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Control # 175

Humoral Immunity to F1 and V Correlates with Protection Against Pneumonic Plague in Mice

Z.N. Llewellyn[1], F. Koide[2], L.E. Bowen[1], L. Nieves-Duran[2], N. Harman-Richardson[3], T. Babas[2], J. Boydston[1], J. Trombley[1], K. Bohannon[1], and P. Silvera[2]

[1]Southern Research Institute, 2000 Ninth Avenue South • Birmingham, AL 35205
[2]Southern Research Institute, 431 Aviation Way, Frederick, Maryland 21701
[3]Alpha StatConsult, LLC, 9820 Warthen Drive • Damascus, MD 20872

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llewellyn@southernresearch.org
Silvera@southernresearch.org

Abstract

Background
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 efficacy study in mice using an F1/V-based vaccine in attempts to identify immune correlates of protection following inhalation challenge with *Y. pestis* CO92.

Methods
On Days 0 and 21, Balb/c mice (N=196) in groups 1-6, were vaccinated with 10, 2.5, 0.625, 0.16, 0.04 and 0µg vaccine plus Alhydrogel, respectively. Blood collected on Days 7, 14, 21, 28, 49 was analyzed for F1 and V-specific antibodies by ELISA (N=136). On Day 49, all remaining mice (N=60) were challenged with a mean aerosol concentration of 1.8E+06 CFU/L *Y. pestis* CO92 via nose-only inhalation and monitored for 14 days. *Y. pestis* CO92 bacterial counts were performed on terminal blood samples using selective agar plates.

Results
Vaccine doses of 10 and 2.5µg induced robust antibody titers to both F1 and V. F1-specific antibodies were detected as early as Day 7 post-vaccination. By contrast, V-specific antibodies were not detected until Day 21. Nevertheless, these titers were significantly boosted following the secondary vaccination on Day 21 ($p<0.001$). On the day of challenge, F1 and V-specific antibody titers ranged between log 2.3-2.5 and 1.9-2.6 assay units, respectively. Following challenge with *Y. pestis* CO92 (mean inhaled dose, 5.3E+05 CFU) on Day 49, survival rates in Groups 1to 6 were 90, 100, 70, 80, 40 and 0%, respectively and all survivors yielded negative cultures for *Y. pestis* CO92. More importantly, regression analysis demonstrated that F1 and V antibody titers correlated with survival.

Conclusions
F1 and V antibodies induced by an F1/V-based vaccine correlated with protection against pneumonic plague.

Introduction

Despite considerable research efforts, no vaccine is currently available to protect humans against pneumonic plague. A formalin-killed (*Yersinia pestis*) *Y. pestis* whole-cell vaccine was widely used by the U.S. Army; however, due to its reactogenicity and failure to protect against pneumonic plague it is no longer used. So far, the F1 and V proteins recombinant vaccines have shown promise. The purpose of this study was to determine the optimal dose of F1/V-based vaccine that will elicit protective immunity against a lethal challenge of *Y. pestis* CO92 in mice.

Methods

Experimental Design

Six groups of mice were vaccinated with decreasing concentrations from 10 µg/0.1 mL in Group 1 to 0 µg/mL in Group 6 of the F1/V-based vaccine as shown in Table 1. Mice were vaccinated on Days 0 and 21. Five mice from each vaccinated group and 2 mice from the control group were euthanized on Days 7, 14, 21, 28, and 49. Ten mice from each group were challenged on Day 49 and then euthanized on Day 63.

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

Dose Group	Test/Vehicle Article Administered	Test/Control Article Dose Level	No. of Animals
1	F1/V-based vaccine	10 µg	35
2	F1/V-based vaccine	2.5 µg	35
3	F1/V-based vaccine	0.625 µg	35
4	F1/V-based vaccine	0.16 µg	35
5	F1/V-based vaccine	0.04 µg	35
6	Vehicle + Adjuvant [a]	0	20

[a] Group 6 received the same concentration of adjuvant (0.11%) as Group 1 (the highest concentration of adjuvant delivered).

Methods

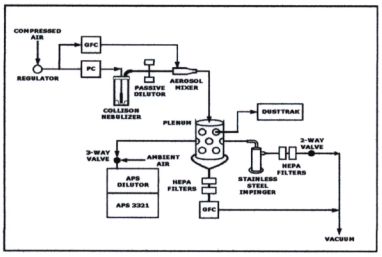
Bacterial Production

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 was 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 Exposure System and Procedure

Mice were exposed to a mean aerosol concentration of 1.8E+06 CFU/L of *Y. pestis* CO92 for 30 minutes in a radial nose-only inhalation exposure system on Day 49 and monitored for up to 14 days post exposure. The inhalation exposure system is shown below in Figure 1.

Figure 1. The inhalation exposure system consisted of five components: an aerosol generation and delivery line, a 36-port rodent nose-only inhalation exposure plenum, an aerosol characterization platform, an exhaust platform, and an air handling station.



Immunological Assays

Serum samples were analyzed for anti-F1 and V IgG and subclass antibodies by ELISA. Immune sera obtained from Day 7, 14, 21, 28, and 49 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 the same time points were diluted in twofold serial dilution from 1:100 to 1:12800 and tested in duplicate wells against rF1 and rV protein by ELISA each day. Pre-vaccinated sera was used as negative control and mouse reference sera (MRS) was tested on each plate as standard curve.

Statistical Analysis

The OD measurements for the pre-vaccinated samples were used to calculate rF1 and rV limits of detection (LoD)[1]. Assay units were calculated using MRS as a standard curve[2]. Assay units ($\log_{10}$) were non-normally distributed (rF1, rV Shapiro-Wilk=0.64,0.76, $P<.0001$). Non-parametric statistics were used to compare rV - and rF1 – specific antibodies across vaccinated groups. Median rV and rF1 specific antibody levels ($\log_{10}$ assay units) on day 49 were correlated (logistic regression) with survival outcome for a challenge group of animals treated with the same vaccine doses. SAS™ v9.2 (Cary, North Carolina) and an alpha value =0.05 was used for all statistical comparisons.

Microbiology

Spleens and blood were collected from animals found dead, euthanized moribund, or at euthanasia on Day 64. Spleens were homogenized. Homogenized spleens and blood were diluted in PBS, and analyzed for the presence and quantification of *Y. pestis*.

Results

Immunological Analysis

Figure 2. The median anti-rF1 and anti-rV titer in log assay unit are shown below up to Day 49. The mice received the first immunization on Day 0 and the second boost immunization on Day 21. The horizontal bar indicates inter-quartile range and dots indicate calculated median for each group per day.

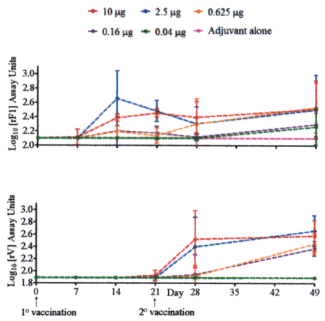
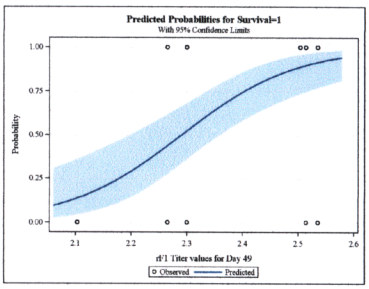
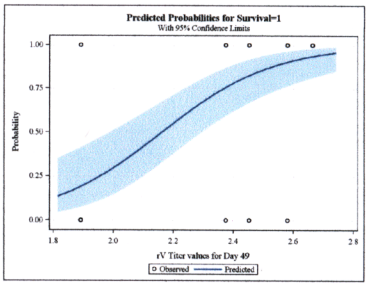


Figure 3. The correlation between anti-rF1 and rV antibodies and murine survival following challenge at Day 49 with *Y. pestis* CO92 are shown below.

Anti -rF1



Anti-rV



Results

Microbiological Analysis

Figure 4. Survivability of mice vaccinated with F1/V-based vaccine and challenged with *Y. pestis* CO92.

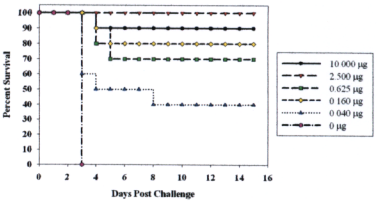
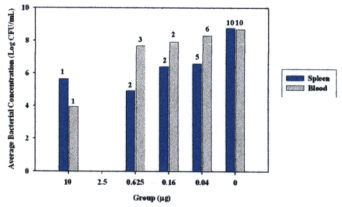


Figure 5. Average concentration of *Y. pestis* CO92 in the blood and spleen collected at euthanasia. The number of samples that were included in the analyses are shown above the bars.



Conclusions

1. F1-specific antibodies were detected as early as Day 7 post-vaccination. By contrast, the appearance of V-specific antibodies was delayed until Day 21. However, V-specific antibody titers were boosted following the second vaccination on Day 21.
2. Low detectable levels of F1-specific antibodies were measured at 0.04µg F1/V-based vaccine dose group on Day 49, but the same dose failed to induce V-specific antibodies.
3. F1 and V-specific antibody titers at the time of challenge (Day 49) correlated with survival. 10µg and 2.5µg immunized groups yield >90% survival probability.

References

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Acknowledgements

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