5

Recent Advancement in the Development of Vaccines Against *Y. pestis* – A Potential Agent of Bioterrorism

Riyasat Ali and D.N. Rao
Department of Biochemistry, All India Institute of Medical Sciences, New Delhi, India

1. Introduction

A bioterrorism attack- is the deliberate release of biological agents such as viruses, bacteria, or toxins used to cause illness or death in people, animals, or plants (CDC). These agents are typically found in nature, but it is possible that they could be changed to increase their ability to cause disease, make them resistant to current therapeutics, or to increase their ability to be spread into the environment. Biological agents can be spread through the air, through water, or in food. Terrorists may use biological agents because they can be extremely difficult to detect and may not cause illness for several hours to several days. Some bioterrorism agents, like Variola major and *Yersinia pestis*, can be spread from person to person, while others e.g. *Bacillus anthracis* are not (Bioterrorism review, 2009). Biological agents make attractive weapons because they are relatively easy to obtain and carry from place to place, can be easily dispersed, and can cause widespread fear and panic beyond the actual physical damage they can cause. Many of the agents that could be used for bioterrorism have been divided into three categories A, B, and C, for public health preparedness based on various characteristics of the microbes or the diseases they cause.

Category A includes the most "dangerous" and highest priority for public health preparedness. Some of these pathogens can be transmitted from person-to-person, cause diseases with a high mortality rate and are likely to cause public panic and social disruption. Category A agents include *B. anthracis* (anthrax), Variola major (smallpox), *Francisella tularensis* (tularemia), *Y. pestis* (plague), *Clostridium botulinum* neurotoxin (botulism), and Viral hemorrhagic fever viruses (e.g. arenaviruses, filoviruses, bunyaviruses, and flaviviruses).

This chapter will discuss the Category A agent *Y.pestis,*the disease it causes,and recent efforts to develop vaccines.

Plague, a zoonotic disease caused by the gram-negative bacillus *Y. pestis* is primarily a disease of rodents, with transmission occurring through infected fleas. Human disease is acquired through rodent flea vectors, as well as respiratory droplets from animal to humans and humans to humans.

2. History of plague

The first reported pandemic of plague has been referred to as the "Great Plague of Justinian" (Sticker, 1908; Hirst,1953). This pandemic began around 532 AD in Egypt and spread through the Middle East and the Mediterranean basin, reaching Turkey, Constantinople, Greece, Italy, and the territories of France and Germany. The second pandemic, which is also known as the Black Death, began in 1334 in China and then spread westward along the trade routes to the Black Sea and eventually to Constantinople. The disease, which spread slowly and inevitably from village to village by infected rats and humans, or more quickly from country to country by ships, eventually killed 20 to 30 million people in Europe (Gottfried,1983). The third pandemic probably originated in the Chinese province of Yunnan around 1855 and spread to the southern coast of China, causing several smaller outbreaks. Larger epidemics occurred when the disease finally reached Canton and Hong Kong in 1894, thus marking the beginning of the third pandemic. Plague spread rapidly throughout the world through all inhabited continents, except Australia. Rats aboard the faster steamships that had replaced slow-moving sailing vessels in merchant fleets carried the disease. Between 1894 and 1903, plague entered 77 ports on 5 continents. During the early years of the third pandemic, the death toll in India and China alone was 12 million. In United State, plague was introduced in 1900. Between 1900 and 1924, most plague cases in the U.S. occurred in port cities along the Pacific and Gulf coasts (Link, 1955). More recently, a plague epidemic caused the death of several hundred residents in the Surat city of India between September and October 1994 (Perry and Fetherstone 1997).

The recent increase in the number of human plague cases together with the reappearance of epidemics in countries such as Malawi, Mozambique, and India has led to its designation by WHO as a re-emerging infectious disease (World Health Organization 2002, World Health Organization 2003). As of 30 July 2010, the Ministry of Health in Peru confirmed a total of 17 cases of pneumonic plague in Ascope province of Department La Libertad. The onset of symptoms for the last reported case of pneumonic plague was on 11 July 2010. During the investigations, 10 strains of *Y. pestis* were isolated from humans, rodents and domestic cats. In 2009, the Chinese Ministry of Health, reported a cluster of pneumonic plague cases in the remote town of Ziketan, Qinghai province. The first case was a 32 year old male herdsman, who developed fever and haemoptysis. Between 1990 and 2005, a total of 107 cases of plague were reported in the U.S.(Centers for Disease Control and Prevention, 2006). Recently there have been reports of 14 deaths potentially due to pneumonic plague in Madagascar (http://www.promed mail.org).

2.2 Plague as a biological weapon

In 1346, during the siege of Kafa (now Feodossia, Ukraine) the attacking Tartar forces catapulted the bodies of warriors who had died of plague into the besieged city as a weapon. It has been speculated that this operation may have been responsible for the advent of the Black Death in Europe (Wheelis, 2002; Lederberg, 2001). In World War II, Unit 731 of the Japanese army, developed plague-infected fleas in China resulting in outbreaks of plague (Harris, 1994). Later, to eliminate dependency on the flea vector, the U.S. and the Soviet Union biological weapons programs developed methods to aerosolize *Y.pestis.* The World Health Organization (WHO) has estimated that, if 50 kg of *Y. pestis* were released as

an aerosol over a city of 5 million people, 150,000 would get pneumonic plague infection of which 36,000 would die (WHO 1970). The plague bacilli would remain viable as an aerosol for 1 hour for a distance of up to 10 km.

3. Clinical characteristics of plague

Plague can be broadly classified into three forms:

3.1 Bubonic plague

This is the classic form of the disease, which is characterised by swollen lymph nodes called buboes. Common symptoms are fever, headache, and chills occurring within 2 to 6 days of exposure to the organism either by flea bite or by contamination of open wounds with infected material. Gastrointestinal complications such as nausea, vomiting, and diarrhoea are common (Iteman et al., 1993, von Reyn et al., 1977). Buboes typically occur in the inguinal and femoral regions but may also occur in other regions of the body (Butler, 1989; Conrad et al., 1968). Bacteraemia or secondary plague septicaemia is frequently seen in patients with bubonic plague (Gage et al., 1992). In humans, the mortality of untreated bubonic plague is approximately 60%, but this is reduced to less than 5% with prompt, effective therapy.

3.2 Septicaemic plague

Primary septicaemic plague occurs mainly in patients with positive blood cultures but no palpable lymphadenopathy. Clinically, septicaemia caused by *Y.pestis* resembles that caused by other gram-negative bacteria. Common symptoms include chills, headache, malaise, and gastrointestinal disturbances. Patients with septicaemic plague are more likely to experience abdominal complications than compared those with bubonic plague. Even with treatment, the mortality of septicaemic plague may range from 30 to 50% (Crook and Tempest 1992, Hull et al., 1987, Poland and Barnes, 1979); untreated septicemic plague is virtually 100% fatal.

3.3 Pneumonic plague

Pneumonic plague is an infection of the lungs due to either inhalation of the organism (i.e., primary pneumonic plague), or dissemination to the lungs via the blood stream (i.e., secondary pneumonic plague). Pneumonic plague is the only form of the disease which is readily spread from person to person via respiratory droplets through close contact (2 to 5 ft) with an infected individual. It progresses rapidly from a flu-like illness to pneumonia with coughing and the production of bloody sputum. The incubation period for primary pneumonic plague is between 1 and 3 days. The last case of pneumonic plague in the U.S., resulting from person-to-person transmission occurred during the 1924 - 1925 epidemic in Los Angeles (Meyer, 1961). Between 1970 and 1993, 12% of U.S. plague patients developed pneumonia secondary to either the bubonic or septicemic form of the disease (Doll, 1994); In recent decades, 28% of human plague cases resulting from exposure to infected domestic cats in the U.S. presented as primary pneumonic plague. The mortality rate for untreated pneumonic plague is nearly 100%. Recent data from Madagascar epidemic indicates that mortality associated with respiratory involvement was 57%.

4. Microbiology

The genus *Yersinia*, a member of the family Enterobacteriaceae, consists of eleven species, of which three are pathogenic for humans: *Y. pestis; Y. pseudutuberculosis* and *Y. enterocolitica. Y. pestis,* is a gram-negative, non-motile, non-spore forming coccobacillus measuring 0.5 to 0.8 μm in diameter and 1 to 3 μm in length. The organism grows at 4 to 40^0 C with optimum growth at 28 to 30 ^{0} C; the optimum pH range is 7.2 to 7.6 but extreme pH (5 to 9.6) can be tolerated (Brubaker, 1972; Holt et al., 1994; Poland and Barnes, 1979; Poland et al., 1994). The lipopolysaccharide of *Y.pestis* is characterized as rough, possessing core components but lacking the extended O-group side chains. There is no true capsule; however, a carbohydrate-protein surface component, termed capsular antigen or fraction 1 (F1) is produced during growth above 33^0C (Barnes and Quan, 1992; Brubaker et al., 1972; Poland et al., 1994).Three biotypes (or biovars) of *Y. pestis* can be differentiated based on the conversion of nitrate to nitrite and fermentation of glycerol: *Antiqua, Orientalis* and *Mediaevalis*. Biotype *Antiqua* is positive for both characteristics, bitype *Orientalis* converts nitrate to nitrite but does not ferment glycerol, and biotype *Mediaevalis* ferments glycerol but does not form nitrite. Strains of the three biotypes exhibit no difference in their virulence or pathology in animals or humans (Brubaker et al., 1972; Poland et al., 1979). It is thought that *Antiqua* was responsible for the First Pandemic, *Mediaevalis* for the Second Pandemic, and *Orientalis* for the Third Pandemic. The majority of *Y. pestis* strains contain three virulence plasmids of 9.5, 70 to 75, and 100 to 110 kb (Ben-Gurion and Shafferman, 1981; Ferber and Brubaker, 1981; Filippov et al., 1990). In strain KIM and its derivatives, these plasmids are referred to as pPCP1 (pesticin, coagulase, plasminogen activator), pCD1 (calcium dependence), and pMT1 (murine toxin).

4.1 Life cycle

Plague primarily affects rodents. Transmission between rodents is associated with their fleas. While infection can occur by direct contact or ingestion, these routes do not normally play a role in the maintenance of *Y. pestis* in animal reservoirs. The rat flea (*Xenopsylla cheopis*), the classic vector for plague, ingests blood from an infected rodent (Hinnebusch and Schwan 1993). A bacterial load of 10^4 CFU/ml of rodent blood would ensure ingestion of at least 300 *Y. pestis* organisms. *Y.pestis* is cleared by some fleas but multiplies in the midgut (stomach) of others. Two days after an infected blood meal, the stomach exhibits clusters of brown specks containing *Y. pestis*. These develop into cohesive dark brown masses containing bacilli, a fibrinoid-like material, and probably hemin which extend throughout the stomach and into the proventriculus and esophagus. By 3 to 9 days after the infected blood meal, the bacterial masses may completely block the proventriculus, extend into the esophagus, and prevent newly ingested blood from reaching the stomach. As the hungry flea repeatedly attempts to feed, the blood sucked from the mammal mixes with bacilli and is regurgitated into the mammalian host (Bacot, 1914; Bacot, 1915; Bahmanyar, 1976; Bibikova 1977; Cavanaugh 1971, 1956). At higher environmental temperatures (> 28 to 30^0 C), blockage of fleas decreases and clearance of the infection increases, possibly due to the temperature regulation of hemin storage and/or Pla protease. (Burroughs, 1947; Cavanaugh, 1971; Cavanaugh, 1980; Kartman, 1969). The normal digestive process of fleas involves maintaining the blood meal as a liquid, which is subsequently degraded primarily by proteolytic enzymes (Wigglesworth, 1984). The fate of the blocked flea is death from starvation and dehydration (Bacot, 1914; Bibikova, 1977).

Y. pestis spreads from the site of the flea bite to the regional lymph nodes and multiplies, resulting in the formation of primary and sometimes secondary buboes (swollen lymph node). The bacilli can spread into the bloodstream (bacteremia), where they are preferentially removed by the spleen and liver. Growth of the organisms continues in the blood, liver, and spleen and eventually spreads to other organs (Pollitzer, 1954). Development of a bacteremia of sufficient degree and duration is essential for effective transmission in nature. Infection of the flea via the blood from a bacteremic rodent completes the cycle. If bubonic plague progresses to the pneumonic form in humans, the potential for respiratory droplet spread and a primary pneumonic plague epidemic occurs (Poland and Barnes, 1979; Poland et al., 1994). This type of epidemic is currently uncommon due to the advent of effective antibiotics and modern public health measures.

5. Diagnosis

Clinical diagnosis of disease is based on patient symptoms and exposure history. Bubonic plague is characterized by painful, swollen lymph node(s), fever, and a history of exposure to fleas, rodents, or other animals. It is very difficult to diagnose septicemic plague without a blood culture because of its resemblance to other gram-negative septicemias. Likewise, pneumonic plague has been mistaken for other pulmonary syndromes (Centers for Disease Control and Prevention. 1994). Recent data indicate that pneumonic plague should be suspected in symptomatic persons with a history of exposure to infected pets, especially cats (Craven et al., 1993; Doll et al., 1994; Gage et al., 1992).

Specimens for laboratory diagnosis include blood, bubo aspirates, and sputum, which can be stained with Gram, Giemsa, Wright, or Wayson stain (Poland et al., 1979). A positive fluorescent-antibody assay directed against purified F1, a capsular antigen expressed predominantly at 37^0 C can be used as presumptive evidence of a *Y. pestis* infection (Du and Forsberg, 1995; Poland et al., 1979). To confirm a diagnosis of plague by bacteriological means, it is necessary to isolate the organism. Other methods for diagnosing plague include: enzyme linked immunosorbent assays (ELISA) (Cavanaugh et al., 1979; Williams et al., 1984), polymerase chain reaction (PCR) assays (Norkina OV 1994), and DNA hybridization (McDonough et al., 1988). ELISAs have been used to measure levels of either F1 antigen or antibodies to F1 in serum (Williams et al.,1984). PCR-gel electrophoresis based methods have been developed for detecting *Y. pestis* in fleas and other specimens (Hinnebusch and Schwan, 1993; Norkina et al., 1994, Tsukano et al., 1996). Real-time PCR assays in various formats have also been developed for detecting and identifying *Y. pestis* (Higgins et al., 1998; Iqbal et al., 2000; Lindler and Tall, 2001; Loiez et al., 2003; Tomaso et al., 2003; Chase et al., 2005; Woron et al., 2006). Real-time PCR based methods are more specific, and require less time and labor than conventional PCR assays. Real-time PCR methods include SYBR Green (Saikaly et al., 2007), molecular beacons (Varma-Basil et al., 2004), TaqMan probes (Loiez et al., 2003; Chase et al., 2005) and minor groove binding (MGB) probes (Skottman et al., 2007), and target specific sequences on the chromosome and (or) plasmids. However, PCR based diagnosis is expensive compared to immunoassays, which may be useful for mass screening during epidemics.

6. Treatment

Patients suspected of having bubonic plague should be placed in isolation until two days after starting antibiotic treatment to prevent the potential spread of the disease. Antibiotics

such as streptomycin, gentamicin, oxytetracyclne, tetracycline and chloramphenicol have been used to treat primary infection (Meyer, 1950). Due to the toxicity associated with streptomycin, patients are not usually maintained on this antibiotic for the full 10-day course but shifted to one of the other antibiotics, usually tetracycline. The tetracyclines are also commonly used for prophylaxis, while chloramphenicol is recommended for the treatment of plague meningitis (Becker et al.,1987). Newer antibiotics have been used to successfully treat experimental plague infections in mice (Bonacorsi et al., 1994). Recently, the quinolone levofloxacin was found to be effective against *Y.pestis, B.anthracis,* and *F. tularensis* (Peterson et al., 2010).

7. Immunology of *Y. pestis*

Yersinia spp like many other gram-negative bacterial pathogens, employ a specialized secretory apparatus called the type III secretion system (TTSS) to interact with host cells (Cornelis et al., 1998; Cornelis, 2000). The TTSS is a multicomponent secretion apparatus that injects specialized proteins (effectors) into the cytosol of the host cell where they interact with a variety of host proteins to manipulate cellular functions to ultimately benefit the pathogen (Galan and Collmer, 1999). The *Yersinia* effector proteins called Yops– (**Y**ersinia **O**uter membrane **P**roteins) and other proteins involved in the TTSS are encoded on a 70-kb plasmid (Cornelis et al., 1998). The functions of the Yops are currently under intense study and fall into two general categories: proteins facilitating the translocation of Yops into the host cells, and those actually secreted into the cytosol. Notably, YopD, YopB, and LcrV (low calcium response protein V) appear to function in the translocation of other Yops into the cytosol whereas YopE, YopH, YopJ (Yop P in *Y. enterocolitica*), YopM, YopO, and YopT function within the host cell. Yops are virulence factors that can interfere with phagocytosis, inhibit the antimicrobial oxidative burst, inhibit the production of inflammatory cytokines (e.g. TNF-α,), and promote apoptosis in macrophages and neutrophils (Cornelis et al.,1998, 2000). Like TTSS effectors of other bacterial pathogens, Yops function by mimicking activities of host cellular proteins and either activate or inhibit cellular processes to promote the pathogen's survival and replication (Staskawicz et al., 2001).

LcrV, is an important virulence factor (Sing et al., 2002; Fields et al., 1999; Lee et al., 2000). It forms the tip of TTSS and helps to translocate effector proteins to host cells. LcrV can also be secreted into the environment (Fields et al., 1999) where it has been shown to down regulate host protective immune responses in an IL-10 mediated manner (Sing et al., 2002). Thus, pretreatment of wildtype peritoneal macrophages with recombinant LcrV (rLcrV) inhibited zymosan induced TNF-α production. The anti-inflammatory effect of macrophages was found to be IL-10 dependent because it could be reversed by neutralizing antibodies specific to IL-10.There was no effect of neutralizing antibodies against IL-4 or TGF-β, two other cytokines known to inhibit inflammatory responses of macrophages (Sing et al., 2002). The cell receptors responsible for LcrV-induced IL-10 production were identified as CD14 and TLR2 (Sing et al., 2002). IL-10 secretion in response to rLcrV was abrogated in CD14- and TLR2- deficient macrophages. Furthermore, the TLR2 stimulating region of LcrV mapped to a short N-terminal 19 amino acid sequence (Sing et al., 2002). CD14 / TLR2 mediated production of IL-10 by LcrV was further established by the observation that TLR2-deficient mice were more resistant to *Y. enterocolitica* infection than their wild-type parents (Sing et al., 2002).

Y. pestis replicates extracellularly; whether its virulence relies upon intracellular replication remains a question of debate. *Y. pestis* replicates within macrophages/ dendritic cells as well as

in vitro (Cavanaugh et al., 1959; Janssen et al., 1969; Straley et al.; 1984; Pujal et al., 2005). Nevertheless, detailed kinetic studies of mice infected intranasally (Lathem et al., 2005) and rats infected intradermally (Sebbane et al., 2005), failed to observe significant numbers of intracellular organisms *in vivo*. However, *Y.pestis* bacilli were detected in spleen cells and in CD11b-expressing macrophages when mice were infected subcutaneously (Lukaszewski et al., 2005). In addition, studies of pneumonic plague in nonhuman primates have documented the presence of intact *Y. pestis* within alveolar macrophages (Finegold et al., 1969; Davis et al., 1996). Electron microscopy confirmed the presence of alveolar macrophages containing intact bacilli in the lungs of aerosol-infected macaques (Finegold et al., 1969).

Although, the growth of *Y. pestis* within phagocytes, plays an important pathogenic role, extracellular bacilli predominate during the late stages of infection although intracellular organisms have also been detected at that time (Finegold et al., 1969; Davis et al., 1996; Lukaszewski et al., 2005). These findings suggested that cells of the monocyte/macrophage lineage offer *Y. pestis* a protected intracellular niche that provides sufficient time for the pathogen to grow within mammals by upregulating expression of capsular F1 protein, LcrV and Yops (Cavanaugh et al., 1959).

One to 4 hours after infection of macrophages, *Y. pestis* rapidly expresses virulence markers such as Yops, F1 antigen, and V antigen. By 1 to 2 days postinfection, the virulence-associated proteins begin to paralyse host immune mechanisms by inducing apoptosis, suppressing the production of proinflammatory cytokines (e.g. TNF-α), inhibiting Fc receptor-mediated phagocytosis, and preventing neutrophil chemotaxis (Perry and Fetherston, 1997). Inside macrophages, *Y. pestis* F1 protein (fraction 1 antigen) forms a capsule around the bacterium. This capsule enhances resistance to engulfment by both macrophages and neutrophils, probably by preventing interactions of receptors that could facilitate uptake of the pathogen (Du et al., 2002). It was also observed that *Y. pestis* produces a less-acylated (tetra-acylated) lipid A at 37 °C, which results in poor induction of host toll-like receptor (TLR) 4-mediated innate immune responses and ultimately poor activation of human macrophages (Kawahara et al., 2002; Kolodziejek et al., 2010). When *Y. pestis* KIM1001, which expresses a poorly TLR4-stimulating LPS, was modified to strongly induce TLR4, it became avirulent (Montminy et al., 2006).

A fimbrial structure in *Y. pestis*, PsaA (pH 6 antigen) is induced at 37°C in acidic media, an environment similar to that of the macrophage phagolysosome (Lindler and Tal, 1993; Price et al., 1995). PsaA selectively binds to apolipoprotein B (*apoB*)-containing lipoproteins (LDL) in human plasma (Makoveichuk et al., 2003), which may prevent recognition by the host immune system (Huang XZ 2004, Makoveichuk et al., 2003).

Infection by *Y.pestis* leads to a global depletion of NK cells and decreased secretion of IFN-γ, resulting in reduced macrophage function. These immunomodulatory effects depend on the effector YopM (Kerschen et al.,2004). Phagocytes (macrophages and neutrophils) are the main target cells of the Yops. YopH, YopE, YopT, and YopO inhibit the phagocytosis of Yersiniae, either by interfering with the host cell actin regulation of Rho GTPases (YopE, YopT, and YopO) or specifically and rapidly inactivating host proteins associated with signalling from the receptor to actin (YopH) (Aepfelbacher and Heesemann, 2001; Aepfelbacher et al., 2005; Andersson et al., 1996; Iriarte et al., 1998; Rosqvist et al., 1990). YopH can suppress the production of reactive oxygen intermediates by macrophages and PMNs (Green et al., 1995). Moreover, Yops also inhibit the proinflammatory responses

elicited by infected cells. YopP inhibits TNF-α and IL-8 release by macrophages, and epithelial, and endothelial cells, respectively (Boland and Cornelis 1998). TNF-α is a potent proinflammatory cytokine, released by activated macrophages and plays a crucial role in limiting the severity of the bacterial infection. In addition to YopP, YopM interacts with protein kinase C-like 2 and ribosomal protein S6 kinase, which are also involved in proinflammatory signalling (McDonald et al., 2003). The suppression of the production of proinflammatory factors not only reduces the activation of NK cells and phagocytes, but also destroys the inflammatory environment needed for adaptive immunity.

7.1 Current vaccine strategy

There is a need for a safe and effective plague vaccine to counter the threat of bioterrorism. Researchers have been trying for more than 100 years to develop such a vaccine (Titball and Williamson, 2004). The first vaccine consisting of a heat-killed broth of densely grown, fully virulent *Y. pestis* was developed in 1897 (Haffkine, 1897; Taylor, 1933). This formulation was found to be effective against bubonic plague but had undesirable side effects, such as high grade fever, in the majority of human recipients and severe adverse reactions limited its acceptance (Taylor, 1933). This vaccine was not effective against the pneumonic form of disease (Taylor, 1933; Lien-Teh, 1926). Later, Meyer and colleagues (1974, 1970) developed a more refined whole-cell plague vaccine comprised of formalin-killed *Y. pestis* organisms suspended in a saline solution. Ultimately, a vaccine of this type was licensed and sold as Plague Vaccine, USP, and was used to protect U.S. military personnel against bubonic plague during the Vietnam War (Meyer, 1970; Cavanaugh et al., 1974). However, these vaccines also caused significant adverse effects, including fever, headache, malaise, lymphadenopathy, erythema and induration at the site of injection (Meyer KF et al., 1974). In addition, they generally failed to protect mice and nonhuman primates against pulmonary *Y. pestis* challenge (Titball and Williamson, 2004; Meyer et al.,1974; Meyer et al.,1970; Kolle and Otto 1904).

In 1904, Kolle and Otto showed that relatively small quantities of live-attenuated *Y. pestis* were sufficient to protect rodents. Later, Strong (1906, 1908) reported that live-attenuated vaccines protected humans from bubonic plague. In subsequent years, this formulation was used to immunize millions of people in Indonesia, Madagascar and Vietnam (Girard, 1963). The results suggested that these vaccines were fully protective in humans against both the bubonic and pneumonic form of plague (Titball and Williamson, 2004; Meyer et al., 1970; Girard, 1963). Unfortunately, the live attenuated vaccines were found to be unstable, sometimes killing experimental animals (i.e., nonohuman primates) due to the retention of significant virulence (Welkos et al., 2002; Meyer et al., 1970; Meyer et al., 1974; Russell et al., 1995). In addition, they also produced frequent side effects in humans such as, debilitating fever, malaise and lymphadenopathy (Meyer et al., 1974). These safety concerns have limited the use of live-attenuated plague vaccines in the U.S. and Europe.

Current vaccines are based on variants of a pigmentation-negative *Y. pestis* strain EV76. Strain EV76 produces a robust T-cell response that contributes to protection against pneumonic plague in a murine model (Sha et al., 2008). Despite safety concerns and a high degree of immune variability among vaccine recipients, the NIIEG line of strain EV 76 is still in use today (Zilinskas, 2006). However, uncertainty about the reversal of virulence makes the EV76 live attenuated option much less appealing than the development of new vaccines.

To overcome the problems associated with the EV76 strain, researchers are trying to find non-pathogenic substitutes by replacing it with a plasmid-expressed gene that could engender protection. In that context, an *Escherichia coli*-derived plasmid encoding the lipopolysaccharide LpxL, which was over-expressed in the EV76 strain, was chosen because of its immunogenicity and ability to activate TLR-4 (Szaba et al., 2008).

The *E.coli lpxL* gene was introduced into the *Y. pestis* chromosome, which encodes a hexa-acylated lipid A. LpxL is a potent TLR-4 agonist, capable of inducing a strong innate immune response. Immunization with this strain resulted in 100 % protection from subsequent subcutaneous and intranasal challenges (Sun et al., 2011). Genes for additonal virulence proteins such as, Ail (attachment invasion locus, also designated as OmpX), plasminogen activator protease (Pla), and pH 6 antigen (Psa) have been deleted in an effort to generate effective live attenuated vaccine strains (Felek et al., 2010). In a pneumonic plague model, animals infected with a *ompX* mutant of *Y. pestis* CO92 survived for two days longer than those infected with the parent strain (Kolodziejek et al., 2010). Moreover, *Δcaf1* mutants and *ΔpsaA* mutants exhibited decreased virulence in a murine infection model (Weening et al., 2011). In a recent study, a *Δcaf1* mutant of *Y. pestis* CO92 was attenuated for virulence in a mouse model of bubonic plague but not in a pneumonic plague mouse model when compared to the WT CO92 strain (Sha et al., 2011).

7.2 Subunit vaccines based on the F1, LcrV and YscF proteins

The F1 antigen plays important role in preventing phagocytosis by macrophages. In 1952, Baker and colleagues purified the capsular F1 protein. F1 specific antibodies produced in rabbits, agglutinated plague bacilli and passively protected mice and rats following subcutaneous challenge with virulent *Y. pestis* (Baker et al., 1952). Passive transfer of F1-specific antibodies also protected macaques against pneumonic plague (Ehrenkranz and Meyer, 1955). Subsequently, vaccination with recombinant F1 was shown to protect mice against aerosolized *Y. pestis* (Andrews et al., 1996). Despite this apparent success, it is now well established that virulent F1-negative *Y. pestis* strains exist (Winter et al., 1960; Friedlander et al., 1995; Welkos et al.,1995, Davis et al., 1996; Worsham et al., 1995). Thus, vaccines based solely upon F1 antigen will likely fail to protect against all strains of *Y.pestis*.

The multifunctional LcrV protein is important for the virulence of *Y.pestis* (Brubaker et al., 2003; Une and Brubaker, 1984; Viboud and Biliska, 2005; Heesemann et al., 2006; Bacon et al., 1956; Janssen et al., 1963; Lawton et al., 1963, Une et al., 1984). Immunization with purified LcrV protected mice against subcutaneous challenge; protection was also observed following the passive transfer of LcrV-specific antibodies (Une T et al., 1984, Lawton et al., 1963, Une et al., 1984, Sato et al., 1991; Nakajima and Brubaker, 1993; Motin et al., 1994). Immunization with recombinant LcrV was shown to protect mice against aerosol infection with both F1-positive and F1-negative strains of *Y. pestis* (Motin et al., 1994; Price et al., 1989; Leary et al., 1995; Anderson et al., 1996; Anderson et al., 1998). In spite of these important findings, a vaccine based on LcrV alone did not fully protect against pneumonic plague, perhaps due to lack of cross-protective immunity against LcrV variants (Roggenkamp et al., 1997).

YscF, a recently identified vaccine candidate, is located on the cell surface and forms the TTSS channel, which is required for the secretion of Yops and toxins (Allaoui et al., 1995, Haddix and Straley, 1992; Hoiczyk et al., 2001; Marenne et al., 2003). Immunization of mice with YscF resulted in a high anti-YscF titer and provided partial protection against intravenous (i.v.) challenge with *Y. pestis* (Matson et al., 2005; Swietnicki et al., 2005).

Vaccines based on recombinant F1 and LcrV provided better protection than vaccines comprised of either subunit alone (Williamson et al., 1995,1996). F1 and LcrV formulations administered with the adjuvant alum provided protection in mice against pulmonary *Y. pestis* challenge (Williamson et al., 1997; Jones et al., 2000). In a similar study, investigators at the US Army Medical Research Institute of Infectious Diseases (USAMRIID) demonstrated that a formulation consisting of a recombinant F1-LcrV fusion protein (rF1V) and alum protected mice against pulmonary challenge with either F1-positive or negative strains of *Y. pestis* (Anderson et al., 1998, Heath et al., 1998).

Yops have also been investigated as protective antigens. Immunization of mice with recombinant Yops (H, E, N, K, or M) engendered no significant protection against *Y. pestis* infection (Andrews et al., 1999; Leary et al., 1999; Nemeth et al., 1997). However, mice immunized with complexes of YopB, YopD, and YopE (BDE) produced high-titers of antibodies specific for Yop B, D, and E, and were protected against lethal intravenous challenge with $F1^-$ but not $F1^+$ *Y. pestis.* Furthermore, mice passively immunized with anti-BDE serum were also protected from lethal challenge with $F1^-$ *Y. pestis* (Ivanov et al., 2008).

Huang et al. (2009) evaluated a vaccine consisting of a spray-freeze dried powder form of a recombinant F1-V fusion protein in a mouse model. The vaccine engendered an antibody response and provided 70-90% protection against lethal subcutaneous challenge with *Y. pestis*. Ren et al. (2009) developed a vaccine consisting of recombinant F1, and V from *Y.pestis,* and the Protective Antigen from *B. anthracis* (rF1 + rV+ rPA). This formulation protected mice from subcutaneous challenge with 10^7 colony-forming units (CFU) of a virulent *Y. pestis* strain, and fully protected rabbits against subcutaneous challenge with 1.2×10^5 spores of virulent *B. anthracis.*

CpG oligodeoxynucleotide (ODN) has been used as an adjuvant together with F1-V antigen to enhance its immune response in mice. CpG ODNs significantly augmented the antibody response even up to 5 months and increased the efficacy of the vaccine in murine model of bubonic and pneumonic plague (Amemiya et al., 2009).

Immunization with flagellin and with F1-V elicited a robust humoral immune response in mice and two species of nonhuman primates. The flagellin-F1-V formulation fully protected mice against intranasal challenge with *Y. pestis* CO92 (Mizel et al., 2009). Oral immunization with cationic liposome–nucleic acid complexes (CLDC) combined with F1 antigen elicited protective immunity against lethal pneumonic plague in C57BL/6J. This formulation protected mice up to 18 weeks post vaccination. Protection mediated by oral CLDC with F1 antigen depends primarily on CD4+ T cells, with a partial contribution from CD8+ T cells (Jones et al., 2010).

Ramirez and Alejandra (2009) constructed an attenuated *Salmonella* Typhi strain that expressed the F1 antigen of *Y. pestis* (*S.* Typhi (F1)), and evaluated its immunogenicity. Newborn mice primed intranasally with a single dose of *S.* Typhi (F1) exhibited a mucosal and cellular immune response one week post immunization. *S.* Typhi(F1) enhanced the activation and maturation of neonatal $CD11c^+$ dendritic cells, and MHC-II cell surface markers and the production of proinflammatory cytokines. The *S.* Typhi(F1)- based formulation improved the capacity of DC for antigen presentation and T cell stimulation in vitro.

The protective efficacy of the F1 + rV270 (an LcrV variant lacking amino acid residues 271–300) vaccines compared to that of EV76 was evaluated. The F1 + rV270 formulation was

tested in both guinea pigs and New Zealand White rabbits by determining the antibody response and protection against subcutaneous challenge with the virulent *Y. pestis* 141 strain (Qi Z et al., 2010).

Xiao et al. (2010) developed an anti-F1-specific human monoclonal antibody (mAb) (m252) and anti-V-specific human mAbs (m253, m254) against the F1 and V antigens, respectively. These monoclonal antibodies were found to be more effective than the corresponding mouse antibodies. Neutralization of TNF-α and IFN-γ interfered with the protective efficacy of F1- or LcrV- specific antibodies against the fully virulent *pgm*-positive *Y. pestis* strain CO92 (Lin et al., 2010). Recently, a recombinant rF1+rV vaccine provided protection in *Cynomolgus macaques* against pneumonic plague following inhalational challenge with a clinical isolate of *Y. pestis* (CO92) (Williamson et al., 2011).

7.3 Plant based vaccines

The use of plant-based oral recombinant vaccines could be an alternative approach for plague immunoprophylaxis. However, F1 and LcrV genes expressed in recombinant plant tissue were relatively less immunogenic due to the lack of signals recognized by the innate immune system through Toll Like receptors. In one such study, Swiss-Webster mice exhibited significant protection following subcutaneous immunization with *Nicotiana tabacum* leaves that expressed a LcrV–F1 (F1-V) translational fusion protein on its surface (Arlen 2008). In a separate study, guinea pigs immunized with a transgenic *Nicotiana benthamiana* tobacco plant expressing the F1-V fusion protein were protected against a subsequent pneumonic plague infection (Del Prete, 2009). However, the amount of recombinant protein produced in plant-based vectors was generally poor. To overcome this problem, the N-terminal of the γ-Zein protein (produced in maize and induces protein body formation) was fused with an F1-V fusion construct, which resulted in up to three times higher accumulation of protein in *Nicotiana tabacum* drived tissues than the F1-V fusion protein alone (Alvarez et al., 2010). Plant-based vaccines have also been evaluated for other Category A agents such as Variola major virus and *B. anthracis* (Rigano et al., 2009). Recently, an F1-V fusion protein expressed in carrot tap roots and lettuce was found to be stable and immunogenic for mice. (Rosales-Mendoza et al., 2010a; Rosales-Mendoza et al., 2010b).

7.4 DNA vaccine straties

DNA vaccines have been developed as an alternative to protein-based vaccines. LcrV- and F1-based DNA vaccines have been developed that contain either all or part of the open reading frames encoding either LcrV, F1, or both. One vaccine containing a portion of *LcrV* that encoded a 127-amino acid peptide, was found to elicit a strong humoral immune response. Furthermore, mice immunized with this vaccine exhibited a 60% survival rate following challenge with *Y.pestis* (Vernazza. et al., 2009). A vaccine consisting of the IL-12 coding sequence and the genes for F1 or LcrV was used to immunize mice intranasally. This formulation enhanced IgA production in the mucosa and showed 80% protection from a subsequent inhalational challenge with *Y.pestis* (Yamanaka et al., 2008)

Recently, a DNA vaccine based on F1 and YscF was constructed by fusion of the gene encoding YscF to the downstream sequence of F1. This strategy enhanced protection resulting from F1 or YscF DNA vaccines alone. This approach suggested a number of ways

to develop protective DNA vaccines (Wang et al., 2010). Immunization with the F1-V based DNA vaccine and the adjuvant, lymphotactin (LTN) resulted in high levels of serum IgG and mucosal IgA antibodies (Yamanaka et al., 2010). The LcrV based DNA vaccine elicited a CD8+ immune response against specific epitopes of this antigen (Wang et al., 2011). Immunization of mice with a DNA vaccine consisting of F1 and V and the gene encoding the heat-labile enterotoxin (LT) of *E. coli* as an adjuvant resulted in 40% protection (Rosenzweig et al., 2011).

7.5 Virus vector based vaccines

Live avirulent or attenuated recombinant viruses expressing genes encoding virulence antigens offer several advantages over their bacterial counterparts. Non-enveloped/ naked viruses may be a better vehicle for vaccine development as these viruses can be stored for a long time without losing their infectivity. In one such case, a recombinant vaccinia virus vector was used to express an F1-V fusion protein. The vaccine was orally administered to C57BL/6J mice and was found to protect against an inhalational challenge of ten times the lethal dose of *Y.pestis* KIM/D27. It provided 100% protection up to 45 weeks post-immunization (Bhattacharya et al., 2010). Moreover, a recombinant raccoon pox virus producing F1 antigen elicited significant protection in orally immunized prairie dogs (*Cynomys spp*.) (Rocke et al., 2008). More recently, two recombinant raccoon pox viruses producing the F1 antigen and a 307-amino-acid truncated form of LcrV engendered a better humoral response and protection in both mice and prairie dogs following subcutaneous challenge with virulent *Y. pestis* CO92 (Rocke et al.,2010a, b).

The route of immunization and booster plays an important role in the immune response and subsequent protection. A recombinant Vaccinia virus Ankara vector producing either the full-length F1 or the truncated 307 amino acid peptide form of LcrV was administered intramuscularly (IM). Vaccines consisting of truncated V antigen and full length F1 antigen provided 85% and 50% protection, respectively, against both intranasal and intraperitoneal challenges with *Y. pestis* CO92 (Brewoo et al., 2010). Recently, modified, non-replicating adenovirus vectors were evaluated for the development of antibodies against both the heavy and light chains of a previously identified anti-LcrV protective antibody. Surprisingly, immunized C57BL/6J male mice showed significant levels of IgG that persisted for up to 12 weeks and exhibited 80% protection in mice after intranasal challenge with a 2×10^4-cfu of fully virulent *Y. pestis* CO92 (Sofer-Podesta et al., 2009).

Human vesicular stomatitis virus (VSV) has been evaluated as an effective vector for the development of a novel plague vaccine. VSV was engineered to express LcrV. Immunized female BALB/c mice showed strong humoral responses with an IgG2a bias dichotomy and exhibited 90% protection from an intranasal challenge with *Y. pestis* CO92 (Chattopadhyaya et al., 2008). These finding highlight the importance of the choice of viral-vectors in the development of plague vaccines.

7.6 Synthetic vaccines based on defined B and T cell epitopes

The concept of synthetic peptide vaccines was laid by the pioneering work of Anderer who demonstrated that a peptide from tobacco mosaic virus (TMV) showed immunoreactivity with antiserum against TMV. In addition, a peptide coupled to a carrier induced specific virus

precipitating and neutralizing antibodies (Deber et al., 1985). The first step in developing a synthetic peptide vaccine for plague is to identify the relevant antigen(s) determine their amino acid sequence, and identify protective B and T cell epitopes. Sabhnani and Rao (2003) identified the immunodominant epitopes of F1 antigen. The immunogenicity of the B cell (B1, B2, and B3) and T cell (T1, T2) peptides was studied in mice using alhydrogel and liposomes as delivery vehicles. B-T constructs of F1 antigen engendered protection in mice. PLGA (poly (DL-lactide-co-glycolide) microsphere delivery of B-T constructs enhanced protection (Tripathi and Rao, 2006). Later, several B and T cell epitopes of V antigen were identified by direct binding, competitive, and T cell proliferation approaches. V antigen peptides a, g and j were found to be pure B cell epitopes and peptides d and k pure T cell epitopes, whereas other peptides b, f and i showed both B and T cell properties (Khan and Rao, 2008). Furthermore, mice immunized intranasally with B-T conjugates of V antigen peptides entrapped in microspheres resulted in high titers of serum and mucosal IgG and IgA upto 120 day postimmunization. Interestingly, some of the conjugates showed enhanced protection in mice challenged with live bacteria (Uppada and Rao, 2009). Gupta et al. (2009) demonstrated the cell mediated immune response of some of the best B-T conjugates in different strains of mice. Surprisingly, some of the B-T conjugates of F1 and V antigen resulted in good lymphocyte proliferation and cytokine production *in vitro* as determined by ELISPOT assay. FACS analysis of some conjugates showed the presence of IFN-γ and perforin secreting CD4+ cells as compared to CD8+ T cells (Gupta et al. 2011), which demonstrated the importance of CD4+ T cells in conferring immunity in the host.

8. Future perspectives

The development of a fully protective vaccine against plague remains a challenge . A perfect vaccine must protect humans against all three biotypes of *Y.pestis*. None of the formulations of F1 and V based vaccines were fully protective against experimental infections. The ideal vaccine would stimulate robust antibody and cell mediated immune response with respect to serum IgG, IgG sub classes and mucosal IgA along with Th1/ Th2 /Th17 cytokines correlation. These parameters could be exploited for protection studies in humans. Standardized procedures will facilitate human clinical trials to determine vaccine formulations, dosages and schedules that best prime protective responses. Incorporating additional antigens such as YscF into F1/LcrV-based vaccines and modifying existing formulations, on both the DNA and protein level, will be more effective and could lead to fully protective vaccine against all strains of *Y.pestis*. Furthermore, using different ways of immunization with novel delivery vehicles and adjuvants could enhance the immune response and efficacy of different formulations. Currently, we have extended our study by designing MAP (Multiple Antigen Peptide) incorporating the relevant protective epitopes of F1, V and YscF antigen in PLGA nanoparticles using CpG, as an adjuvant to activate Toll Like Receptor 9 (TLR-9) of the innate immune system (Uppada et al. 2011). This preparation gave a better immunogenicity profile than that of single epitope based immunogens.

A better understanding of virulence mechanisms, host pathogen interactions that operate within the body and especially the lungs during infection, could provide some new alternative targets for vaccines and therapeutics. The focus should be on the pneumonic form of disease rather than the bubonic and septicemic forms. Identifying agonists of TLR-2 or TLR-4 is also an important area of research for plague vaccine. Synthetic microbial

products that activate the Th1 and Th17 pathways are also beneficial to host immunity. However, given present concerns for bioterrorism, which may involve the release of aerosolized *Y. pestis*, there is now a greater need to explicitly characterize virulence factors that impact pulmonary disease.

9. References

Aepfelbacher M, and Heesemann J (2001). Modulation of Rho GTPases and the actin cytoskeleton by *Yersinia* outer proteins (Yops). Int. J. Med. Microbiol. 291:269–276.

Aepfelbacher M, Zumbihl R, and Heesemann J (2005). Modulation of Rho GTPases and the actin cytoskeleton by YopT of *Yersinia*. Curr. Top. Microbiol. Immunol. 291:167–175.

Allaoui A, Schulte R, Cornelis G R, (1995). Mutational analysis of the *Yersinia enterocolitica virC* operon: characterization of *yscE, F, G, I, J, K* required for Yop secretion and *ysc*H encoding YopR. Mol Microbiol 1995, 18:343-355.

Alvarez ML, Topal E, Martin F, Cardineau GA (2010). Higher accumulation of F1-V fusion recombinant protein in plants after induction of protein body formation. Plant Mol Biol 72:75–89.

Amemiya K, Meyers JL, Rogers TE et al. (2009). CpG oligodeoxynucleotides augment the murine immune response to the *Yersinia pestis* F1-V vaccine in bubonic and pneumonic models of plague. Vaccine 27;16: 2220–2229.

Anderson GW Jr , Heath DG, Bolt CR, Welkos SL, Friedlander AM, (1998). Short- and long-term efficacy of single-dose subunit vaccines against *Yersinia pestis* in mice. Am. J. Trop. Med. Hyg ;58(6): 793–799.

Anderson GW Jr, Leary SE, Williamson ED, et al. (1996). Recombinant V antigen protects mice against pneumonic and bubonic plague caused by F1-capsule-positive and -negative strains of Yersinia pestis. Infect. Immun ; 64(11):4580–4585.

Andersson K, Carballeira N, Magnusson KE, Persson C, Stendahl O, Wolf-Watz H, and Fallman M (1996). YopH of *Yersinia pseudotuberculosis* interrupts early phosphotyrosine signaling associated with phagocytosis. Mol. Microbiol. 20:1057–1069.

Andrews GP, Heath DG, Anderson GW Jr, Welkos SL, Friedlander AM (1996). Fraction 1 capsular antigen (F1) purification from *Yersinia pestis* CO92 and from an *Escherichia coli* recombinant strain and efficacy against lethal plague challenge. Infect. Immun: 64; (6):2180–2187.

Andrews GP, Strachan ST, Benner GE, Sample AK, Anderson GW Jr., Adamovicz JJ, Welkos SL, Pullen JK, and Friedlander AM (1999). Protective efficacy of recombinant *Yersinia* outer proteins against bubonic plague caused by encapsulated and nonencapsulated *Yersinia pestis*. Infect. Immun: 67;1533–1537.

Arlen PA, Singleton M, Adamovicz JJ, Ding Y, Davoodi-Semiromi A, Daniell H (2008) Effective plague vaccination via oral delivery of plant cells expressing F1-V antigens in chloroplasts. Infect Immun: 76;3640–3650.

Bacon GA, Burrows TW (1956). The basis of virulence in *Pasteurella pestis*: an antigen determining virulence. Br. J. Exp. Pathol. 37:481–493.

Bacot AW, and Martin CJ (1914). LXVII. Observations on the mechanism of the transmission of plague by fleas. J. Hyg: 13 (Plague Suppl. 3):423–439.

Bahmanyar M, and Cavanaugh DC (1976). Plague manual. World Health Organization, Geneva, Switzerland.

Baker EE, Sommer H, Foster LE, Meyer E, Meyer KF (1952). Studies on immunization against plague. I. The isolation and characterization of the soluble antigen of *Pasteurella pestis*. J. Immunol: 68;131–145.

Barnes AM, and Quan TJ (1992). Plague, p. 1285–1291. *In* S. L. Gorbach, J. G. Bartlett, and N. R. Blacklow (ed.), Infectious diseases. The W. B. Saunders Co., Philadelphia, Pa.

Becker TM, Poland JD, Quan TJ, White ME, Mann JM, and Barnes AM (1987). Plague meningitis—a retrospective analysis of cases reported in the United States, 1970–1979. West. J. Med. 147:554–557.

Ben-Gurion, R, and Shafferman A (1981). Essential virulence determinants of different *Yersinia* species are carried on a common plasmid. Plasmid: 5 (2); 183–187.

Bhattacharya D, Mecsas J, Hu LT (2010). Development of a vaccinia virus based reservoir-targeted vaccine against *Yersinia pestis*. Vaccine: 28;7683–7689.

Bibikova VA (1977). Contemporary views on the interrelationships between fleas and the pathogens of human and animal diseases. Annu. Rev. Entomol. 22:23–32.

Bioterrorism Overview, Centers for Disease Control and Prevention, 2008-02-12, retrieved 2009-05-22.

Boland A, and Cornelis GR (1998). Role of YopP in suppression of tumor necrosis factor alpha release by macrophages during *Yersinia* infection. Infect. Immun. 66;1878–1884.

Bonacorsi SP, Scavizzi MR, Guiyoule A, Amouroux JH, and Carniel E (1994). Assessment of a fluoroquinolone, three b-lactams, two aminoglycosides, and a cycline in treatment of murine *Yersinia pestis* infection. Antimicrob. Agents Chemother. 38:481–486.

Brewoo JN, Powell TD, Stinchcomb DT, Osorio JE (2010). Efficacy and safety of a modified vaccinia Ankara (MVA) vectored plague vaccine in mice. Vaccine: 28;5891–5899.

Brubaker RR (2003). Interleukin-10 and inhibition of innate immunity to yersiniae: roles of Yops and LcrV (V antigen). Infect. Immun ;71(7):3673–3681.

Brubaker RR (1972). The genus *Yersinia*: biochemistry and genetics of virulence. Curr. Top. Microbiol. Immunol. 57:111–158.

Burroughs AL (1947). Sylvatic plague studies. The vector efficiency of nine species of fleas compared with *Xenopsylla cheopis*. J. Hyg. 45:371–396.

Butler T (1989). The black death past and present. 1. Plague in the 1980s. Trans. R. Soc. Trop. Med. Hyg. 83:458–460.

Cavanaugh DC, Elisberg BL, Llewellyn CH, et al. (1974). Plague immunization. V. Indirect evidence for the efficacy of plague vaccine. J. Infect. Dis; 129(Suppl):S37–S40.

Cavanaugh DC, Randall R (1959).The role of multiplication of *Pasteurella pestis* in mononuclear phagocytes in the pathogenesis of flea-borne plague. J. Immunol; 83:348–363.

Cavanaugh DC (1971). Specific effect of temperature upon transmission of the plague bacillus by the oriental rat flea, *Xenopsylla cheopis*. Am. J. Trop. Med. Hyg. 20:264–272.

Cavanaugh DC, and Williams JE (1980). Plague: some ecological interrelationships, p. 245–256. In R. Traub and H. Starcke (ed.), Fleas. Proceedings of the International Conference on Fleas. A. A. Balkema, Rotterdam, The Netherlands.

Cavanaugh DC, Fortier MK, Robinson DM, Williams JE, and Rust JH Jr. (1979). Application of the ELISA technique to problems in the serologic diagnosis of plague. Bull. Pan Am. Health Org. 13:399–402.

Cavanaugh DC, Wheeler CM, Suyemoto W, Shimada T, and Yamakawa Y (1956). Studies on *Pasteurella pestis* in various flea species. II. Simplified method for the experimental infection of fleas. J. Infect. Dis. 98:107–111.

Centers for Disease Control and Prevention (1994). Human plague-United States, 1993–1994. Morbid. Mortal. Weekly Rep. 43:242–246.

Centers for Disease Control and Prevention (2006). Human plague-four states. MMWR Morb Mortal Wkly Rep.006; 55(34):940-943.

Chase CJ, Ulrich MP, Wasieloski LP Jr, Kondig JP, Garrison J, et al. (2005). Real-time PCR assays targeting a unique chromosomal sequence of *Yersinia pestis*. Clin Chem 51: 1778–1785.

Chattopadhyaya A, Park S, Delmas G, Suresh R, Senina S, Perlin DS, Rose JK (2008). Single-dose, virus-vectored vaccine protection against *Yersinia pestis* challenge: CD4+ cells are required at the time of challenge for optimal protection. Vaccine 26:6329–6337

Conrad, FG, FR LeCocq, and R Krain (1968). A recent epidemic of plague in Vietnam. Arch. Int. Med. 122:193–198.

Cornelis GR (2000). Molecular and cell biology aspects of plague. Proc. Natl. Acad. Sci. USA. 97:8778–8783.

Cornelis GR, Boland A, Boyd AP, Geuijen C, Iriarte M, Neyt C, Sory MP, and Stainier I. (1998). The virulence plasmid of *Yersinia*, an antihost genome.Microbiol. Mol. Biol. Rev. 62:1315–1352.

Craven RB, Maupin GO, Beard ML, Quan TJ, and Barnes AM (1993). Reported cases of human plague infections in the United States, 1970–1991. J. Med. Entomol. 30:758–761.

Crook LD, and. Tempest B. (1992). Plague: a clinical review of 27 cases. Arch. Intern. Med. 152:1253–1256.

Davis KJ, Fritz DL, Pitt ML, et al.(1996). Pathology of experimental pneumonic plague produced by fraction 1-positive and fraction 1-negative *Yersinia pestis* in African green monkeys (*Cercopithecus aethiops*). Arch. Pathol. Lab. Med ;120(2):156–163.

Deber CM, Hruby VJ and Kopple KD (eds) (1985). Peptides: Structure and function. (Rockford:PierceChemical Co.) 23p.

Del Prete G, Santi L, Andrianaivoarimanana V, Amedei A, Domarle O, D' Elios MM, Arntzen CJ, Rahalison L, Mason HS (2009). Plant-derived recombinant F1, V, and F1-V fusion antigens of *Yersinia pestis* activate human cells of the innate and adaptive immune system. Int J Immunopathol Pharmacol 22:133–143.

Doll JM, Zeitz PS, Ettestad P, Bucholtz AL, Davis T, and Gage K (1994). Cat-transmitted fatal pneumonic plague in a person who traveled from Colorado to Arizona. Am. J. Trop. Med. Hyg. 51:109–114.

Du Y, Galyov E, and Forsberg A (1995). Genetic analysis of virulence determinants unique to *Yersinia pestis*. Contrib. Microbiol. Immunol. 13:321–324.

Du Y, Rosqvist R, and Forsberg A (2002). Role of fraction 1 antigen of *Yersinia pestis* in inhibition of phagocytosis. Infect. Immun. 70:1453–1460.

Ehrenkranz NJ, Meyer KF (1955). Studies on immunization against plague. VIII. Study of three immunizing preparations in protecting primates against pneumonic plague. J. Infect. Dis ; 96:138–144.

Felek S, Tsang TM, Krukonis ES (2010). Three *Yersinia pestis* adhesins facilitate Yop delivery to eukaryotic cells and contribute to plague virulence. Infect Immun 78:4134–4150.

Ferber DM, and Brubaker RR (1981). Plasmids in *Yersinia pestis*. Infect. Immun. 31:839–841.

Fields KA, Nilles ML, Cowan C, and Straley SC (1999). Virulence role of V antigen of *Yersinia pestis* at the bacterial surface. Infect. Immun. 67:5395–5408.

Filippov AA, Solodovnikov NS, Kookleva LM, and Protsenko OA (1990). Plasmid content in *Yersinia pestis* strains of different origin. FEMS Microbiol. Lett. 67:45–48.

Finegold MJ (1969). Pneumonic plague in monkeys. An electron microscopic study. Am. J. Pathol ;54 (2):167–185.

Friedlander AM, Welkos SL, Worsham PL, et al. (1995). Relationship between virulence and immunity as revealed in recent studies of the F1 capsule of *Yersinia pestis*. Clin. Infect. Dis ; 21(Suppl 2):S178–S181.

Gage KL, Lance SE, Dennis DT, and Montenieri JA (1992). Human plague in the United States: a review of cases from 1988–1992 with comments on the likelihood of increased plague activity. Border Epidemiol. Bull. 19:1–10.

Galan JE, and Collmer A (1999). Type III secretion machines: bacterial devices for protein delivery into host cells. Science. 284:1322–1328.

Girard G (1963) Immunity in plague. Results of 30 years of work on the *Pasteurella pestis* Ev' (Girard and Robic) strain. Biol Med (Paris);52:631–731.

Gottfried RS (1983). The black death. Natural and human disaster in medieval Europe. The Free Press, New York, N.Y.

Green SP, Hartland EL, Robins-Browne RM, and Phillips WA (1995). Role of YopH in the suppression of tyrosine phosphorylation and respiratory burst activity in murine macrophages infected with *Yersinia enterocolitica*. J. Leukoc. Biol. 57:972–977.

Gupta G, Khan AA & Rao DN (2009). Cell-Mediated Immune Response and Th1/Th2 Cytokine Profile of B-T Constructs of F1 and V Antigen of *Yersinia pestis*. Scandinavian Journal of Immunology 71, 186–198.

Gupta G, et al (2011). Evaluation of CD4+/CD8+ T-cell expression and IFN-γ, perforin secretion for B–T constructs of F1and V antigens of *Yersinia pestis*. Int Immunopharmacol. doi:10.1016/j.intimp.2011.10.012

Haddix PL, Straley SC (1992). Structure and regulation of the *Yersinia pestis yscBCDEF* operon. J Bacteriol 174(14):4820-4828.

Haffkine WM (1897). Remarks on the plague prophylactic fluid. Br. Med. J ;1:1461.

Harris SH (1994). *Factories of Death*. New York, NY: Routledge; 1994:78, 96.

Heath DG, Anderson GW Jr, Mauro JM, et al. (1998). Protection against experimental bubonic and pneumonic plague by a recombinant capsular F1-V antigen fusion protein vaccine. Vaccine ;16(1112): 1131–1137.

Heesemann J, Sing A, Trulzsch K (2006). Yersinia's stratagem: targeting innate and adaptive immune defense. Curr. Opin. Microbiol ; 9 (1) : 55–61.

Higgins JA, Ezzell J, Hinnebusch BJ, Shipley M, Henchal EA, et al. (1998), 5' nuclease PCR assay to detect *Yersinia pestis*. J Clin Microbiol 36: 2284–2288.

Hinnebusch J, Schwan TG (1993). New method for plague surveillance using polymerase chain reaction to detect *Yersinia pestis* in fleas. J Clin Microbiol 31: 1511–1514.

Hirst LF (1953). *The Conquest of Plague: A Study of the Evolution and Epidemiology.* Oxford, UK: Clarendon Press.

Hoiczyk E, Blobel G (2001). Polymerization of a single protein of the pathogen *Yersinia enterocolitica* into needles punctures eukaryotic cells. Proc Natl Acad Sci U S A, 98(8):4669-4674.

Holt JG, Krieg NR, Sneath PHA, Staley JT, and Williams ST (ed.). (1994). Bergey's manual of determinative bacteriology, 9th ed., p. 175–289. The Williams & Wilkins Co., Baltimore, Md.

Huang XZ, and Lindler LE (2004). The pH 6 antigen is an antiphagocytic factor produced by *Yersinia pestis* independent of Yersinia outer proteins and capsule antigen. Infect. Immun. 72:7212–7219.

Huang J, D'Souza AJ, Alarcon JB, Mikszta JA et al. (2009). Protective Immunity in Mice Achieved with Dry Powder Formulation and Alternative Delivery of Plague F1-V Vaccine. Clin Vacc Immunol:16 719–725

Hull HF, Montes JM, and Mann JM (1987). Septicemic plague in New Mexico. J. Infect. Dis. 155:113–118.

Iqbal SS, Chambers JP, Goode MT, Valdes JJ, Brubaker RR (2000). Detection of *Yersinia pestis* by pesticin fluorogenic probe-coupled PCR. Mol Cell Probes 14: 109–114.

Iriarte M, and GR Cornelis (1998). YopT, a new *Yersinia* Yop effector protein, affects the cytoskeleton of host cells. Mol. Microbiol. 29:915–929.

Iteman I, Guiyoule A, De Almeida AMP, Guilvout I, Baranton G, and Carniel E (1993). Relationship between loss of pigmentation and deletion of the chromosomal iron-regulated *irp2* gene in *Yersinia pestis*: evidence for separate but related events. Infect. Immun. 61:2717–2722.

Ivanov MI, Noel BL, Rampersaud R, Mena P, Benach JL, and Bliska JB (2008). Vaccination of Mice with a Yop Translocon Complex Elicits Antibodies That Are Protective against Infection with F1-*Yersinia pestis*. Infect. Immun. 76;11, 5181–5190.

Janssen WA, Lawton WD, Fukui GM, Surgalla MJ (1963). The pathogenesis of plague. I. A study of the correlation between virulence and relative phagocytosis resistance of some strains of *Pasteurella pestis*. J. Infect. Dis ;113:139–143.

Janssen WA, Surgalla MJ (1969). Plague bacillus: survival within host phagocytes. Science ;163:950–952.

Jones A, Catharine Bosio (2010). Protection against pneumonic plague following oral immunization with a non-replicating vaccine. Vaccine: 16; 28(36):5924-9.

Jones SM, Day F, Stagg AJ, Williamson ED (2000). Protection conferred by a fully recombinant sub-unit vaccine against *Yersinia pestis* in male and female mice of four inbred strains. Vaccine;19(23): 358–366.

Kartman L. (1969). Effect of differences in ambient temperature upon the fate of *Pasteurella pestis* in *Xenopsylla cheopis*. Trans. R. Soc. Trop. Med. Hyg. 63:71–75.

Kawahara K, Tsukano H, Watanabe H, Lindner B, Matsuura M (2002). Modification of the structure and activity of lipid A in *Yersinia pestis* lipopolysaccharide by growth temperature. Infect Immun 70:4092–4098.

Kerschen EJ, Cohen DA, Kaplan AM, and Straley SC (2004). The plague virulence protein YopM targets the innate immune response by causing a global depletion of NK cells. Infect. Immun. 72:4589–4602.

Khan AA, Rao DN (2008). Identifying B and T cell epitopes and studying humoral, mucosal and cellular immune responses of peptides derived from V antigen of *Yersinia pestis*.Vaccine: 26; 316—332.

Kolle W, Otto R (1904). Weitere Untersuchungen uber die Pestimmunitat. Zeitschr. F. Hyg;48:399–428.

Kolodziejek AM, Schnider DR, Rohde HN, Wojtowicz AJ, Bohach GA, Minnich SA, Hovde CJ (2010). Outer membrane protein X (Ail) contributes to *Yersinia pestis* virulence in pneumonic plague and its activity is dependent on LPS core length. Infect Immun;78:5233–5243.

Lathem WW, Crosby SD, Miller VL, Goldman WE (2005). Progression of primary pneumonic plague: a mouse model of infection, pathology, and bacterial transcriptional activity. Proc. Natl Acad. Sci. USA; 102(49):17786–17791.

Lawton WD, Erdman RL, Surgalla MJ (1963). Biosynthesis and purification of V and W antigen in *Pateurella pestis*. J. Immunol; 91:179–184.

Leary SE, Williamson ED, Griffin KF, et al. (1995). Active immunization with recombinant V antigen from *Yersinia pestis* protects mice against plague. Infect. Immun;63(8):2854–2858.

Leary SE, Griffin KF, EE Galyov, J Hewer, ED Williamson, A Holmstrom, A Forsberg, and RW Titball (1999). *Yersinia* outer proteins (YOPS) E, K and N are antigenic but non-protective compared to V antigen, in a murine model of bubonic plague. Microb. Pathog. 26:159–169.

Lederberg J., ed. (2001).*Biological Weapons limiting the threat.*, MIT Press.

Lee VT, Tam C, and Schneewind O (2000). LcrV, a substrate for *Yersinia enterocolitica* type III secretion, is required for toxin targeting into the cytosol of HeLa cells. J. Biol. Chem. 275:36869–36875.

Lien Teh WA (1926). Treatise on Pneumonic Plague. League of Nations Health Organisation; Geneva, Switzerland.

Lindler LE, Fan W, Jahan N (2001). Detection of ciprofloxacin-resistant *Yersinia pestis* by fluorogenic PCR using the LightCycler. J Clin Microbiol 39: 3649–3655.

Lindler LE, and Tall BD (1993). *Yersinia pestis* pH 6 antigen forms fimbriae and is induced by intracellular association with macrophages. Mol. Microbiol. 8:311–324.

Lin JS, et al. (2010).TNF-α and IFN-γ contribute to F1/LcrV-targeted immune defense in mouse models of fully virulent pneumonic plague.Vaccine: 16;29(2):357-62.

Link VB (1955). A History of Plague in the United States of America [Public Health Service Monograph No. 26]. Washington, DC: Government Printing Office, 1955.

Loiez C, Herwegh S, Wallet F, Armand S, Guinet F, et al. (2003). Detection of *Yersinia pestis* in sputum by real-time PCR. J Clin Microbiol 41: 4873–4875.

Lukaszewski RA, Kenny DJ, Taylor R, et al. (2005). Pathogenesis of *Yersinia pestis* infection in BALB/c mice: effects on host macrophages and neutrophils. Infect. Immun;73(11):7142–7150.

Makoveichuk E, Cherepanov P, Lundberg S, Forsberg A and Olivecrona G. (2003). pH6 antigen of *Yersinia pestis* interacts with plasma lipoproteins and cell membranes. J. Lipid Res. 44:320–330.

Marenne MN, Journet L, Mota LJ, Cornelis GR (2003). Genetic analysis of the formation of the Ysc-Yop translocation pore in macrophagesby *Yersinia enterocolitica*: role of LcrV, YscF and YopN. Microb Pathog. 35(6):243-258.

Matson JS, Kelly A Durick, David S Bradley and Matthew L Nilles (2005). Immunization of mice with YscF provides protection from *Yersinia pestis* infections. BMC Microbiology, 5:38.

McDonald C, Vacratsis PO, Bliska JB, and Dixon JE (2003). The yersinia virulence factor YopM forms a novel protein complex with two cellular kinases. J. Biol. Chem. 278:18514–18523.

McDonough KA, Schwan TG, Thomas RE, and Falkow S (1988). Identification of a *Yersinia pestis*-specific DNA probe with potential for use in plague surveillance. J. Clin. Microbiol. 26:2515–2519.

Meyer KF, Cavanaugh DC, Bartelloni PJ, Marshall JD Jr. (1974). Plague immunization. I. Past and present trends. J. Infect. Dis 1974;129 (Suppl):S13–S18.

Meyer KF, Smith G, Foster L, Brookman M, Sung M (1974). Live attenuated *Yersinia pestis* vaccine: virulent in nonhuman primates, harmless to guinea pigs. J. Infect. Dis;129(Suppl):S85–S112.

Meyer KF (1970). Effectiveness of live or killed plague vaccines in man. Bull. World Health Org. 42 (5):653–666.

Meyer KF (1950). Modern therapy of plague. JAMA 144:982–985.

Meyer KF (1961). Pneumonic plague. Bacteriol. Rev. 25:249–261.

Mizel SB, Graff AH et al. (2009). Flagellin-F1-V fusion protein is an effective plague vaccine in mice and two species of nonhuman primates. Clin Vac Immunol: 16;1: 21–28.

Montminy SW, Khan N, McGrath S, Walkowicz MJ, Sharp F, Conlon JE, Fukase K, Kusumoto S, Sweet C, Miyake K, Akira S, Cotter RJ, Goguen JD, Lien E (2006). Virulence factors of *Yersinia pestis* are overcome by a strong lipopolysaccharide response. Nat Immunol 7:1066–1073.

Motin VL, Nakajima R, Smirnov GB, Brubaker RR (1994). Passive immunity to yersiniae mediated by antirecombinant V antigen and protein A-V antigen fusion peptide. Infect. Immun 62(10):4192–4201.

Nakajima R, Brubaker RR (1993). Association between virulence of *Yersinia pestis* and suppression of gamma interferon and tumor necrosis factor alpha. Infect. Immun. ;61(1):23–31.

Nemeth J, and Straley SC (1997). Effect of *Yersinia pestis* YopM on experimental plague. Infect. Immun. 65:924–930.

Norkina OV, Kulichenko AN, Gintsburg AL, Tuchkov IV, Popov Yu A, et al. (1994) Development of a diagnostic test for *Yersinia pestis* by the polymerase chain reaction. J Appl Bacteriol 76: 240–245.

Perry RD, and Fetherston JD (1997). *Yersinia pestis*: etiologic agent of plague. Clin. Microbiol. Rev. 10:35–66.

Peterson JW, Walberg KG, Pawlik J, Bush K, Taormina J, Hardcastle J, Moen S, Thomas J, Lawrence W, Ponce C, Parham T, Chatuev BM, Sower L, Klimpel G, Eaves-Pyles T, Chopra AK (2010). Evaluation of protection afforded by fluoroquinolones against respiratory infections with *Bacillus anthracis*, *Yersinia pestis*, and *Francisella tularensis*. Open Microbiology Journal 4:34–46.

Poland JD, Quan TJ, and AM Barnes (1994). Plague, p. 93–112. *In* G. W. Beran (ed.), Handbook of zoonoses. Section A. Bacterial, rickettsial, chlamydial, and mycotic, 2nd ed. CRC Press, Inc., Ann Arbor, Mich.

Poland JD, and Barnes AM (1979). Plague, p. 515–559. *In* J. H. Steele (ed), CRC handbook series in zoonoses. Section A. Bacterial, rickettsial, and mycotic diseases, vol. I. CRC Press, Inc., Boca Raton, Fla.

Pollitzer R (1954). Plague. W. H. O. Monogr. Ser. 22:1–698.

Price SB, Leung KY, Barve SS, Straley SC (1989). Molecular analysis of *lcrGVH*, the V antigen operon of *Yersinia pestis*. J. Bacteriol ;171:5646–5653.

Price SB, Freeman MD and Yeh KS (1995). Transcriptional analysis of the *Yersinia pestis* pH 6 antigen gene. J. Bacteriol. 177:5997–6000.

Pujol C, Bliska JB (2005). Turning *Yersinia* pathogenesis outside in: subversion of macrophage function by intracellular yersiniae. Clin. Immunol ;114(3):216–226.

Qi Z, Zhou L, Zhang Q (2010). Comparison of mouse, guinea pig and rabbit models for evaluation of plague subunit vaccine F1 + rV270. Vaccine 28 1655–1660.

Ramirez K et. al. (2009). Mucosally delivered *Salmonella typhi* expressing the *Yersinia pestis* F1 antigen elicits mucosal and systemic immunity early in life and primes the neonatal immune system for a vigorous anamnestic response to parenteral F1 boost. J. Immunol. 182; 1211-1222.

Ren J, Dong D, Zhang J, Zhang J, et al. (2009). Protection against anthrax and plague by a combined vaccine in mice and rabbits. Vaccine. 9; 27(52):7436-41

Rigano MM, Manna C, Giulini A, Vitale A, Cardi T (2009). Plants as biofactories for the production of subunit vaccines against biosecurity- related bacteria and viruses. Vaccine 27:3463–3466

Rocke TE, Smith SR, Stinchcom DT, Osorio JE (2008). Immunization of black-tailed prairie dog against plague through consumption of vaccine-laden baits. J Wildl Dis 44:930–937.

Rocke TE, Iams KP, Dawe S, Smith SR, Williamson JL, Heisey DM, Osorio JE (2010a). Further development of raccoon poxvirus vectored vaccines against plague (*Yersinia pestis*). Vaccine 28:338–344.

Rocke TE, Pussini N, Smith SR, Williamson J, Powell B, Osorio JE (2010b). Consumption of baits containing raccoon pox-based plague vaccines protects black-tailed prairie dogs (*Cynomys ludovicianus*). Vector Borne Zoonotic Dis 10:53–58.

Roggenkamp A, Geiger AM, Leitritz L, Kessler A, Heesemann J (1997). Passive immunity to infection with *Yersinia* spp. mediated by anti-recombinant V antigen is dependent on polymorphism of V antigen. Infect. Immun ;65:446–451.

Rosales-Mendoza S, Soria-Guerra RE, Moreno-Fierros L, Alpuche-Solis AG, Martinez-Gonzalez L, Korban SS (2010a). Expression of an immunogenic F1-Vfusion protein in lettuce as a plant-based vaccine against plague. Planta 232:409–416.

Rosales-Mendoza S, Soria-Guerra RE, Moreno-Fierros L, Han Y, Alpuche-Solís AG, Korban SS (2010b). Transgenic carrot tap roots expressing an immunogenic F1-V fusion protein from *Yersinia pestis* are immunogenic in mice. J Plant Physiol 168:174–180.

Rosenzweig JA, Olufisayo Jejelowo, Jian Sha,Tatiana E. Erova, Sheri M. Brackman, Michelle L. Kirtley, Cristina J. van Lier, Ashok K. Chopra(2011).Progress on plague vaccine development. Appl Microbiol Biotechnol DOI 10.1007/s00253-011-3380-6.

Rosqvist R, Forsberg A, Rimpilainen M, Bergman T, Wolf-Watz H (1990). The cytotoxic protein YopE of *Yersinia* obstructs the primary host defense. Mol. Microbiol. 4:657–667.

Russell P, Eley SM, Hibbs SE, et al. (1995). A comparison of Plague vaccine, USP and EV76 vaccine induced protection against *Yersinia pestis* in a murine model. Vaccine ;13:1551–1556.

Sabhnani L, & Rao DN (2003). Developing subunit immunogens using B and T cell epitopes and their constructs derived from the F1 antigen of *Yersinia pestis* using novel delivery vehicles. FEMS Immunol Med Microbiol. 15;38(3):215-29.

Saikaly PE, Barlaz MA, de Los Reyes FL, 3rd (2007). Development of quantitative real-time PCR assays for detection and quantification of surrogate biological warfare agents in building debris and leachate. Appl Environ Microbiol 73: 6557–6565.

Sato K, Nakajima R, Hara F, Une T, Osada Y (1991). Preparation of monoclonal antibody to V antigen from *Yersinia pestis*. Contrib. Microbiol. Immunol;12:225–229.

Sebbane F, Gardner D, Long D, Gowen BB, Hinnebusch BJ (2005). Kinetics of disease progression and host response in a rat model of bubonic plague. Am. J. Pathol ;166(5):1427–1439.

Sha J, Agar SL, Baze WB, Olano JP, Fadl AA, Erova TE, Wang S, Foltz SM, Suarez G, Motin VL, Chauhan S, Klimpel GR, Peterson JW, Chopra AK (2008). Braun lipoprotein (Lpp) contributes to the virulence of yersiniae: potential role of Lpp in inducing bubonic and pneumonic plague. Infect Immun 76:1390–1409.

Sha J, Endsley JJ, Kirtley ML, Foltz SM, Huante MB, Erova TE, Kozlova EV, Popov VL, Yeager LA, Zudina IV, Motin VL, Peterson JW, Chopra AK (2011). Characterization of an F1 deletion mutant of *Yersinia pestis* CO92, pathogenic role of F1 antigen in bubonic and pneumonic plague, and evaluation of the sensitivity and specificity of F1 antigen capture-based dipsticks. J Clin Microbiol 49:1708–1715.

Sing A, Roggenkamp A, Geiger AM, and Heesemann J (2002). *Yersinia enterocolitica* evasion of the host innate immune response by V antigen-induced IL-10 production of macrophages is abrogated in IL-10-deficient mice. J. Immunol. 168:1315–1321.

Sing A, Rost D, Tvardovskaia N, Roggenkamp A, Wiedemann A, Kirschning CJ, Aepfelbacher M, and Heesemann J (2002). Yersinia V-antigen exploits toll-like receptor- 2 and CD14 for interleukin 10–mediated immunosuppression. J. Exp. Med.: 21;196(8):1017-24.

Skottman T, Piiparinen H, Hyytiainen H, Myllys V, Skurnik M, et al. (2007). Simultaneous real-time PCR detection of *Bacillus anthracis*, *Francisella tularensis* and *Yersinia pestis*. Eur J Clin Microbiol Infect Dis 26: 207–211.

Sofer-Podesta C, Ang J, Hackett NR, Senina S, Perlin D, Crystal RG, Boyer JL (2009). Adenovirus-mediated delivery of an anti-V antigen monoclonal antibody protects mice against a lethal *Yersinia pestis* challenge. Infect Immun 4:1561–1568.

Staskawicz BJ, Mudgett MB, Dangl JL, and Galan JE (2001). Common and contrasting themes of plant and animal diseases. Science. 292:2285–2289.

Sticker G (1908). *Abhandlungen aus der Seuchengeschichte und Seuchenlehre. Band I. Die Geschichte der Pest.* Giessen: A Toepelmann Verlag.

Straley SC, Harmon PA (1984). Growth in mouse peritoneal macrophages of *Yersinia pestis* lacking established virulence determinants. Infect. Immun; 45(3):649–654.

Strong RP (1908). Protective inoculation against plague. J. Med. Res; 18:325–346.

Strong RP (1906). Vaccination against plague. Phillipine J. Sci; 1:181–190.

Sun W, Six D, Kuang X, Roland KL, Raetz CR, Curtiss R III (2011). A live attenuated strain of *Yersinia pestis* KIM as a vaccine against plague. Vaccine 29:2986–2998.

Swietnicki W, Powell BS, Goodin J (2005). *Yersinia pestis* Yop secretion protein F: Purification, characterization, and protective efficacy against bubonic plague. Protein Exp Purif: 42; 166–172

Szaba FM, Kummer LW, Wilhelm LB, Lin JS, Parent MA, Montminy-Paquette SW, Lien E, Johnson LL, Smiley ST (2008). D27-pLpxL, an avirulent strain of *Yersinia pestis*, primes T cells thatprotect against pneumonic plague. Infect Immun 77:4295–4304.

Taylor J (1933). Haffkine's plague vaccine. Indian Med. Res. Memoirs; 27:1–125.

Titball RW, Williamson ED (2004). *Yersinia pestis* (plague) vaccines. Expert Opin. Biol. Ther; 4(6): 965–973.

Tomaso H, Reisinger EC, Al Dahouk S, Frangoulidis D, Rakin A, et al. (2003). Rapid detection of *Yersinia pestis* with multiplex real-time PCR assays using fluorescent hybridisation probes. FEMS Immunol Med Microbiol 38: 117–126.

Tripathi V and Rao DN (2006). Inducing systemic and mucosal immune responses to B-T construct of F1 antigen of *Yersinia pestis* in microsphere delivery. Vaccine. 12; 24(16):3279-89.

Tsukano H, Itoh K, Suzuki S, Watanabe H (1996). Detection and identification of *Yersinia pestis* by polymerase chain reaction (PCR) using multiplex primers. Microbiol Immunol 40: 773–775.

Une T, Brubaker RR (1984). In vivo comparison of avirulent Vwa- and Pgm- or Pstr phenotypes of yersiniae. Infect. Immun; 43(3):895–900.

Une T, Brubaker RR (1984). Roles of V antigen in promoting virulence and immunity in yersiniae. J. Immunol; 133(4):2226–2230.

Uppada JB and DN Rao (2009). Humoral immune responses and protective efficacy of sequential B- and T-cell epitopes of V antigen of *Yersinia pestis* in microparticle delivery by intranasal immunization. Med. Microbiol. Immunnol.198; 4, 247-256.

Uppada JB and Rao DN (2011). Enhanced humoral and mucosal immune responses after intranasal immunization with chimeric multiple antigen peptide of LcrV antigen epitopes of *Yersinia pestis* coupled to palmitate in mice. Vaccine 29 (50):9352-60.

Varma-Basil M, El-Hajj H, Marras SA, Hazbon MH, Mann JM, et al. (2004). Molecular beacons for multiplex detection of four bacterial bioterrorism agents. Clin Chem 50: 1060–1063.

Vernazza C, Lingard B, Flick-Smith HC, Baillie LW, Hill J, Atkins HS (2009). Small protective fragments of the *Yersinia pestis* V antigen. Vaccine 27:2775–2780.

Viboud GI, Bliska JB (2005). *Yersinia* outer proteins: role in modulation of host cell signaling responses and pathogenesis. Annu. Rev. Microbiol; 59:69–89.

Von Reyn CF, Weber NS, Tempest B, Barnes AM, Poland JD, Boyce JM, and Zalma V (1977). Epidemiologic and clinical features of an outbreak of bubonic plague in New Mexico. J. Infect. Dis. 136:489–494.

Wang S, et al. (2011). Involvement of CD8+ T cell-mediated immune responses in LcrV DNA vaccine induced protection against lethal *Yersinia pestis* challenge. Vaccine doi:10.1016/j.vaccine.2010.12.062.

Wang S, Mboudjeka I, Goguen JD, Lu S (2010). Antigen engineering can play a critical role in the protective immunity elicited by *Yersinia pestis* DNA vaccines. Vaccine 28: 2011–2019.

Weening EH, Cathelyn JS, Kaufman G, Lawrenz MB, Price P, Goldman WE, Miller VL (2011). The Dependence of *Yersinia. pestis* capsule for pathogenesis is influenced by mouse background. Infect Immun 79:644–652.

Welkos S, Pitt ML, Martinez M, et al. (2002). Determination of the virulence of the pigmentation-deficient and pigmentation-/plasminogen activator-deficient strains of *Yersinia pestis* in non-human primate and mouse models of pneumonic plague. Vaccine; 20(1718):2206–2214.

Welkos SL, Davis KM, Pitt LM, Worsham PL, Freidlander AM (1995). Studies on the contribution of the F1 capsule-associated plasmid p*Fra* to the virulence of *Yersinia pestis*. Contrib. Microbiol. Immunol; 13:299–305.

Wheelis M. (2002), Biological warfare at the 1346 siege of Caffa, Emerg Infect Dis 8:971-975.
Wigglesworth V B (1984). Insect physiology, p. 54–68, Chapman & Hall, New York.
Williams JE, Gentry MK, Braden CA, Leister F and Yolken RH (1984). Use of an enzyme-linked immunosorbent assay to measure antigenaemia during acute plague. Bull. W. H. O. 62:463–466.
Williamson ED, Eley SM, Griffin KF, et al. (1995). A new improved sub-unit vaccine for plague: the basis of protection. FEMS Immunol. Med. Microbiol; 12(34):223–230.
Williamson ED, Eley SM, Stagg AJ, et al.(1997). A sub-unit vaccine elicits IgG in serum, spleen cell cultures and bronchial washings and protects immunized animals against pneumonic plague. Vaccine;15 (10):1079–1084.
Williamson ED, Sharp GJ, Eley SM, et al.(1996). Local and systemic immune response to a microencapsulated sub-unit vaccine for plague. Vaccine; 14(1718):1613–1619.
Williamson ED,et al. (2011). Recombinant (F1+V) vaccine protects cynomolgus macaques against pneumonic plague. Vaccine; 24; 29(29-30):4771-7.
Winter CC, Cherry WB, Moody MD (1960). An unusual strain of *Pasteurella pestis* isolated from a fatal human case of plague. Bull. W.H.O.; 23:408–409.
World Health Organization (2002). Plaguein Malawi and India. Available at http://www.who.int/csr/don/archive/disease/plague/en/; accessed January 25, 2005.
World Health Organization (2003). Plague in Algeria. Available at http //www.who.int/csr/don /2003_06_24a/ en/; accessed January 25, 2005.
World Health Organization (1970). *Health Aspects of Chemical and Biological Weapons*. Geneva, Switzerland; 1970:98-109.
Woron AM, Nazarian EJ, Egan C, McDonough KA, Cirino NM, et al. (2006). Development and evaluation of a 4-target multiplex real-time polymerase chain reaction assay for the detection and characterization of *Yersinia pestis*. Diagn Microbiol Infect Dis 56: 261–268.
Worsham PL, Stein MP, Welkos SL (1995). Construction of defined F1 negative mutants of virulent *Yersinia pestis*. Contrib. Microbiol. Immunol; 13:325–328.
Xiao X, Zhu Z, Dankmeyer JL, Wormald MM, Fast RL, et al. (2010). Human anti-plague monoclonal antibodies protect mice from *Yersinia pestis* in a bubonic plague model. PLoS ONE 5(10): e13047.
Yamanaka H, Teri Hoyt, Xinghong Yang (2010). A parenteral DNA vaccine protects against pneumonic plague. Vaccine 28 : 3219–3230.
Yamanaka H, Hoyt T, Yang X, Golden S, Bosio CM, Crist K, Becker T, Maddaloni M, Pascual DW (2008). A nasal interleukin-12 DNA vaccine coexpressing *Yersinia pestis* F1-V fusion protein confers protection against pneumonic plague. Infect Immun 76:4564–4573.
Zilinskas RA (2006). The anti-plague system and the Soviet biological warfare program. Crit. Rev. Microbiol; 32(1):47–64.

Bioterrorism

Edited by Dr. Stephen Morse

ISBN 978-953-51-0205-2
Hard cover, 192 pages
Publisher InTech
Published online 28, March, 2012
Published in print edition March, 2012

This book consists of nine chapters, written by international authorities, discussing various aspects of bioterrorism preparedness and response. Five of the chapters are agent-specific and highlight the pathogenesis, prevention and treatment, and the potential of specific organisms (Rickettsia and Yersinia pestis) or toxins (ricin, botulinum neurotoxins, and staphylococcal enterotoxins) to be used for nefarious purposes. Four chapters discuss different aspects of detecting and responding to a bioterrorism attack. These include methods for spatio-temporal disease surveillance, international laboratory response strategies, detection of botulinum neurotoxins in food and other matrices, and the use of physical methods (ie Raman spectroscopy) to detect spores.

InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

IntechOpen