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

Yersinia pestis, the causative agent of plague, is an aerobic, non-motile, gram-negative bacillus belonging to the family Enterobacteriaceae. 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 Fedosia, 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 LD50 of aerosol-delivered plague is likely to be 100-fold lower than that for anthrax spores (LD50 of 5 x 10^8 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/LcrV-based vaccines protect cynomolgus macaques against aerosolized Y. pestis but fail to adequately protect African green monkeys (efficacy ranging 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 NIEG 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).
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 immunogenicity 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 (Figure 1 and 2) [70,71].

Based on previous investigations, we constructed 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+).

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

<table>
<thead>
<tr>
<th>Mutant strains</th>
<th>LD50 (cfu)</th>
<th>LD90 (cfu)</th>
<th>s.c. challenge dose (cfu)</th>
<th>Survival of s.c. challenge</th>
<th>i.n. challenge dose (cfu)</th>
<th>Survival of i.n. challenge</th>
<th>Reference</th>
</tr>
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<tr>
<td>Δdam Y. pestis</td>
<td>2.3 × 10^7</td>
<td>ND</td>
<td>7.5 × 10^2</td>
<td>100%</td>
<td>ND</td>
<td>ND</td>
<td>[62]</td>
</tr>
<tr>
<td>Y. pestis EV NIIEG</td>
<td>&gt;2.5 × 10^4</td>
<td>ND</td>
<td>2 × 10^4</td>
<td>No protection</td>
<td>ND</td>
<td>ND</td>
<td>[61]</td>
</tr>
<tr>
<td>ΔlpxM Y. pestis EV NIIEG</td>
<td>&gt;2.5 × 10^4</td>
<td>ND</td>
<td>2 × 10^4</td>
<td>71%</td>
<td>ND</td>
<td>ND</td>
<td>[61]</td>
</tr>
<tr>
<td>ΔyopH Y. pestis</td>
<td>&gt;10^7</td>
<td>&gt;10^7</td>
<td>10^5</td>
<td>70%</td>
<td>10^5</td>
<td>60%</td>
<td>[65]</td>
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<tr>
<td>Y. pestis pBR-lpxL</td>
<td>-10^7</td>
<td>ND</td>
<td>10^6</td>
<td>100%</td>
<td>5 × 10^4</td>
<td>100%</td>
<td>[66]</td>
</tr>
<tr>
<td>ΔsmpB-ssrAΔpgm Y. pestis</td>
<td>ND</td>
<td>&gt;10^7</td>
<td>ND</td>
<td>ND</td>
<td>2 × 10^5</td>
<td>100%</td>
<td>[68]</td>
</tr>
<tr>
<td>ΔguaBA Y. pestis</td>
<td>7×10^4</td>
<td>ND</td>
<td>66</td>
<td>100%</td>
<td>ND</td>
<td>ND</td>
<td>[69]</td>
</tr>
<tr>
<td>ΔrelAΔspoT Y. pestis</td>
<td>5.8×10^4</td>
<td>ND</td>
<td>1.5×10^5</td>
<td>100%</td>
<td>2 × 10^4</td>
<td>60%</td>
<td>[70]</td>
</tr>
<tr>
<td>ΔPcrpt::TT araC PBAD crp Y. pestis</td>
<td>4.3×10^5</td>
<td>10^4</td>
<td>1.4×10^5</td>
<td>100%</td>
<td>1.4×10^4</td>
<td>70%</td>
<td>[71]</td>
</tr>
</tbody>
</table>

Note: a, LD50 for s.c. inoculation; b, LD50 for i.n. inoculation; c, using Δpgm Y. pestis as challenge.

### Figure 1. Mouse survival after Y. pestis KIM5+ Challenge.

(A) Swiss Webster mice vaccinated s.c. with 2.5 × 10^4 CFU of Y. pestis KIM5+ 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 Y. pestis KIM5+ 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.
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.

References


