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Current Vaccine Development against Plague

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Abstract

Yersinia pestis, the causative organism of the plague, has played an important role in shaping human history. Plague is an illness that may manifest in bubonic, pneumonic, or septicemic form. Natural outbreaks devastated entire populations in medieval times and an estimated 200 million humans were killed by plague throughout history. The organism can still be found today throughout the world, including the United States. Approximately 2,000 cases of plague are reported each year to the World Health Organization. The potential use of the bacteria in modern times as an agent of bioterrorism makes understanding this organism a priority. The author reviews the current vaccine development against plague after a brief description of the history of plague pandemics.

Keywords: *Yersinia pestis*; plague; vaccines.

1. Introduction

Y*ersinia pestis*, the causative agent of plague, is an aerobic, non-motile, gram-negative bacillus belonging to the family *Enterobacteriacea*. It is a zoonotic infection transmitted to humans via the bite of a flea. The natural reservoir of *Y. pestis* is rodents, squirrels, and prairie dogs. It is usually transmitted via the flea *Xenopsylla cheopis* [1]. Large reservoirs of *Y. pestis* still exist on all major inhabited continents, except Australia, with a large reservoir being in the southwestern US [2].

In human history, plague are endemic in many parts of the world, including the US, and have resulted in horrible disaster [3]. The first outbreak of plague was documented in 1320 before the common era (BCE) among the Philistines. Biblical reference to this outbreak is found in I Samuel 5:6. In the last two millennia, the plague has reached pandemic proportions affecting many countries on most continents [4]. The first plague pandemic, also referred to as the Justinian Plague, began in Pelusium, Egypt in 541 common era (CE) [2,4,5]. Outbreaks in Europe, Central and Southern Asia, and Africa killed an estimated 100 million people [2,4]. The second pandemic of the fourteenth century (1347 - 1350 CE) began in Sicily and rapidly spread throughout Europe over the next several years, killing an estimated one third of the European population [2,4,5]. During that time, plague became known as "Black Death". Outbreaks of plague continued to occur sporadically in Europe over the next several centuries. The third pandemic began in 1894 in Hong Kong and Canton and in Bombay in 1898. It spread around the world over a ten year period, predominantly from infected rats and their fleas that were aboard steamships [2,4]. An estimated 12 million deaths occurred. Between 1898 and 1908 approximately six million deaths occurred in India alone [6]. Since 1954, an average of ~1,800 confirmed plague cases worldwide per year were recorded by the WHO. The number of yearly cases is highly dependent upon epidemic outbreaks and the reporting of cases to WHO; cases reported to WHO likely represent only a fraction of the total number of cases. The 1994 outbreak of pneumonic plague in India (876 cases with 54 fatalities) highlighted both the potential for serious epidemics and the lack of adequate training of health care personnel in recognizing and dealing with plague [2,7].

2. Materials and Methods

2.1 Study sites and sampling

Three clinical forms of human plague exist: bubonic, pneumonic, and septicemic. *Y. pestis* cells spread from the site of the infected flea bite to the regional lymph nodes, grow to high numbers causing formation of a bubo, and spill into the blood stream where bacteria are removed in the liver and spleen. Growth continues in the liver and spleen, spreads to other organs, and causes a septicemia. Fleas feeding on septicemic or bacteremic animals complete the life cycle. Humans are accidental hosts that are highly susceptible to plague. In humans bubonic plague can develop into an infection of the lung (secondary pneumonic plague); this can lead to aerosol transmission (primary pneumonic plague) [2,8].

In addition to the potential for natural infections, *Y. pestis* is generally considered to be among the top five potential biological weapons [9]. One of the earliest recorded biological warfare attempts used plague - Tartar forces, laying siege to 14th-century Kaffar (now called Feodosia, Ukraine), catapulted their plague victims into the city in an attempt to start an epidemic among the defending forces.

During World War II, Japanese forces released plague-infected fleas from aircraft over Chinese cities. More recently, an Ohio man with extremist connections tried to obtain *Y. pestis* from the American Type Culture Collection and evidence indicates that *Y. pestis* was being developed for potential biological warfare use in the former Soviet Union. To be effective, a bioterrorism/biowarfare agent must be easily propagated and prepared, easily dispersed with high infectivity, and must cause a rapidly developing, severe disease [9-13]. Plague clearly fulfills these criteria. The organism is readily cultured in a variety of common laboratory growth media. Aerosol dispersal is a proven route of infection that causes pneumonic plague - aerosol delivery of doses as low as ~100 organisms to African green monkeys caused fatal infections [14]. Thus the LD_{50} of aerosol-delivered plague is likely to be 100-fold lower than that for anthrax spores (LD_{50} of 5 x 10^4 in rhesus monkeys) [15]. Pneumonic plague has a short incubation period and progresses rapidly to a highly fatal infection. Victims often become sources of secondary infections as indicated by plague epidemics throughout history [2,8].

Three other factors make *Y. pestis* an attractive potential biological agent for terrorists or governments. The organism can be easily obtained from any of the numerous and widely dispersed animal reservoirs of plague [2]. Additionally, the emergence of *Y. pestis* strains resistant to multiple drugs has been isolated from plague patients in Madagascar, which may spread character of multiple antibiotic resistances to plague reservoirs [16,17]. Second, *Y. pestis* is easily genetically manipulated to create strains with specific engineered traits, such as constructing a super *Y. pestis* strains resistant to the top 5-6 antibiotics of choice in prompt treatment of plague. Third, currently there is no plague vaccine available for vaccination of the public or military personnel. All those factors make *Y. pestis* with high potential for use as a bioterrorism/biowarfare agent. Especially in its pneumonic form, *Y. pestis* has the dubious distinction of being one of the most highly virulent and rapidly fatal causes of acute bacterial infections.

3. Results

Although antibiotics have been used for plague prophylaxis, they are useful only in the setting of a known case of plague. In addition, Although plague infection is associated with high mortality, bubonic plague survivors were considered immune to subsequent infection [18]. The apparent ability to resist infection was the basis for Haffkine's first plague vaccine [19]. The promise of plague immunity continues to fuel research into the development of safe and efficacious vaccines. The first FDA licensed plague vaccine, USP (Cutter Biological) or Killed Whole-Cell (KWC) vaccine, was a formalin preparation of the fully virulent strain *Y. pestis* 195/P. The vaccine is considered safe and efficacious at preventing bubonic plague [20,21]. Adverse reactions such as fever, headache, lymphadenopathy and the need for booster injections eventually diminished interest in the USP vaccine. KWC immunization does not generate protective immunity against pneumonic plague [22,23]. As vaccine development has shifted towards preventing pneumonic plague, USP would not be considered efficacious [9]. Therefore, Greer laboratories had stopped manufacturing the only U.S. licensed vaccine [2]. Recent efforts to create a safe and effective plague vaccine have focused on the development of recombinant subunit vaccines that elicit antibodies against two well characterized *Y. pestis* antigens, the F1 capsule and the virulence protein LcrV [24-27]. But USAMRIID demonstrated that F1/LcrVbased vaccines protect cynomolgus macaques against aerosolized *Y. pestis* but fail to adequately protect African green monkeys (efficacy ranged from 0% to 75% in five trials) [28].

A number of approaches are underway to improve the efficacy of F1/LcrV-based vaccines [29]. Some researchers are genetically modifying the antigens [30,31], while others are exploring the use of alternate adjuvants [32-35] and delivery platforms using attenuated *Salmonella*, *Y. pseudotuberculosis* or virus [36-48]. These approaches are certainly promising. However, as already noted, F1-negative *Y. pestis* strains exist [14,49-53], and pathogenic *Yersinia* species express multiple LcrV variants, including some that may not confer cross-protective immunity [54].

Most of the live vaccine strains are derivatives of virulent *Y. pestis* that contain spontaneously arising mutations within the pigmentation (*pgm*) locus. Unfortunately, these vaccines can be unstable and sometimes display virulence in non-human primates, even killing experimental animals [20,55-57]. In addition, they frequently cause debilitating fever, malaise, and lymphadenopathy in humans [22]. Safety concerns have limited enthusiasm for the development of live attenuated vaccines in the United States and Europe. However, live attenuated vaccines were administered to tens of millions of humans in Indonesia, Madagascar, and Vietnam, apparently without causing any deaths [58]. Live attenuated vaccines also were studied extensively in the former Soviet Union [22,59,60], and the NIIEG line of pgm-negative strain EV 76 is still in use today in Russia [59,61]. As recently as 2002, USAMRIID researchers noted, 'Despite their drawbacks, there is ample evidence that live attenuated strains of *Y. pestis* should be considered as potential vaccine candidates' [57].

Currently, several groups recently described attenuated *Y. pestis* strains with well-defined genetic modifications that may be useful as live vaccines (Table 1).

Table 1. Current progress of live attenuated *Y. pestis* vaccines.

Mutant strains	LD_{50} (cfu) [a]	LD_{50} (cfu) [b]	s.c. challenge dose (cfu)	Survival of s.c. challenge	i.n. challenge dose(cfu)	Survival of i.n. challenge	Reference
Δ*dam Y. pestis*	2.3×10^3	ND	7.5×10^3	100%	ND	ND	[62]
Y. pestis EV NIIEG	$>2.5\times 10^4$	ND	2×10^4	No protection	ND	ND	[61]
Δ*lpxM Y. pestis* EV NIIEG	$>2.5\times 10^4$	ND	2×10^4	71%	ND	ND	[61]
Δ*yopH Y. pestis*	$>10^7$	$>10^7$	10^5	70%	10^5	60%	[65]
Y. pestis pBR-LpxL	$\sim 10^7$	ND	10^6	100%	5×10^4	100%	[66]
Δ*smpB-ssrA* Δ*pgm Y. pestis*	ND	$>10^8$	ND	ND	2×10^5 [c]	100%	[68]
Δ*guaBA Y. pestis*	7×10^4	ND	66	100%	ND	ND	[69]
Δ*relA* Δ*spoT Y. pestis*	5.8×10^5	ND	1.5×10^5	100%	2×10^4	60%	[70]
ΔP_{crp21}::*TT araC* P_{BAD} *crp Y. pestis*	4.3×10^5	10^4	1.4×10^5	100%	1.4×10^4	70%	[71]

Note: a, LD_{50} for s.c. inoculation; b, LD_{50} for i.n. inoculation; c, using Δ*pgm Y. pestis* as challenge.

A *dam* strain of *Y. pestis* is attenuated and induces protection against plague [62]. The *pgm* mutant strain primes CD4 and CD8 T cells that synergistically protect against lethal pulmonary *Y. pestis* infection [63]. A strain with mutations in both the *pgm* and *pla* loci safely induces humoral responses in monkeys [57]. Vaccination with strains harboring mutations in both *pgm* and *lpxM* loci or only the *pgm* locus protects mice against subcutaneous challenge [61,64]. Vaccination with a *yopH* mutant protects mice against both subcutaneous and pulmonary challenge [65]. Vaccination with strains engineered to constitutively produce LPS bearing hexa-acylated lipid A generate very good immungenicity against pneumonic and bubonic plague [66,67]. A *smpB- ssrA* mutant of *Y. pestis* functions as a live attenuated vaccine to protect mice against pulmonary plague infection [68] and a *guaBA* mutant of *Y. pestis* is attenuated in virulence and provides protection against plague in a mouse model of infection [69]. In our lab, we have constructed *relA* and *spoT* double mutant *Y. pestis* strain and introduced regulated delay system (arabinose regulated Crp expression) into *Y. pestis*. These two mutants have shown good protective immunogenicity against plague (Figure1 and 2) [70,71].

Based on previous investigations, we construct a live attenuated *Y. pestis* strain that express *E. coli lpxL* from the chromosome and in combination with the arabinose-regulated *crp* mutation and show that the double mutant is safer and also provides better protection against bubonic and pneumonic plague than the Pgm-negative mutant. In our investigations, our mutant strain immunization generates complete protection against huge dose of subcutaneous challenge (10^7 CFU of KIM5+).

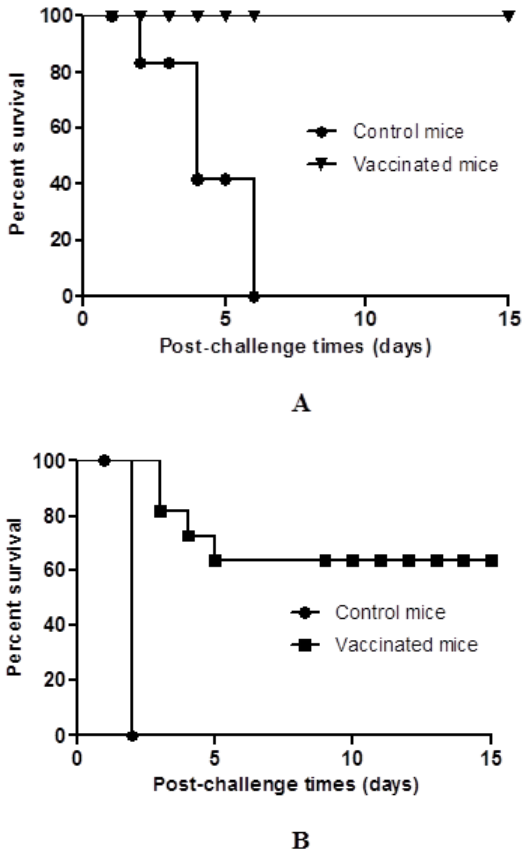

Figure 1. Mouse survival after *Y. pestis* KIM5$^+$ Challenge. (A) Swiss Webster mice vaccinated s.c. with 2.5×10^4 CFU of χ10004(pCD1Ap) and a were challenged with 1.5×10^5 CFU of *Y. pestis* KIM5$^+$ via the s.c. route. (B) Swiss Webster mice vaccinated s.c. with 2.5×10^4 CFU of χ10004(pCD1Ap) were challenged via the i.n. route with 2×10^4 CFU of *Y. pestis* KIM5$^+$. Immunization provided significant protection against both challenge routes (P<0.001). For each experiment, there were 10 mice in the vaccinated group and 4 mice in the control group. The experiment was performed twice.

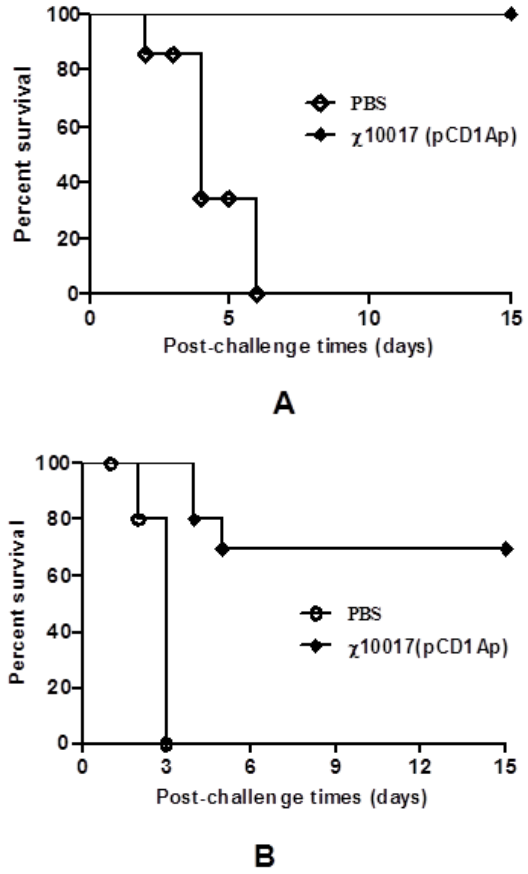

Figure 2. Mouse survival after *Y. pestis* KIM5^{+} Challenge. (A) Swiss Webster mice vaccinated s.c. with 3.0×10^4 CFU of χ10017(pCD1Ap) were challenged with 1.4×10^5 CFU of *Y. pestis* KIM5+ via the s.c. route. (C) Swiss Webster mice vaccinated s.c. with 3.0×10^4 CFU of χ10017(pCD1Ap) were challenged via the i.n. route. with 1.4×10^4 CFU of *Y. pestis* KIM5+. For panels A and B, survival of immunized mice was significantly greater than PBS controls in all experiments (P<0.001). There were 10 mice per vaccination group and 4 mice per control group for each experiment. The experiment was performed twice.

We also notice that our mutant strain mutant can give good protection (80% survival) against 100 $LD_{50}$of KIM5+ by intranasal challenge (intranasal LD_{50}, ~100 CFU) (Unpublished data). Our results indicate that this is a very promising finding toward the goal of designing a live safe *Y. pestis* vaccine that is highly protective against bubonic and pneumonic plague with minimal side effects. Additionally, the potential safety and efficacy of this mutant strain could be enhanced further with additional defined attenuating mutations.

4. Conclusion

Live attenuated versions of virulent pathogens as vectors or vaccines generate broad humoral immune response, but also most effectively prime cellular immunity [72]. Plague vaccines based on live attenuated *Y. pestis* provide the theoretical advantage of simultaneously priming immunity against many antigens, thereby reducing the likelihood of circumvention by weapon engineers [28]. Therefore, research on the development of new improved live attenuated *Y. pestis* vaccines should continue and will be strongly encouraged.

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