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Control # 106

Longevity of Vaccine Protection Against Pneumonic Plague in BALB/c Mice

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Abstract

Background
Yersinia pestis is the etiological agent of bubonic and pneumonic plague. Subunit vaccine approaches comprising recombinant forms of the fraction 1 (F1) and V (virulence) proteins have demonstrated protection against pneumonic plague in mice. At last year's meeting, we reported on protection conferred by rYP vaccine at Day 49 post-vaccination and that F1 and V antibody levels correlated with protection against challenge with *Y. pestis* CO92. Here, using a similar vaccination regimen we have extended our studies to investigate the longevity of protection induced by rYP vaccine.
Methods
On day 0 and 21, three sets of Balb/c mice (N=110/set) in Groups 1-6, were vaccinated with 2.50, 0.63, 0.16, 0.04, 0.01 and 0µg vaccine plus Alhydrogel, respectively. Blood collected on various times post-vaccination was analyzed for F1 and V antibodies by ELISA. On day 79, 109 and 139 all mice were challenged with 1.1E+06, 1.4E+06, or 2.0E+06 CFU/L *Y. pestis* CO92 via nose-only inhalation exposure and monitored for 14 days. *Y. pestis* CO92 bacterial counts were performed on terminal blood samples using selective agar plates.
Results
On Day 21, mean F1 and V antibody titers in Groups 1-5 were 2.58, 2.42, 2.22, 1.23, 1.05 and 1.08, 1.06, 1.01, 1.04, 1.03 $\log_{10}$ assay units, respectively. F1 and V antibodies continued to rise in Groups 1-5 following the 2° vaccination on Day 21. Notably, V-specific antibodies were significantly boosted in Groups 1-5 by Day 29 ($p<0.01$). As expected, F1 and V antibodies were not detected in Group 6. Following challenge with *Y. pestis* CO92 (mean inhaled dose, 6.7x10^5 CFU) on Day 79, survival rates in Groups 1-6 were 95, 90, 80, 40, 0 and 0%, respectively.
Conclusions
F1 and V antibodies induced by an F1/V-based vaccine correlated with protection against pneumonic plague. Pending data will shed further light on the ability of rYP vaccine to confer protection on Day 109 and 139.

Introduction

Yersinia pestis (*Y. pestis*) is the etiological agent of bubonic and pneumonic plague. Subunit vaccine approaches comprising recombinant forms of the fraction 1 (F1) and virulence (V) proteins have demonstrated protection against pneumonic plague in mice. Here, we performed a dose-sparing immune duration efficacy study in mice using an F1/V-based vaccine in attempts to further evaluate correlates of immunity in rYP vaccinated mice following extended times to lethal inhalation challenge with *Yersinia pestis* CO92.

Methods

Experimental Design

Groups of Balb/c mice were vaccinated with decreasing concentrations from 2.5 µg to 0 µg of the rYP vaccine as shown in Table 1. Mice were vaccinated on Days 0 and 21. Twenty (20) mice from each vaccinated group and ten (10) mice from the control group were bled on Days 7, 14, 21, 28, 49, 73, and 103. Group A (sub group 1~6) were challenged on Day 79 and group B (sub group 7~12) were challenged on day 109.

Table 1. Description of test article administration to each group.

Group	Sub Group	Test/Vehicle Article Administered	Dose Level	Dose Volume	Challenge Article	Challenge Dose (LD_{50})	No. of Core Animals
A	1	rYP Vaccine	2.5 µg	0.1 mL	YpC092	50	20
	2	rYP Vaccine	0.625 µg	0.1 mL	YpC092	50	20
	3	rYP Vaccine	0.16 µg	0.1 mL	YpC092	50	20
	4	rYP Vaccine	0.04 µg	0.1 mL	YpC092	50	20
	5	rYP Vaccine	0.01µg	0.1 mL	YpC092	50	20
	6	Vehicle + Adjuvant	0	0.1 mL	YpC092	50	10
B	7	rYP Vaccine	2.5 µg	0.1 mL	YpC092	50	20
	8	rYP Vaccine	0.625 µg	0.1 mL	YpC092	50	20
	9	rYP Vaccine	0.16 µg	0.1 mL	YpC092	50	20
	10	rYP Vaccine	0.04 µg	0.1 mL	YpC092	50	20
	11	rYP Vaccine	0.01µg	0.1 mL	YpC092	50	20
	12	Vehicle + Adjuvant	0	0.1 mL	YpC092	50	10

Methods

Bacterial Production

Y. pestis CO92 working stocks were thawed and inoculated on tryptose blood agar base plates. Plates were incubated at 28 ± 3 °C for approximately 48 to 72 hours and bacterial growth harvested in 1% peptone. The concentration of the bacterial suspension was estimated based on an optical density (OD) reading with the actual concentration of the challenge material determined by retrospective plate counts.

Inhalation Challenge System and Procedure

Mice were challenged with 1.1E+06, 1.4E+06, or 2.0E+06 CFU/L of *Y. pestis* CO92 for 30 minutes by nose-only inhalation on Days 79 (Group A), 109 (Group B), and 139 (Group C), respectively. Mice were monitored for 14 days post challenge. The inhalation challenge system is shown below in Figure 1.

Figure 1. The murine nose-only inhalation challenge system consisted of six components: a compressed air source, an aerosol generation and delivery line, a 48-port radial nose-only inhalation challenge plenum, an aerosol characterization platform, an air handling station, and an exhaust platform.

Immunological Assays

Serum samples were analyzed for anti-F1 and V IgG antibodies by ELISA. Immune sera obtained from Day 7, 14, 21, 28, 49, 73, and 103 post primary immunization were screened for anti-F1 and anti-V total immunoglobulin G (IgG) antibodies using the Protein G Peroxidase Conjugated system. Sera from each time-point were diluted in two-fold serial dilution steps from 1:100 to 1:12800 and tested in duplicate against rF1 and rV protein by ELISA. Pre vaccinated sera was used as negative control and a mouse reference sera (MRS) was tested on each plate as standard curve.

Statistical Analysis

OD measurements for the pre-vaccinated samples were used to calculate rF1 and rV limits of detection (LoD). Assay units were calculated using MRS as a standard curve[1], where MRS was assigned a value of 1000 assay units. Animal rV and rF1 specific antibody levels ($\log_{10}$ assay units) on Days 73 and 103 were correlated (logistic regression) with the survival outcomes of animals in Groups A and B, respectively. SAS™ v9.2 (Cary, North Carolina) and an alpha value = 0.05 was used for all statistical comparisons.

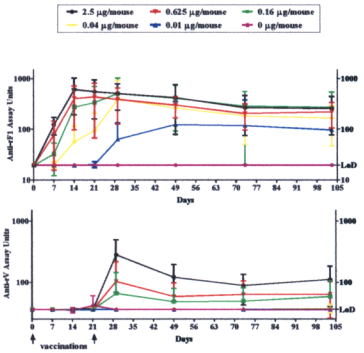
Microbiology

Bacterial clearance of surviving animals were confirmed by a spleen bacterial load assay. Briefly, spleens were collected from animals found dead, euthanized moribund, or survived and euthanized on Day 94 (for Group A) and Day 124 (for Group B) . Homogenized spleens were diluted in PBS, and analyzed for the presence and quantification of *Y. pestis*.

Results

Immunological Analysis

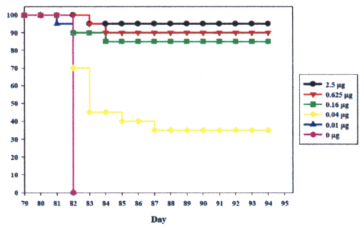
Figure 2. Mean anti-rF1 and anti-rV titers are shown below for dose groups 0 - 2.5µg/mouse up to 103 days post-vaccination. Mice received the 1st vaccination on Day 0 followed by a 2nd boost vaccination on Day 21. Vertical bars indicate the standard deviation for each group/time-point. The LoD (limit of detection) for the assay is indicated on the right y axis.



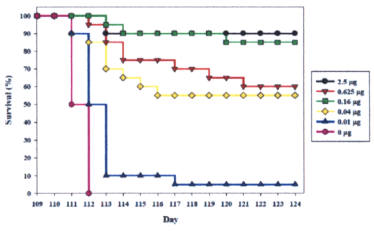
Post Challenge Survival

Figure 3. Survivability of Mice Vaccinated with rYP plague vaccine and challenged on Day 79 (Group A) and 109 (Group B) with *Y. pestis* CO92

Group A Survival Day 79 Challenge



Group B Survival Day 109 Challenge



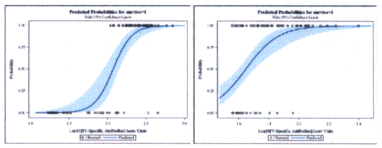
Results

F1 and V Antibody Titers Correlate with Survival

Figure 4. Correlation between anti-rF1 and rV antibodies and survival following challenge at Day 79 and 109 with *Y. pestis* CO92.

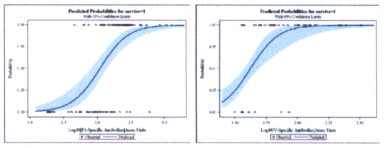
Day 79 Challenge

Anti -rF1 **Anti -rV**



Day 109 Challenge

Anti -rF1 **Anti -rV**



Conclusions

1. F1-specific antibodies were detected as early as Day 7 post-vaccination reaching high titers by Day 14 for mice vaccinated with higher doses of rYP vaccine. While the appearance of V-specific antibodies was delayed, titers were boosted following the 2° vaccination on Day 21.
2. rYP vaccine induced higher levels of antibodies to the F1 protein compared to the V protein. Nevertheless, both F1 and V antibodies titers were maintained up to the time of *Y. pestis* challenge on Day 79 and 109.
3. More importantly, logistic regression analysis demonstrated that F1 and V-specific antibody titers measured just prior to challenge on Day 73 and Day 103 correlated with survival against lethal inhalation challenge with *Y. pestis* CO92.

References

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2. Guyton, AC. *Measurement of the respiratory volumes of laboratory animals (1947).* American Journal of Physiology. 1947, 150: 70-77.

Acknowledgements

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