Poster HMB-1021 | ASM Microbe | 15-19-2023, Houston, TX.

Layered and integrated medical countermeasures to protect against pneumonic plague in mice after exposure to aerosolized non-encapsulated *Yersinia pestis*.

Yuli Talyansky, Christopher P. Klimko, Jennifer L. Shoe, Nathaniel O. Rill, Melissa Hunter, Jennifer L. Dankmeyer, Ronald Toothman, Sergei S. Biryukov, Joel Bozue, David DeShazer, and Christopher K. Cote.

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

Abstract

Yersinia pestis is the bacterium that causes plague, a disease of historical significance that continues to be both a public health and a biodefense threat. A significant effort has focused on identifying protective vaccine strategies to protect against *Y. pestis* infections; however, there are no approved vaccines to prevent or ameliorate disease. When used as individual medical countermeasures monoclonal antibodies, antibiotic treatment plans (therapeutics or post-exposure prophylaxes), and vaccine strategies remain suboptimal. Our objective was to improve overall disease outcomes by layering vaccines with antibiotic regimens. We define a "layered" approach as multiple medical countermeasures that are delivered at distinct times, whereas a "combination" strategy would deliver multiple medical countermeasures concurrently. In these studies, we focused on pneumonic plague initiated after exposure to non-encapsulated *Y. pestis* because when disease is initiated after exposure to encapsulated *Y. pestis* the mice are substantially easier to protect. BALB/c mice that were naïve or vaccinated with two doses of the live attenuated *Y. pestis* Δ*yscN* vaccine were exposed to aerosolized *Y. pestis* C12 (non-encapsulated) approximately 30 days after the last vaccination. Streptomycin (Q6) or ciprofloxacin (Q12) was initiated at 60 h post-infection and the regimen was for five days. Mice were observed for 21 days post-cessation of antibiotics. Administering an anti-LcrV monoclonal antibody was also examined as a possible component of a layered defense strategy. When used in a layered approach, current vaccine strategies to combat plague (*Y. pestis* CO92 Δ*yscN*, C12 Δ*yscN*, or recombinant F1V) and antibiotic regimens or monoclonal antibodies result in significantly improved protection in the mouse models of pneumonic plague due to synergy associated with the medical countermeasures. These results were similar when examining several intentionally sub-optimal antibiotic regimens. Initial studies also demonstrate the utility of layering monoclonal antibodies with sub-optimal vaccination strategies. Importantly, this layered strategy worked similarly when either protein subunit or live attenuated vaccines were evaluated. Layered and integrated medical countermeasures will provide novel treatments not only to combat *Y. pestis* infections but also diseases caused by other microbial pathogens that are refractory to individual strategies, particularly in the case of engineered or emerging bacterial biothreat agents.

Introduction

Medical countermeasures (MCM), including vaccines, prophylactics, and therapeutics, are often studied independent of each other in animal models of infectious disease. Our Layered and Integrated Medical Intervention Technologies (LIMIT) program has allowed us to evaluate the interplay of multiple MCM in animal models of infection. The primary objectives of the LIMIT program are to lengthen the therapeutic dosing window and identify beneficial MCM combinations that will provide the best protection for service members. Combination therapy may also identify potential MCM dose sparing options, such as decreasing the amount of MCM delivered and/or decreasing the duration of MCM treatment. We have examined vaccination plus antibiotic or monoclonal antibody (mAb), to treat BALB/c mice infected with a lethal subcutaneous or aerosolized dose of *Y. pestis* C12. This unencapsulated isolate of *Y. pestis* CO92 has been shown to be refractory to many vaccine strategies including rF1-V as well as some live vaccine platforms. Additionally, delayed antibiotic treatment can result in significant mortality in unvaccinated animals and the window of opportunity to protect is short (within approximately 60-72 hours after exposure to aerosolized *Y. pestis*). Thus, the layered defense strategy is required to provide full protection using suboptimal medical countermeasures and offers flexible strategies to deal with emerging or engineered threats.

Methods

Animals and vaccination studies. Female BALB/c mice were 7-10 weeks of age at time of vaccination. Vaccinated mice were administered the second dose 20-28 days after the initial vaccine dose. Sera and organs were collected from a cohort of mice to assess immune responses to the vaccines and the effect on bacterial burden. Mice were challenged 27-29 days post final vaccination. Mice were exposed to aerosolized (pneumonic) or SC (bubonic) challenge doses of virulent *Y. pestis* C12.

Treatment of vaccinated mice. Streptomycin was prepared in water for injection and a dose of 20 mg/kg administered to mice by the intraperitoneal (IP) route every six hours for five days. The mice were injected with the antibiotic beginning 60 h after exposure to aerosolized *Y. pestis* C12. The monoclonal antibody 7.3 was provided by Dstl, Porton Down, UK and was administered via IP injection approximately 60 h post infection at 50 ug per mouse.

Results

Live attenuated *Y. pestis* vaccines could be an important component of layered defense strategies against plague: antibiotics

The protection afforded BALB/c mice against aerosol challenge with *Y. pestis* C12 by a suboptimal vaccination and delayed post-challenge antibiotic treatment strategy was examined. The mice were either vaccinated twice with mutant CO92 Δ*yscN* (21 days apart) or administered buffer alone, and then challenged 4 weeks after the second vaccine dose with a lethal aerosol dose of *Y. pestis* C12. They were subsequently treated with 20 mg streptomycin/kg (or buffer alone) beginning 48 or 60 hours after challenge, and the effects on survival determined, as shown in **Figure 1A** and **Figure 1B**. The vaccination had no effect on the survival of mice after challenge with *Y. pestis* C12, while the administration of the antibiotic was associated with 90% and 100% of the treated (only) and vaccinated, treated mice, respectively (**Figure 1A**). However, increasing the time between challenge and treatment to 60h revealed the enhanced effect on survival of the layered approach. Whereas all mice treated with 20 mg/kg streptomycin alone or vaccinated alone succumbed, 80% of the vaccinated and treated mice survived (p = 0.0007 versus treated and p = 0.0011 versus vaccinated alone); the median TTM of the treated group was extended compared to the unvaccinated and vaccinated control groups (**Figure 1B**), *i.e.*, 7.0 days versus 5.0 days (p = 0.0044) or versus 5.5 days (p = 0.044), respectively. The benefits accrued from this vaccination-treatment scheme was further illustrated in **Figure 1C**. To improve the survival of unvaccinated mice treated at a delayed time after challenge, animals were given a higher dose of streptomycin. A total of 20% of mice receiving 40 mg/kg streptomycin 60 h after challenge survived. In contrast, 70% of the CO92 Δ*yscN* -vaccinated, treated mice survived, an increase that approached significance at days 7 and 21 (p = 0.070). All mice in the two control groups succumbed by day 6, p = 0.0044 versus vaccine and KPhos controls compared to vaccinated and treated mice (**Figure 1C**). The C12 Δ*yscN* vaccine was evaluated similarly in the context of antibiotic treatment (**Figure 2**). Vaccination and treatment with 20 mg/kg streptomycin 60 h post-challenge with C12 elicited significantly better protection against aerosol infection. In addition, the C12 challenge dose was higher in this experiment (1.67 x 10^6 CFU, 21 LD_{50}s) than that used in the **Figure 1** study (GM 3.62 x 10^5 CFU, 5 LD_{50}s). Half of the vaccinated and treated mice survived and none of the vaccinated and untreated or unvaccinated treated controls survived (p = 0.0325 for survival at days 7 and 21 after challenge). This layered therapy revealed that the combinations of vaccination and post-exposure antibiotic treatment were synergistic in the challenge experiments shown in **Figure 1B** (synergy score 5.28, p = 0.0013), **Figure 1C** (synergy score 2.91, p = 0.0040), and **Figure 2** (synergy score 3.16, p = 0.009). These synergistic effects allowed the treatment to be delayed after exposure to aerosolized *Y. pestis* and may facilitate a sparing effect on the amount of antibiotic required.

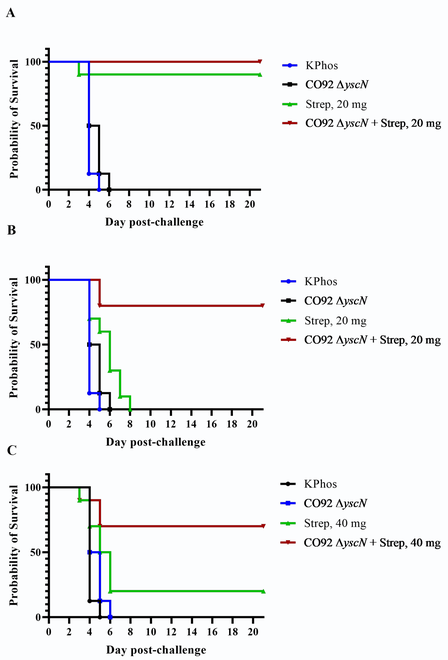

Figure 1. The protection of BALB/c mice against lethal aerosol challenge with *Y. pestis* strain C12 by a vaccination and post-challenge antibiotic treatment strategy: The impact on survival of antibiotic dose and time of treatment. In each experiment, two groups of mice were vaccinated twice (20 days apart) with mutant CO92 Δ*yscN* (0.92 x10^7 CFU and 0.48 x 10^7 CFU/mouse, respectively), and two groups were unvaccinated. All mice were challenged by the aerosol route with *Y. pestis* C12 four weeks after the boost dose. The challenge doses were administered in two runs, with doses of 5.6 x 10e^5 CFU/mouse (6 LD_{50}s) for vaccinated groups and 3.11 x 10^5 CFU (4 LD_{50}s) for unvaccinated mice. The mice were treated with either 20 mg streptomycin starting at 48h (**A**) or 60h (**B**) after challenge; or 40 mg starting at 60h after challenge (**C**). n = 8 mice/group (KPhos alone and vaccine alone) or 10 mice/group (streptomycin alone and vaccine + streptomycin).

Live attenuated *Y. pestis* vaccines could be an important component of layered defense strategies against plague: monoclonal antibodies

As demonstrated in **Figure 3**, the delayed treatment with the anti-LcrV mAb 7.3 did offer an added benefit to mice receiving a suboptimal LAV. This experiment is proof-of-concept and additional follow-on experiments are planned. While the survival rates did not reach statistical significance in this experiments, there was a statistically significant increase in time-to-death or euthanasia when the mAb and vaccines were used in a layered approach ($p \leq$ 0.001)

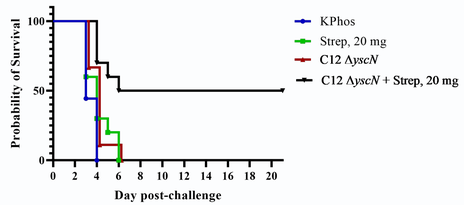

Figure 2. The protection of BALB/c mice against lethal aerosol challenge with *Y. pestis* strain C12 by a vaccination and post-challenge antibiotic treatment strategy. The mice were vaccinated as described in Figure 6 but with mutant C12 Δ*yscN* (0.48 x 10^7 and 0.70 x 10^7 CFU for doses 1 and 2 respectively); controls received KPhos alone. They were either untreated or treated with 20 mg/kg streptomycin 60 h after challenge with *Y. pestis* strain C12 four weeks after the boost dose. The challenge dose was 1.67 x 10^6 CFU/mouse (21 LD_{50}s). n = 9 mice/group (KPhos alone and vaccine alone) or 10 mice/group (streptomycin alone and vaccine + streptomycin).

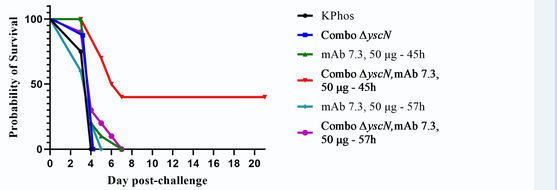

Figure 3 A comparison of protection afforded by vaccination of mice with the Combo Δ*yscN* vaccine and treatment with anti-V mAb 7.3. BALB/c mice were vaccinated twice with the Combo LAV or with KPhos and challenged four weeks later with *Y. pestis* strain C12 by the aerosol route. 45 or 57 h post-challenge, the mice were treated intraperitoneally with 50 µg of the anti-V mAb 7.3 or with KPhos alone. The mice were challenged with approximately 5 LD_{50} of *Y. pestis* C12 and monitored daily for 21 days after challenge.

SUMMARY

1. Live attenuated vaccines are an important part of the layered and integrated medical intervention (LIMIT) strategy.
2. Live attenuated vaccines offer numerous advantages to other vaccination strategies and offer additional tools to combat emerging or engineered threats.
3. The LIMIT strategy offers new opportunities to test medical counter measures that were previously discarded because they failed to meet protection thresholds as stand-alone countermeasures.

ACKNOWLEDGEMENTS AND DISCLAIMERS

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

Chris Cote
Christopher.k.cote.civ@health.mil