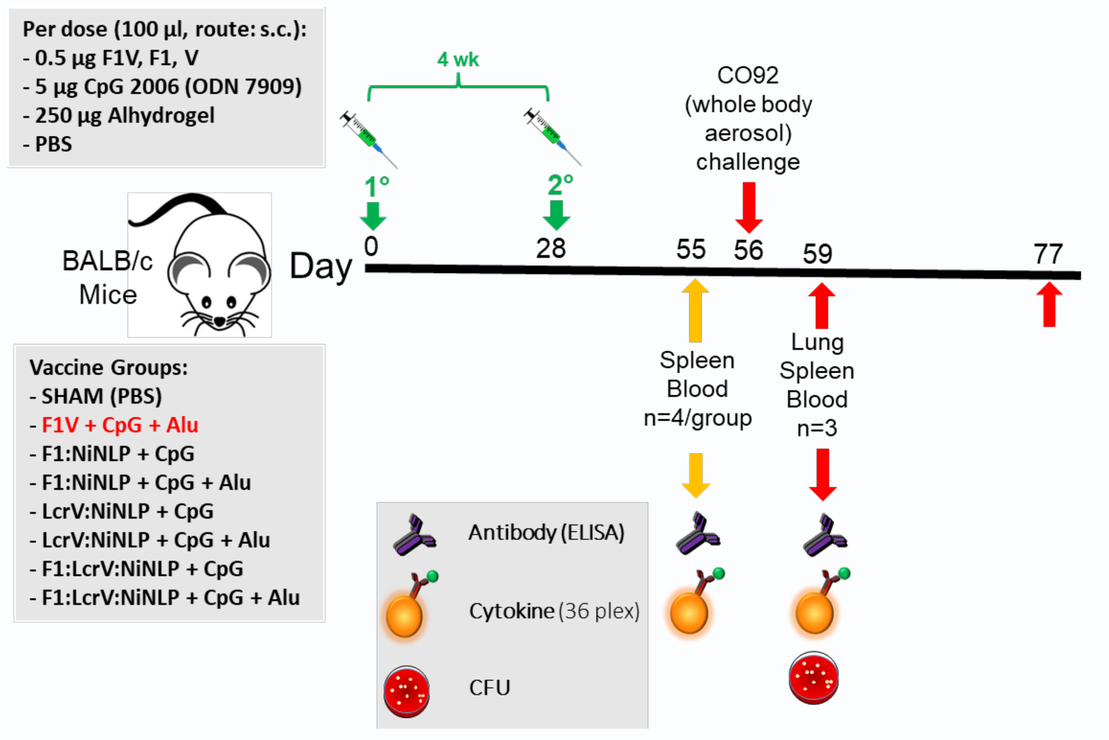

Per dose (100 µl, route: s.c.):
- 0.5 µg F1V, F1, V
- 5 µg CpG 2006 (ODN 7909)
- 250 µg Alhydrogel
- PBS
4 wk
1°
2°
CO92
(whole body
aerosol)
challenge
BALB/c
Mice
Day
0
28
55
56
59
77
Spleen
Blood
n=4/group
Lung
Spleen
Blood
n=3
Vaccine Groups:
- SHAM (PBS)
- F1V + CpG + Alu
- F1:NiNLP + CpG
- F1:NiNLP + CpG + Alu
- LcrV:NiNLP + CpG
- LcrV:NiNLP + CpG + Alu
- F1:LcrV:NiNLP + CpG
- F1:LcrV:NiNLP + CpG + Alu
Antibody (ELISA)
Cytokine (36 plex)
CFU

Figure 2. Overview of the immunization and challenge strategy for direct comparison of *Yersinia pestis* vaccine candidates that were challenged with *Y. pestis CO92*. The numbers with the degree sign (°) denote vaccine prime and consecutive boost(s).

Bacteriology: The tissues collected were weighed and homogenized with disposable PRECISION™ homogenizers (Covidien, Dublin, Republic of Ireland); the CFU of the homogenate were determined on sheep blood agar plates. Undiluted homogenate and 10-fold dilutions in PBS (Dulbecco's phosphate buffered saline, without Ca++ or Mg++) were plated in duplicate to determine sterility. The limit of detection was ~10–100 CFU/ml blood (depending upon the experiment) or 5 CFU/organ.

Antibody Titers: Immunoglobulin (IgG) antibody responses were determined by semi-quantitative endpoint ELISA using sera from vaccinated BALB/c mice, as previously described (1). The pure proteins (F1, V, the F1-V recombinant fusion) and γ-radiation inactivated whole cells of *Y. pestis* CO92 were used as capture antigens at a concentration of 2 µg/ml and 10 µg/ml, respectively. Two-fold dilutions of the serum were made in triplicate and the results are reported as the geometric mean (geometric SEM) of the reciprocal of the highest dilution giving a mean OD of at least 0.1 ± 1 SD at 450nm with a reference filter (570nm). Samples with an antibody titer of 50 or less were considered negative.

Cytokine Levels: Analyte levels were assayed in restimulated splenocytes and in lung homogenates. Supernatant or homogenates were examined for cytokine expression by Luminex MagPix 36-plex mouse panel (Thermo Fisher Scientific, Grand Island, NY, USA) as per manufacturer directions.

Results

Table 1. Fold change in cytokines response in splenocytes prior to challenge from vaccinated mice relative to sham treated mice that were stimulated with F1V, F1, or V (Day 55)

F1V (25 ug/ml)

Cytokine	F1V + CpG + Alu	F1:NiNLP + CpG	F1:NiNLP + CpG + Alu	LcrV:NiNLP + CpG	LcrV:NiNLP + CpG + Alu	F1:LcrV:NiNLP + CpG	F1:LcrV:NiNLP + CpG + Alu
IL-5	374.70	1.03	671.68	1.23	2,194.98	2.13	1,254.75
IL-13	99.49	1.00	242.00	1.27	453.72	1.96	309.22
IL-2	73.44	3.82	66.42	2.51	107.54	6.25	70.95
IFN gamma	41.98	3.56	80.31	2.32	175.53	5.91	81.42
IL-6	38.59	2.72	63.80	3.62	42.56	7.55	46.40
IL-4	36.36	0.87	24.42	1.11	69.72	3.53	55.39
IL-3	35.12	1.94	41.56	1.41	46.54	5.91	43.33
GM-CSF	31.50	1.00	41.52	1.25	47.04	2.00	37.71
IL-17A (CTLA-8)	24.19	1.13	4.99	1.66	4.96	2.47	3.87
IL-22	14.61	1.00	16.36	1.01	12.29	1.86	12.23
TNF alpha	12.34	0.79	11.31	0.95	18.85	0.91	12.55
MIP-1 alpha (CCL3)	11.65	1.10	17.55	1.35	19.45	1.89	21.03
MCP-3 (CCL7)	8.03	1.58	9.17	1.39	9.49	3.86	9.60
IL-10	7.73	1.00	5.27	1.23	28.94	1.66	15.63
Eotaxin	6.43	1.00	1.59	1.00	1.30	1.07	2.02
IP-10 (CXCL10)	6.18	1.61	7.91	1.24	5.86	4.79	7.18
MIP-1 beta (CCL4)	5.04	1.10	7.78	1.31	8.80	1.58	10.15
MIP-2 alpha (CXCL2)	4.45	1.28	4.91	1.15	4.96	1.45	4.74
LIF	3.89	0.98	1.49	0.98	1.02	1.00	2.54
IL-1 beta	2.97	1.26	3.63	1.34	4.54	1.44	3.62
IL-12p70	2.76	0.96	2.10	0.62	4.45	0.99	3.05
IL-15	2.37	1.00	1.81	1.00	3.03	0.96	2.75
IL-27	2.26	1.00	1.09	1.00	1.13	1.00	1.87
RANTES (CCL5)	1.44	0.87	1.72	1.01	1.62	1.07	1.55
GRO-alpha (CXCL1)	1.35	1.09	1.20	1.02	0.74	1.25	1.28
IL-9	1.21	1.00	1.00	1.00	1.14	1.00	1.25
IFN alpha	1.03	1.00	1.00	1.00	1.00	1.00	0.95
ENA-78 (CXCL5)	1.02	1.00	1.00	1.00	1.00	1.00	0.90
G-CSF	1.00	1.00	1.00	1.00	1.00	1.00	0.95
IL-18	1.00	1.00	1.30	1.00	2.16	1.00	1.80
IL-31	1.00	1.00	1.00	1.00	1.00	1.00	0.95
IL-23	1.00	1.00	1.00	1.00	1.00	1.00	0.95
IL-28	0.93	1.00	1.00	1.00	1.00	1.00	0.93
IL-1 alpha	0.88	1.00	1.00	1.00	1.00	1.00	0.77
M-CSF	0.64	1.00	0.92	1.00	1.00	1.00	0.75

F1 (25 ug/ml)

Cytokine	F1V + CpG + Alu	F1:NiNLP + CpG	F1:NiNLP + CpG + Alu	LcrV:NiNLP + CpG	LcrV:NiNLP + CpG + Alu	F1:LcrV:NiNLP + CpG	F1:LcrV:NiNLP + CpG + Alu
IL-5	51.18	4.98	2,346.70	0.89	2.13	5.39	1,107.76
IL-17A (CTLA-8)	21.02	11.46	128.09	1.66	1.21	12.57	26.74
IL-13	20.42	4.14	410.55	0.84	2.85	4.11	233.80
IL-4	20.23	3.17	65.10	0.97	2.64	4.82	54.85
IL-6	17.18	5.46	72.93	0.96	1.54	3.98	35.64
IL-2	15.67	6.90	128.05	1.08	2.25	6.36	37.46
IL-3	14.61	4.65	93.87	0.57	1.32	7.27	42.50
GM-CSF	9.02	2.16	67.95	1.13	1.09	2.89	35.04
IL-22	5.33	5.38	63.51	1.43	1.23	5.31	28.07
Eotaxin	5.17	0.99	2.21	0.99	1.00	1.15	1.93
IL-10	3.21	1.93	17.49	1.00	1.02	2.71	11.30
MCP-3 (CCL7)	2.73	1.82	3.21	1.06	1.24	1.93	2.79
IL-27	2.22	0.65	6.79	0.74	0.76	1.73	4.26
MIP-1 alpha (CCL3)	1.57	1.09	7.53	0.88	1.06	1.10	3.23
IL-1 beta	1.47	1.41	3.04	1.21	1.57	1.58	2.21
IFN gamma	1.40	2.71	30.21	0.21	0.94	1.46	13.95
TNF alpha	1.40	1.09	3.93	1.00	1.22	0.90	2.40
LIF	1.36	1.00	9.73	1.00	1.00	1.03	4.09
MIP-1 beta (CCL4)	1.32	1.10	9.23	1.02	1.04	1.30	4.28
IL-12p70	1.30	0.56	4.26	0.82	0.64	1.32	2.99
IP-10 (CXCL10)	1.24	2.16	3.49	0.68	0.73	1.63	2.18
IFN alpha	1.18	1.00	1.00	1.00	1.00	1.00	0.91
IL-9	1.12	1.00	7.52	1.00	1.00	1.05	3.57
ENA-78 (CXCL5)	1.09	1.00	1.00	1.00	1.00	1.00	0.97
IL-28	1.00	1.00	1.00	1.00	1.00	1.00	0.76
IL-31	1.00	1.00	1.00	1.00	1.00	1.00	0.90
G-CSF	1.00	1.00	0.83	1.00	1.00	1.00	0.86
IL-23	1.00	1.00	1.00	1.00	1.00	1.00	0.90
MIP-2 alpha (CXCL2)	0.99	0.95	2.54	1.10	0.93	0.77	1.49
GRO-alpha (CXCL1)	0.92	1.02	1.29	0.90	0.68	0.90	0.98
IL-15	0.89	0.81	3.92	0.68	0.57	1.40	2.30
RANTES (CCL5)	0.82	0.92	1.39	0.90	0.93	0.96	0.98
IL-1 alpha	0.78	1.00	0.96	1.00	1.00	1.00	0.94
IL-18	0.60	0.60	2.39	0.60	0.60	0.60	2.88
M-CSF	0.51	1.00	0.93	1.00	1.00	1.00	0.71

V (25 ug/ml)

Cytokine	F1V + CpG + Alu	F1:NiNLP + CpG	F1:NiNLP + CpG + Alu	LcrV:NiNLP + CpG	LcrV:NiNLP + CpG + Alu	F1:LcrV:NiNLP + CpG	F1:LcrV:NiNLP + CpG + Alu
IL-5	217.70	1.00	24.65	2.24	3,394.56	2.06	1,070.63
IL-13	49.86	1.00	15.65	1.84	598.55	2.33	267.90
IL-4	32.97	1.29	4.91	2.92	147.14	4.44	74.32
IL-6	25.72	0.56	1.89	5.19	52.44	4.18	31.37
GM-CSF	16.05	1.09	2.44	2.13	74.36	2.78	23.80
IL-2	15.58	1.30	4.81	2.97	85.80	2.45	22.61
IL-3	13.75	1.09	3.64	1.69	43.96	2.62	19.41
IL-17A (CTLA-8)	8.42	0.88	1.48	2.70	21.37	4.47	6.94
IL-22	7.12	0.95	2.60	2.51	35.92	3.69	15.60
IFN gamma	6.68	0.45	1.59	2.40	94.83	2.20	18.91
Eotaxin	4.96	1.00	1.07	1.23	1.99	1.07	1.76
IL-10	4.94	1.00	1.07	1.94	33.06	2.16	14.95
MCP-3 (CCL7)	4.42	1.06	1.85	1.67	5.80	1.80	4.25
MIP-1 alpha (CCL3)	3.72	0.77	1.79	1.39	13.81	1.12	6.60
IL-1 beta	3.18	0.80	1.83	2.24	7.10	1.54	4.29
TNF alpha	3.02	0.61	1.34	1.29	7.95	0.49	3.83
IP-10 (CXCL10)	2.34	0.90	1.22	1.74	4.14	1.58	2.53
MIP-1 beta (CCL4)	1.92	0.88	1.34	1.26	8.92	1.14	4.78
IL-12p70	1.48	0.87	0.74	0.90	4.76	1.18	2.12
LIF	1.39	1.00	1.00	1.14	3.77	0.97	2.27
IL-15	1.23	0.85	0.78	1.14	3.41	0.86	2.47
IL-27	1.20	1.00	1.00	1.26	2.23	1.14	1.71
MIP-2 alpha (CXCL2)	1.10	0.72	1.03	0.94	1.67	0.66	1.14
GRO-alpha (CXCL1)	1.06	0.78	0.99	1.15	0.85	0.87	0.84
IL-9	1.00	1.00	1.00	0.99	2.36	1.00	1.51
G-CSF	1.00	1.00	1.00	1.00	1.00	1.00	0.97
IL-31	1.00	1.00	1.00	1.00	1.00	1.00	0.97
IL-18	1.00	1.00	1.00	1.00	3.72	1.00	1.77
IL-1 alpha	1.00	1.00	1.00	1.00	1.00	1.00	0.82
IL-28	1.00	1.00	1.00	1.00	1.00	1.00	0.97
IL-23	1.00	1.00	1.00	1.00	1.00	1.00	0.97
IFN alpha	0.96	1.00	1.00	1.00	1.06	1.00	0.97
ENA-78 (CXCL5)	0.88	1.00	1.00	1.00	1.00	0.96	0.79
RANTES (CCL5)	0.87	0.77	1.16	0.94	1.30	0.95	1.00
M-CSF	0.76	1.00	1.00	1.00	0.91	1.00	0.87

- Extensive upregulation of numerous cytokines (e.g., IL-2, IL-3, IL-4, IL-5, IL-6, IL-13, IL-17, GM-CSF, IFN-γ) after F1V, F1, and V restimulation in mice vaccinated with F1V or NiNLP vaccines, but only when formulated with Alhydrogel.
  - Cytokine response induced by F1V, F1, and V is greater in the NiNLP relative to F1V vaccinated groups.
  - Inclusion of both F1 and LcrV in the vaccine formulation may diminish the cytokine response to the respective antigens in the F1:LcrV:NiNLP + CpG + Alu vaccinated mice.

Table 2: Total IgG response 28 days post-vaccination in BALB/c mice (Day 55)

Vaccine Groups [b]	IgG Titer [a]		
	Anti-F1V	Anti-F1	Anti-LcrV
PBS (Sham)	50 (1.00)	50 (1.00)	50 (1.00)
F1V + CpG + Alu	508,973 (1.56)	127,243 (1.23)	320,000 (1.56)
LcrV:NiNLP + CpG	8,492 (4.03)	50 (1.00)	13,481 (4.65)
F1:NiNLP + CpG	30,204 (1.61)	47,946 (1.79)	50 (1.00)
F1:LcrV:NiNLP + CpG	50,797 (1.49)	25,398 (1.53)	28,509 (1.55)
LcrV:NiNLP + CpG + Alu	227,620 (1.79)	211 (3.02)	341,044 (1.91)
F1:NiNLP + CpG + Alu	85,430 (1.26)	111,934 (1.37)	50 (1.00)
F1:LcrV:NiNLP + CpG + Alu	115,489 (1.71)	113,137 (1.32)	142,544 (1.22)

[a] Values represent geometric mean (Geo Mean) with geometric standard error (GSE).
[b] n = 4 animal sera per group.

- NiNLP vaccines, when formulated with Alhydrogel, induce a robust antibody response against F1V, F1, and LcrV antigens that is comparable to the F1V vaccine.

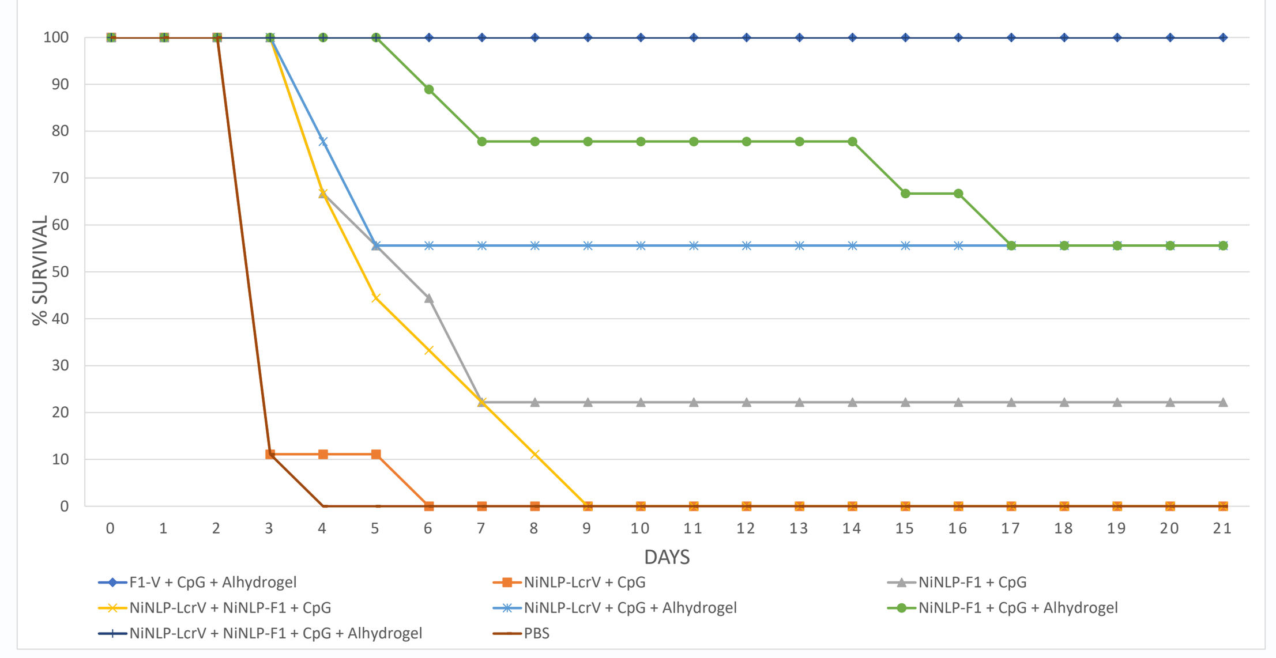


Figure 3. Survival curves of vaccinated and control BALB/c mice (n=9/group) challenged with 7.65 LD_{50} of *Y. pestis* CO92 by the aerosol route. (Day 56-77)

- The F1V and F1:LcrV:NiNLP + CpG + Alu vaccinated mice were fully protected following challenge with CO92. The single antigen F1:NiNLP and LcrV:NiNLP + CpG + Alu protected ~50% of the mice.

Table 3: Fold change relative to PBS in cytokine responses in lung and spleen homogenates from mice three days post-challenge with *Y. pestis* CO92. (Day 59)

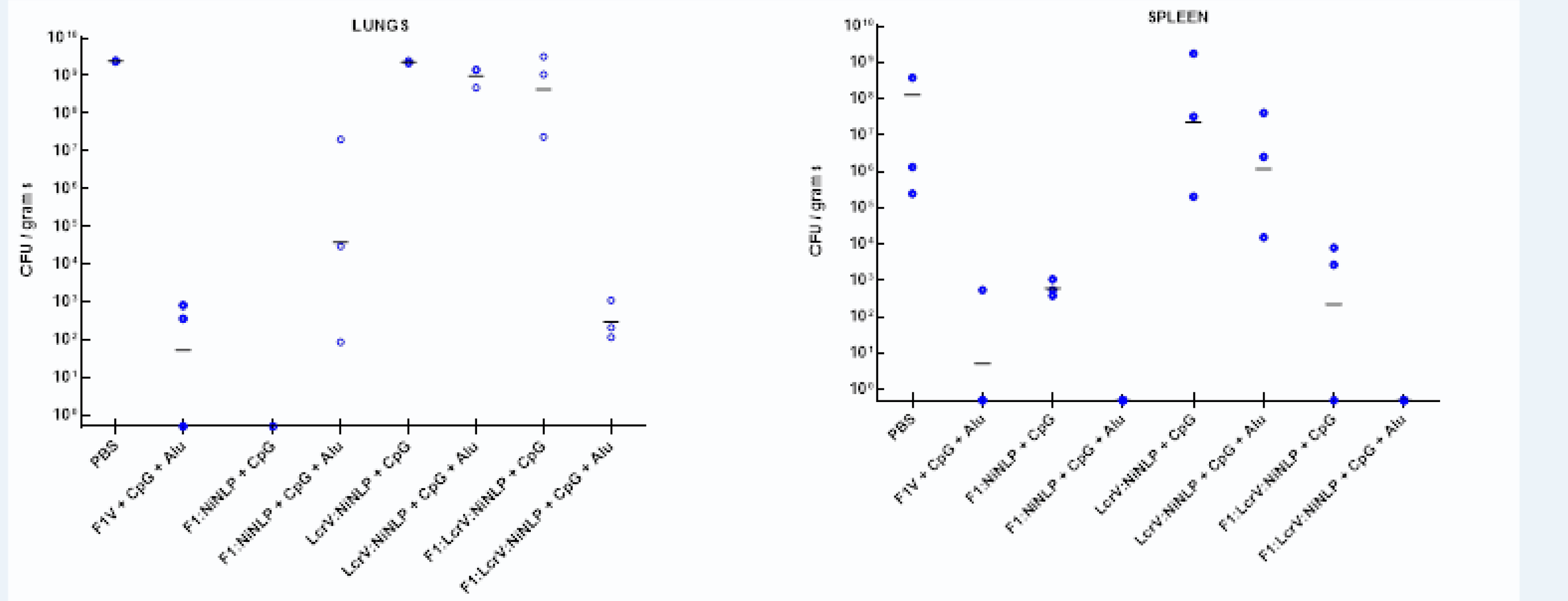
Lung homogenates 3 days post challenge with *Yp* CO92

Cytokines	F1V + CpG + Alu	F1:NiNLP + CpG	F1:NiNLP + CpG + Alu	LcrV:NiNLP + CpG	LcrV:NiNLP + CpG + Alu	F1:LcrV:NiNLP + CpG	F1:LcrV:NiNLP + CpG + Alu
IL-5	8.928	1.018	16.126	2.434	26.004	1.064	3.116
Eotaxin	1.740	1.191	1.215	1.274	1.014	0.634	0.930
IL-28	1.484	1.595	1.533	0.663	1.322	0.872	1.563
RANTES (CCL5)	1.049	0.954	1.130	1.115	1.172	0.893	0.967
IFN alpha	1.011	1.000	1.195	1.197	1.908	1.187	1.000
IL-31	1.000	1.000	0.685	1.000	0.458	0.434	1.000
IL-3	1.000	1.000	0.826	1.000	0.476	0.734	1.000
IL-2	0.893	0.722	0.769	1.032	0.880	0.698	0.575
IL-23	0.784	0.930	1.083	0.989	1.562	1.173	0.743
IL-4	0.678	0.442	3.848	1.548	1.517	0.951	0.854
IL-15	0.675	0.571	0.805	1.300	1.620	0.799	0.297
ENA-78 (CXCL5)	0.587	0.605	0.281	1.183	1.512	0.569	0.589
IL-9	0.494	0.353	0.719	1.426	1.157	0.844	0.310
IL-10	0.477	0.507	0.641	1.301	0.869	0.678	0.465
M-CSF	0.454	0.405	0.775	1.595	2.383	1.142	0.199
IP-10 (CXCL10)	0.348	0.323	0.718	1.320	1.345	0.896	0.237
IL-13	0.317	0.491	1.230	1.497	1.468	0.883	1.134
IL-27	0.224	0.220	0.337	1.556	0.802	0.591	0.163
MCP-3 (CCL7)	0.210	0.121	0.503	1.032	1.182	0.763	0.068
IL-12p70	0.170	0.203	0.694	1.050	1.431	0.829	0.170
MIP-1 beta (CCL4)	0.108	0.091	0.351	1.587	1.412	0.988	0.078
IL-1 alpha	0.085	0.066	0.257	1.048	1.137	0.800	0.055
GRO-alpha (CXCL1)	0.080	0.046	0.294	2.275	2.537	1.418	0.037
IL-1 beta	0.049	0.041	0.187	1.337	1.550	0.947	0.030
MIP-1 alpha (CCL3)	0.048	0.040	0.216	1.195	1.682	0.927	0.031
MCP-1 (CCL2)	0.039	0.023	0.122	1.836	1.224	0.450	0.004
TNF alpha	0.021	0.015	0.113	1.011	0.749	0.576	0.013
GM-CSF	0.021	0.022	0.100	0.904	0.999	0.548	0.013
G-CSF	0.019	0.014	0.090	2.162	1.569	1.143	0.005
IL-17A (CTLA-8)	0.017	0.004	0.209	1.552	1.215	1.160	0.003
IFN gamma	0.013	0.015	0.133	1.289	1.105	0.242	0.006
IL-22	0.007	0.013	0.239	1.136	1.384	0.586	0.005
IL-6	0.006	0.004	0.021	1.943	1.414	0.519	0.002

Spleen homogenates 3 days post challenge with Yp CO92

Cytokines	F1V + CpG + Alu	F1:NiNLP + CpG	F1:NiNLP + CpG + Alu	LcrV:NiNLP + CpG	LcrV:NiNLP + CpG + Alu	F1:LcrV:NiNLP + CpG	F1:LcrV:NiNLP + CpG + Alu
RANTES (CCL5)	2.716	2.745	1.063	1.103	1.135	1.131	1.633
IL-4	1.772	1.288	0.870	2.104	4.657	0.365	1.155
IL-5	1.318	1.000	1.000	1.083	9.810	1.000	1.000
IL-9	1.216	1.000	1.000	1.366	1.236	1.000	1.000
IL-15	1.025	0.665	0.664	1.723	2.256	0.664	0.664
IFN alpha	1.000	1.000	1.000	1.085	1.000	1.000	1.000
IL-31	1.000	1.000	1.000	1.000	1.000	1.000	1.000
IL-3	1.000	1.000	1.000	1.000	1.000	1.000	1.000
IL-23	1.000	1.000	1.000	1.000	1.000	1.000	1.000
M-CSF	0.979	1.250	0.430	1.777	1.792	0.281	1.266
ENA-78 (CXCL5)	0.914	0.525	0.954	0.904	1.201	1.794	0.393
GM-CSF	0.901	0.627	0.627	2.472	2.028	0.627	0.627
IL-13	0.752	0.522	0.522	1.963	1.312	0.522	0.522
IL-10	0.748	0.278	0.655	1.577	2.394	0.316	0.542
IL-28	0.648	1.452	1.857	3.230	7.356	0.899	0.576
IL-2	0.624	0.621	0.621	1.975	1.882	0.621	0.621
IL-27	0.470	0.391	0.239	2.260	2.083	0.202	0.257
IL-17A (CTLA-8)	0.384	0.454	0.454	2.980	0.725	0.454	0.454
IL-12p70	0.326	0.199	0.197	1.899	2.934	0.201	0.107
MIP-1 beta (CCL4)	0.320	0.629	0.461	2.109	1.036	0.379	0.502
TNF alpha	0.259	0.191	0.191	1.520	0.936	0.191	0.191
IL-22	0.194	0.184	0.184	2.406	0.530	0.184	0.184
Eotaxin	0.149	0.186	0.156	1.440	0.509	0.231	0.216
IP-10 (CXCL10)	0.125	0.144	0.093	1.208	1.517	0.099	0.114
MIP-1 alpha (CCL3)	0.122	0.166	0.102	2.223	0.826	0.091	0.137
IL-1 beta	0.120	0.135	0.152	1.736	0.673	0.188	0.086
IL-1 alpha	0.059	0.045	0.055	0.856	0.669	0.093	0.025
MCP-1 (CCL2)	0.042	0.024	0.024	2.015	1.841	0.024	0.024
MCP-3 (CCL7)	0.039	0.040	0.035	3.373	0.875	0.069	0.034
IL-6	0.028	0.028	0.012	3.075	0.919	0.053	0.012
IFN gamma	0.026	0.026	0.006	2.821	4.438	0.007	0.007
GRO-alpha (CXCL1)	0.015	0.014	0.030	1.185	0.353	0.227	0.009
G-CSF	0.009	0.009	0.015	1.870	0.482	0.164	0.009

- Following challenge cytokine attenuation was greatest in mice vaccinated with F1:LcrV:NiNLP + CpG + Alu followed by mice vaccinated with F1V + CpG+ Alu. Surprisingly, mice vaccinated with F1:NiNLP + CpG had a similar level of cytokine modulation as the F1V + CpG + Alu vaccinated mice and yet the level of protection was only ~22%.



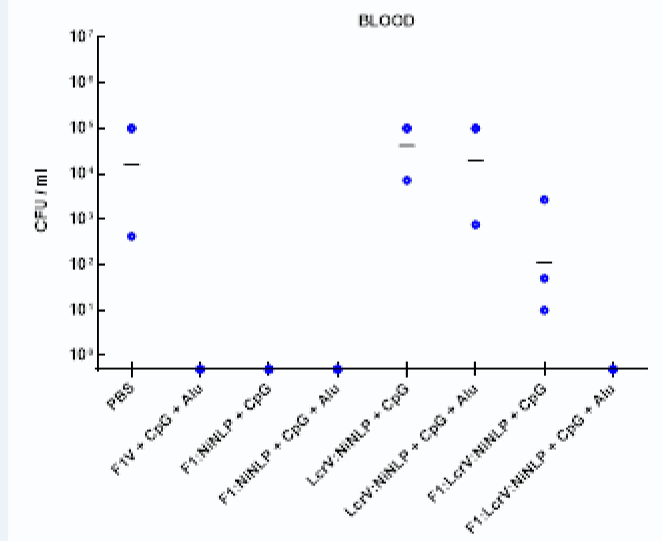


Figure 4. The recovery of bacteria as determined by CFU from lungs, blood, and spleens of BALB/c mice 3 days post-challenge with *Y. pestis* CO92. The samples were from 3 mice/group. The left axis represents CFU/ml (blood) or CFU/g (lung and spleen). The individual points represent one animal. The dash symbol is the geometric mean. The limits of detection were 100 CFU/ml blood and 5 CFU/g spleen or lung homogenate. (Day 59)

- Bacterial load in the lungs is a good indicator of protection provided by the F1V + CpG + Alu and F1:LcrV:NiNLP + CpG + Alu vaccines. Similarly, bacterial load in the spleen is comparable to that of the lungs but multiple logs lower. Surprisingly, there was no recoverable bacteria in the lungs of F1:NiNLP + CpG vaccinated mice but a significant bacterial load was present in the spleen.

Conclusions

- The most protective vaccine formulations (F1V + CpG + Alu and F1:LcrV:NiNLP + CpG + Alu) induced a strong total IgG response against F1, V, and F1V proteins.
- Both vaccine formulations induced a strong cell-mediated immune response with induction of Th1-, Th2-, and Th17-related cytokines, although the NLP-based vaccine induced a stronger cytokine response relative to F1V vaccine.
- Much like with the F1V vaccine, the inclusion of Alhydrogel in the NLP vaccine formulations was critical for enhanced immunogenicity and protection.
- Bacterial load in the lungs correlated with hypercytokinemia at 3 days post-challenge with *Y. pestis* CO92. The two most protective vaccines also prevented bacteremia and mitigated bacterial dissemination into the spleens.

Summary

The modularity and lipid bilayer structure of NLPs may offer additional advantages relative to the chimeric protein formulation with potential for incorporation of additional protective antigens with adjustable ratios (to manipulate the resulting immune response) and against multiple pathogens, as well as incorporation of transmembrane proteins.

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Opinions, interpretations, conclusions, and recommendations are those of the authors and are not necessarily endorsed by the U.S. Army or the Department of Defense Health Agency.

Research was conducted in compliance with the Animal Welfare Act and other federal statutes and regulations relating to animals and experiments involving animals and adheres to principles stated in the *Guide for the Care and Use of Laboratory Animals*, National Research Council, 2011. The facility where this research was conducted is fully Accredited by the Association for Assessment and Accreditation Of Laboratory Animal Care International.

POINTS OF CONTACT

Sergei Biryukov
sergei.s.biryukov.civ@health.mil