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

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Abstract

Background
Jerivme pestis is the etiological agent of bubonic and pneumonic plague. Subunit vaccine approaches comprising recombinant forms of the fraction 1 (F) and V (virulence) proteins have demonstrated protection against pneumonic plague in mice. At last year's meeting, we reported on protection conferred by F97p vaccine at Day 49 post-vaccination and that F97 and V antibody titers correlated with protection against challenge with J. pestis CC925. Here, using a similar vaccination regimen we have extended our studies to investigate longevity of protection induced by F97p vaccine.

Methods
On Day 0 and Day 21, three sets of Balb/c mice (N=1106) in groups 1-6, were vaccinated with 2.50, 0.25, 0.08, 0.04, 0.02 and non-vaccine placebo, respectively. Blood collected on various times post-vaccination was analyzed for F and V antibodies by ELISA. On Day 79, 109 and 139 mice were challenged with 1.1E6, 1.4E6, or 2.1E6CFU of J. pestis CC925 via intra-nasal instillation exposure and monitored for 14 days. J. pestis CC925 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 2.8, 2.42, 2.22, 1.23, 1.05 and 1.06, 1.01, 1.04, 1.03 log2, respectively. F1 and V antibodies continued to decline in Groups 1-5 following the 2nd vaccination on Day 21. Notably, V-specific antibodies were significantly boosted in Groups 1-5 by Day 29 (p<0.05). As expected, F1 and V antibodies were not detected in Group 6. Following challenge with J. pestis CC925 (mean lethal dose, 6E6 CFU) on Day 79, survival rates in Groups 1-5 were 95, 90, 80, 45, 20 and 0%, respectively.

Conclusions
F1 and V antibodies induced by an F79p-based vaccine correlated with protection against pneumonic plague. Preclinical data will be used to develop a vaccine for clinical protection on Day 109 and 139.

Introduction
Jerivme pestis (J. 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. Here, we report on a one-year longitudinal antigen exposure study in mice using an F79p-based vaccine in attempts to further evaluate correlates of immunity in F97p vaccinated mice following extended times to lethal challenge infection with J. pestis CC925.

Methods

Bacterial Production
J. pestis CC925 working stocks were thawed and inoculated on tryptic blood agar base plates. Plates were incubated at 28 ± 1 °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.

Immunological Analysis
Mice were challenged with 1.1E6, 1.4E6, or 2.1E6CFU of J. pestis CC925 for 30 minutes via nose-only instillation on Days 79 (Group A), 109 (Group B) and 139 (Group C), respectively. Mice were monitored for 14 days post-challenge. The immunological challenge system is provided below in Figure 1.

Figure 1. The murine nose-only immunological challenge system consisted of six components: a compressed air source, an aerosol generation and delivery line, a 49-port radial nose-only immunological challenge platform, an aerosol characterization platform, an edifice handling station, and an exhaust platform.

Immunological Assays
Serum samples were analyzed for anti-F1 and V IgG antibodies by ELISA. Serum samples obtained from Days 7, 14, 28, 38, 93, 105, and 105 post-primary immunization were assayed for anti-F1 and anti-V total immunoglobulin G (IgG) antibodies using the Protein G ProteinA Coated system. Serum from each time-point were diluted in two-fold serial dilution steps from 1:100 to 1:1280 and tested in duplicate against F1 and V protein by ELISA. Pre-vaccinated sera were used as a negative control and a mouse reference sera (MRS) was tested on each plate as standard curves.

Statistical Analysis
OD measurements for the pre-vaccinated samples were used to calculate F1 and V limits of detection (LOD). Assay units were calculated using MRS as a standard curve, where MRS was assigned a value of 1000 assay units. Analyte F1 and V specific antibody levels (ng/mL, assay units) on Days 79 and 109 were correlated logistically with the survival outcomes of animals in Groups A and B, respectively. SASSM-2.2 (Cary, North Carolina) and an alpha value = 0.05 was used for all statistical comparisons.

Microbiology
Bacterial clearance of surviving animals was confirmed by a spleen bacterial load assay. Briefly, spleens were isolated from animals (standard dissection of submandibular, auricular and mesenteric lymph nodes) on Day 94 (for Group A) and Day 124 (for Group B). Homogenized organs were plated on PDS, and analyzed for the presence and quantification of J. pestis.

Results

Immunological Analysis
Figure 2. Mice anti-F1 and anti-V antibody titers are shown below for dose groups 1-2.5ug/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/timepoint. The LOD (limit of detection) for the assay is indicated on the right y-axis.

Post Challenge Survival
Figure 2. Survivability of mice vaccinated with F97p vaccine at the challenge on Day 79 (Group A) and 109 (Group B) with J. pestis CC925.

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 F97p vaccine. While the appearance of V-specific antibodies was delayed, titers were boosted following the 2nd vaccination on Day 21.

2. F97p vaccine induced higher levels of antibodies to the F1 protein compared to the V protein. Nevertheless, both F1 and V antibodies were maintained up to the time of T. pesti 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 challenge infection with J. pestis CC925.

Acknowledgements
This contract was funded by the National Institutes of Health, National Institute of Allergy and Infectious Diseases, Contract N01-AI-30960.

References


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