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Immune defense against pneumonic plague

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Summary

Yersinia pestis is one of the world's most virulent human pathogens. Inhalation of this Gram-negative bacterium causes pneumonic plague, a rapidly progressing and usually fatal disease. Extensively antibiotic-resistant strains of *Y. pestis* exist and have significant potential for exploitation as agents of terrorism and biowarfare. Subunit vaccines comprised of the *Y. pestis* F1 and LcrV proteins are well-tolerated and immunogenic in humans but cannot be tested for efficacy, because pneumonic plague outbreaks are uncommon and intentional infection of humans is unethical. In animal models, F1/LcrV-based vaccines protect mice and cynomolgus macaques but have failed, thus far, to adequately protect African green monkeys. We lack an explanation for this inconsistent efficacy. We also lack reliable correlate assays for protective immunity. These deficiencies are hampering efforts to improve vaccine efficacy. Here, I review the immunology of pneumonic plague, focusing on evidence that humoral and cellular defense mechanisms collaborate to defend against pulmonary *Y. pestis* infection.

Keywords

Yersinia pestis; phagocytes; neutrophils; macrophages; antibodies; humoral immunity; cellular immunity; vaccine

Bubonic, septicemic, and pneumonic plague

Pandemics of plague have ravaged human populations throughout recorded history (1–6). Fortunately, improved sanitation and public health surveillance, coupled with effective therapeutics and a better understanding of transmissibility, greatly reduce the likelihood of a natural, modern day pandemic. Nevertheless, isolated plague outbreaks continue to this day (6–8). Moreover, *Yersinia pestis*, the etiologic agent of plague, displays natural genetic plasticity (9,10), can acquire antibiotic resistance (11–14), and has been weaponized (15–17). Thus, plague has considerable potential to re-emerge as infectious disease threat during the 21st century.

Y. pestis is a Gram-negative, non-motile, facultative intracellular bacterium (3,5). Sylvatic rodent populations are the primary natural reservoirs for *Y. pestis* (1,4). In that setting, blood-feeding ectoparasites, primarily fleas, transmit the bacilli from one rodent to another. The most common form of human disease, bubonic plague, is transmitted from rodent reservoirs to humans via infected fleas (1–6).

Y. pestis evolved from *Y. pseudotuberculosis*, an enteropathogen, within the last 20,000 years (18). Transmission by insect vector is one of many adaptations that accompanied this recent evolution (3–6). In addition, evolution selected for traits that enable *Y. pestis* to

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breach tissue barriers and achieve high titer within the mammalian bloodstream, thereby enabling flea-borne dissemination. Evolution also may have selected for the exceptional virulence of *Y. pestis*, since the death of infected mammals presumably facilitates transmission by compelling infected fleas to seek new hosts (5).

After a 2–6 day incubation period, infected humans usually display a sudden onset illness characterized by headache, chills, fever, malaise, and the appearance of a painful bubo, which results from the swelling of lymph nodes draining the fleabite (1–6). Without prompt antibiotic treatment, 50–60% of these bubonic infections are lethal. Late stage pathology includes sepsis, disseminated intravascular coagulopathy, and multiple organ failure (1–6,19). Up to 30% of fleabites lead directly to septicemic plague, without prior evidence of a bubo (2,20).

Occasionally, the bubonic and septicemic infections progress to secondary pneumonic infections, which can be transmitted directly between humans via infectious respiratory droplets (1,2,4,21–23). The course of primary pneumonic plague is even more fulminant than bubonic plague. Following an incubation period of 1–6 days, symptoms typically begin with rigor, severe headache, nausea, and malaise, and then advance quickly to fever, cough, and difficult breathing. The cough becomes increasingly productive, sometimes yielding frothy, infectious, bright red sputum teeming with bacilli. Pneumonitis progresses to lobar pneumonia and bilateral lung involvement. Deaths typically result from respiratory failure and/or sequelae of severe sepsis, including circulatory collapse, coagulopathy, and hemorrhage. Pneumonic plague is nearly always fatal unless treated with antibiotics within 20 hours of symptom onset (2). As noted by Wu Lien-Teh (21) in his extensive review of early 20th century autopsy findings, ‘Considering the acute and quickly developing nature of the pulmonary process, the surprising thing is not that so many patients die but that even a few recover’.

Plague is now entrenched in rodent populations on every continent except Australia (1,4). Plague foci are maintained as enzoonoses in populations of disease-resistant rodent species and epizootics sporadically decimate populations of disease susceptible rodent species. Prior human pandemics, such as the ‘Black Death’ of the 14th century, likely resulted from cohabitation of humans with rodent populations experiencing disease outbreaks (1,4). Measures that reduce contact with rodents and fleas should limit the spread of bubonic plague. However, human plague outbreaks occasionally bring high frequencies of pneumonic disease and person-to-person transmission (1,7,8,21). Gauze masks can prevent person-to-person transmission pneumonic plague (21,22). Nevertheless, as recently as 2005, a pneumonic plague outbreak in the Congo killed 54 of 114 infected individuals (8).

Plague as a weapon of war and terror

The heightened modern day interest in plague stems primarily from bioterrorism concerns (6,15,16,23). The United States Centers for Disease Control classified *Y. pestis* a Category A Select Agent due to its potential to pose a severe threat to public health and safety. Unlike many Select Agents, *Y. pestis* already has a long history as a biowarfare agent (6,15,16,23). In 1347, the Tartars catapulted plague-ridden corpses into the besieged city of Kaffa, causing residents to flee and spread the Black Death to Italy. During World War II, the Japanese initiated local outbreaks of bubonic plague in Chinese cities by dropping ceramic bomblets containing *Y. pestis*-infected fleas. During the Cold War, both American and Soviet scientists devised means to effectively aerosolize *Y. pestis*, thereby removing the need for the flea vector (23). Despite signing the Biological Weapons Convention in 1972, Soviet scientists continued offensive bioweapons research and reportedly developed the requisite technology to deploy large quantities of aerosolized *Y. pestis* (15,16). Miniature

cities in the Soviet Union were devoted to the production of weapons-grade biologics, including *Y. pestis* (15), and one of today's primary concerns is that rogue scientists from the Cold War era may be willing to share the knowledge required to produce and deploy *Y. pestis* (6,15). Moreover, antibiotic-resistant *Y. pestis* strains are now known to exist (11–14). Covertly aerosolized, antibiotic-resistant *Y. pestis* would be a formidable weapon of terror (6,16,23).

Evasion of innate host defense mechanisms

A number of excellent reviews have previously discussed molecular aspects of *Yersiniae* virulence and the impacts of *Yersiniae* virulence factors on host biology (3,24–27). This review focuses primarily on pneumonic plague. The exceptionally rapid course of pneumonic plague suggests that *Y. pestis* virulence in susceptible mammalian hosts results primarily from an inadequate innate immune response. Many studies support this concept, yet few studies have validated specific impairments of host defense *in vivo*.

Targeting neutrophils

Phagocytes are one of the innate immune system's primary defenses against extracellular bacterial infections. Autopsies of human pneumonic plague victims note abundant extracellular bacteria but little evidence of phagocytosis (21). In non-human primate models, the robust cellular responses that typically characterize other bacterial pneumonias are delayed and ineffective during pneumonic plague (28). Mouse models reveal steadily progressive bacterial growth in pulmonary tissues, with dissemination to other organs by 36 h post infection (29–31). The lungs exhibit remarkably little evidence of inflammation at 24 h post infection and only later do neutrophil numbers increase. Thus, phagocytes appear unable to adequately control the replication of extracellular *Y. pestis* in humans and in animal models of pneumonic plague.

Y. pestis bacilli grown at 28°C, to mimic the environment within fleas, express a distinct repertoire of genes from *Y. pestis* bacilli grown at 37°C (3,5,32). *In vitro*, the capacity of *Y. pestis* to resist phagocyte-mediated destruction is most notable when the bacilli are grown at 37°C (33). Initially, this resistance to phagocytosis was attributed to temperature-regulated production of a gel-like capsule (33–37). However, we now know that the capsule's primary constituent, the F1 protein, is dispensable for virulence in mice, primates, and humans (38–42).

Current dogma stipulates that a temperature-regulated, plasmid-encoded, type III secretion system accounts for much of the capacity of *Y. pestis* to evade destruction by phagocytes (3,24,26,27). This secretion system, which is critical for virulence, produces an 'injectisome' that facilitates the translocation of a set of *Yersinia* outer proteins (Yops) from the bacilli into host cells. *In vivo*, the Yops primarily target neutrophils, macrophages, and dendritic cells (43). The most critical Yops for *Y. pestis* virulence include a potent protein tyrosine phosphatase (YopH) and a guanosine triphosphatase-activating protein (YopE). *In vitro* studies suggest that these factors function, in part, by impairing phagocytosis and oxidative burst, thus antagonizing the uptake and killing of *Y. pestis* by phagocytes (26). Another plasmid-encoded protein that is critical for virulence, LcrV, facilitates delivery of the Yops (44–48). LcrV also suppresses neutrophil chemotaxis directly, independent of its role in Yop translocation (49).

Hijacking phagocytes as a niche for intracellular growth

In addition to evading destruction by phagocytes, *Y. pestis* also appears to commandeer macrophages and use them as a protected niche for intracellular replication (50). However, the extent to which intracellular growth contributes to virulence during plague remains a

subject of considerable debate. Detailed kinetic analyses of mice infected intranasally (29) or rats infected intradermally (51) failed to observe significant numbers of intracellular organisms at any time. Nevertheless, *Y. pestis* certainly replicates within macrophages *in vitro* (33,50,52,53), and multiple studies of pneumonic plague in non-human primates documented intact *Y. pestis* organisms within alveolar macrophages (28,42). Moreover, a recent flow cytometry-based study detected viable *Y. pestis* within spleen cells of infected mice (54). In that study, nearly all the bacilli appeared to reside within CD11b-expressing macrophages for the first several days of infection (54).

Y. pestis may have evolved to persist within macrophages. *Y. enterocolitica*, an enteropathogenic relative of *Y. pestis*, efficiently activates macrophage apoptosis. By comparison, *Y. pestis* exhibits greatly diminished cytolytic activity, apparently due to less efficient translocation of YopJ (55). Moreover, the *Y. pestis ripA* gene, which is absent in *Y. enterocolitica*, suppresses production of antimicrobial nitric oxide, thereby facilitating *Y. pestis* survival within macrophages that have been activated with interferon γ (IFNγ) after infection (56). PhoP-regulated genes, which are required for intracellular survival and replication of *Salmonella enterica* within macrophages, likewise promote the survival of *Y. pestis* in macrophages (57,58), and a *Y. pestis phoP* mutant is attenuated 75-fold in a mouse model of bubonic plague (57). *Y. pestis lpp* mutants also display reduced survival in macrophages and significant attenuation in mouse models of bubonic and pneumonic plague (59).

Although the *in vivo* significance of intracellular bacteria has yet to be demonstrated decisively, the available data strongly suggest that *Y. pestis* growing within phagocytes plays an important, perhaps critical, pathogenic role during plague (50). Extracellular bacilli undoubtedly dominate the late stages of infection, but intracellular organisms have even been detected at that time (28,42,54). During bubonic plague, cells of the monocyte/macrophage lineage may provide a protected intracellular niche that allows time for flea-transmitted *Y. pestis* bacilli to adjust to growth within mammals (33), in part by upregulating expression of capsular F1 protein, LcrV and Yops, and thus enabling subsequent growth as extracellular, phagocyte-resistant bacteria. Further studies are required to better define functional roles for intracellular replication during the pneumonic form of plague.

Dampening inflammation

In comparison with other Gram-negative bacteria, pulmonary infection by *Y. pestis* elicits a notably delayed inflammatory response (29–31). LcrV and several Yops have been implicated as key suppressors of cytokine and chemokine production during plague (3,24–27). It is important to note, however, that much of the LcrV and Yop literature is derived from studies of *Y. pseudotuberculosis* and *Y. enterocolitica*, and a growing body of work suggests that genetic differences among homologous *Yersiniae* virulence factors significantly impact their activities. For example, LcrV activates Toll-like receptor 2 (TLR2)-mediated production of anti-inflammatory interleukin-10 (IL-10) (25,27), but a number of recent studies question the importance of this pathway during plague, in part because the *Y. pestis* LcrV activates TLR2-mediated IL-10 production far less efficiently than the LcrV of other *Yersiniae* (60–62) and because IL-10-deficient mice are fully susceptible to *Y. pestis* infection (63). Likewise, the cytolytic impacts of *Y. pestis* YopJ are far less pronounced than those of its *Y. enterocolitica* homolog, and yet the capacity of YopJ to suppress production of tumor necrosis factor α (TNFα) is conserved (55). These observations suggest that some mechanisms used by enteric *Yersiniae* to establish chronic, localized gastrointestinal infections may not be conserved in *Y. pestis*, which overwhelms innate host defense and rapidly causes lethal disease. Thus, caution must be exercised when extending specific results from the enteric *Yersiniae* to *Y. pestis*, and future studies will need to explicitly evaluate the anti-inflammatory activities of individual *Y. pestis* Yops.

Lipopolysaccharide (LPS) with tetra-acylated forms of lipid A are weakly inflammatory in comparison with LPS with hexa-acylated lipid A. *Y. pestis* primarily produces hexa-acylated lipid A at 26°C, but switches to tetra-acylated forms when grown at 37°C (64,65). To investigate whether the expression of a relatively weak LPS contributes to virulence, Lien and colleagues (66) engineered a *Y. pestis* strain that produces hexa-acylated lipid A at 37°C. They found this strain to be more than 100,000-fold attenuated in a mouse model of bubonic plague. The attenuated phenotype is accompanied by increased production of TNFα and depends upon host expression of TLR4, an innate receptor for LPS (66). These findings strongly suggest that wildtype *Y. pestis* evades innate immunity, at least in part, by avoiding TLR-mediated activation of innate immunity. With help from Lien and colleagues, we recently generated our own hexa-acylated lipid A-producing *Y. pestis* strain and assessed its virulence. This strain is more than 350-fold attenuated in our mouse model of pneumonic plague (STS, unpublished data). Importantly, these studies indicate that all other *Y. pestis* virulence mechanisms can be overcome by a strong LPS response (66).

Other impacts on innate immunity

Y. pestis virulence mechanisms also target non-phagocytic cells of the innate immune system. Straley and colleagues (67) discovered that YopM, which is critical for *Y. pestis* virulence, promotes systemic depletion of natural killer (NK) cells in a mouse model of septic plague. We also observe reduced numbers of splenic NK cells in our mouse model of pneumonic plague (STS, unpublished data). However, further studies are required to establish that NK cells combat plague and that YopM-mediated depletion of NK cells actually contributes to *Y. pestis* virulence, rather than reflecting a vestigial activity of the enteric yersiniae.

It seems likely that a number of synergistic mechanisms likely contribute to the exceptional virulence of *Y. pestis*. For example, recent studies indicate that *Y. pestis* produces factors that antagonize host production and use of reactive nitrogen. Specifically, mutation of the *ripA* gene increases nitric oxide production and decreases survival of *Y. pestis* bacilli within activated macrophages *in vitro* (56), and mutation of the *hmp* gene, which encodes a nitric oxide detoxifying flavohemoglobulin, attenuates *Y. pestis* in a mouse model of bubonic plague (68). Together, these genes likely help the bacilli replicate within macrophages and evade destruction by neutrophils. In addition to targeting cells classically associated with innate immunity, *Y. pestis* also invades epithelial cells (69), and the *Yersiniae* Yops impact the capacity of epithelial cells to upregulate proinflammatory cytokines, chemokines, and adhesion molecules (70,71). Thus, parallel targeting of both leukocytes and stromal cells may synergistically suppress innate responses during plague.

Many bacteria have evolved means to convert host plasminogen to plasmin, a protease that degrades extracellular matrix (72). *Y. pestis* produces a plasmid-encoded plasminogen activator, denoted Pla. In mouse models, Pla deficiency attenuates *Y. pestis* delivered by the intradermal and subcutaneous routes, but not by the intravenous route, suggesting an important role for Pla in *Y. pestis* dissemination (20,73,74). Lathem *et al.* (30) recently demonstrated an important role for Pla in the development of primary pneumonic plague in mice. Surprisingly, dissemination to and growth within the spleen was relatively unaffected by Pla deficiency, whereas bacterial growth in the lung was greatly reduced. Further studies are required to discern the specific mechanisms by which Pla impacts *Y. pestis* virulence during pneumonic plague.

The available data certainly support the dogma that *Y. pestis* virulence results from its capacity to evade, incapacitate, and altogether overwhelm innate immune defenses. A number of *in vitro* studies have suggested plausible virulence mechanisms. However, there still remain huge gaps in our understanding of *Y. pestis* virulence, and, in particular, there is

a great need for studies that precisely define the virulence mechanisms that operate within the lung during pneumonic plague.

Vaccine primed, acquired host defense against plague

Y. pestis readily overcomes host defense in susceptible animal species, but convalescent animals effectively resist re-infection (1). This acquired ability to resist infection suggests it should be possible to develop vaccines that confer protection against plague by pre-priming immune defense mechanisms. However, as reviewed previously (75–77), over 100 years of research have yet to generate a safe and effective pneumonic plague vaccine.

Killed whole cell plague vaccines

Haffkine (78,79) described the first widely used plague vaccine in 1897. Vaccine recipients were injected with heat-killed cultures of *Y. pestis* organisms (78). These vaccinations likely protected against bubonic plague, but they were highly reactogenic (79) and probably did not protect against pneumonic plague (21). In the mid-20th century, Meyer and colleagues (75,76) championed the development of more refined whole-cell vaccines comprised of formalin-killed *Y. pestis* bacilli. A vaccine of this type was licensed and sold in the United States as Plague Vaccine (USP), and a similar vaccine is still manufactured today by Australia's Commonwealth Serum Laboratories. Controlled clinical trials have not been reported, but studies of United States military personnel during the Vietnam War strongly suggest that formalin-killed, whole-cell vaccines protect against bubonic plague (75,80). However, these vaccines cause significant adverse reactions, particularly after booster injections, which are needed to maintain protection (2). Moreover, they generally fail to protect mice and non-human primates against pulmonary *Y. pestis* challenge, and several humans contracted pneumonic plague despite immunization with formalin-killed vaccines (75–77,81). Thus, killed whole-cell vaccines are probably not suitable for defense against weaponized pneumonic plague.

Subunit plague vaccines

In 1952, Baker and colleagues (82) demonstrated that vaccination with F1, the *Y. pestis* capsular protein, protects mice and rats from subcutaneous challenge with virulent bacilli. Ehrenkranz and Meyer (83) subsequently demonstrated that vaccination with F1 protects macaques against pneumonic plague, as does passive transfer of serum collected from F1-vaccinated rabbits. Vaccination with recombinant F1 likewise protects mice against aerosolized *Y. pestis* (84), as does passive transfer of an F1-specific monoclonal antibody (85,86). However, virulent F1-negative *Y. pestis* strains exist, so vaccines based solely on F1 may fail to protect against weaponized pneumonic plague (38–42,87).

In contrast to F1, LcrV is critical for virulence (44–48). Vaccination with purified LcrV protects mice against subcutaneous *Y. pestis* challenge, as does passive transfer of LcrV-specific antibodies (46–48,88–90). Vaccination with recombinant LcrV protects mice against aerosol challenge with either F1-positive or F1-negative *Y. pestis* strains (91,92). Moreover, an LcrV-specific monoclonal antibody passively protects mice against aerosolized *Y. pestis*, even when administered 48 h post infection (86).

Vaccines based on LcrV alone also may fail to protect against weaponized pneumonic plague, because pathogenic *Yersinia* species express LcrV variants that may not confer cross-protective immunity (93). Vaccines containing both F1 and LcrV will be more difficult to circumvent. They also provide greater protection than vaccines comprised of either subunit alone (94,95). The United Kingdom's defense department demonstrated that an alum formulation of the *Y. pestis* F1 and LcrV proteins protects mice against pulmonary *Y. pestis* challenge (96,97). In parallel studies, the United States Army Medical Research

Institute of Infectious Diseases (USAMRIID) demonstrated that an alum formulation of an engineered F1-LcrV fusion protein protects mice against pulmonary challenge with either F1-positive or F1-negative *Y. pestis* strains (92,98). Both vaccines appear to be safe, well-tolerated, and immunogenic in humans (99,100).

It is not ethical to challenge humans with pneumonic plague, so the United State's Food and Drug Administration (FDA) plans to license plague vaccines in accordance with the 'Animal Rule', which requires safety and immunogenicity data in humans and robust efficacy data in animal models that mimic the human disease (101). Mouse and non-human primate models have received the most attention thus far. At an FDA-sponsored Plague Vaccine Workshop held in 2004, USAMRIID presented data from a series of F1/LcrV vaccine studies in two types of non-human primates (102). With regard to the chosen models, USAMRIID researcher Dr. Louise Pitt stated, 'In comparing the African green monkey and the cynomolgus macaque to date, based on clinical signs, the disease progression, and the pathology, as well as the susceptibility in terms of an LD50, they are very similar…and both are very similar to what is known about human disease' (102). USAMRIID then demonstrated that F1/LcrV-based vaccines protect cynomolgus macaques against aerosolized *Y. pestis* but fail to adequately protect African green monkeys (efficacy ranged from 0–75% in five trials) (102). Presently, it is unclear whether F1/LcrV-based vaccines will provide humans with the effective protection observed in cynomolgus macaques or the inadequate protection observed in African green monkeys.

A number of approaches are underway to improve the efficacy of F1/LcrV-based vaccines (77). Some researchers are genetically modifying the antigens (103,104), while others are exploring the use of alternate adjuvants (105–108) and delivery platforms (109–116). These approaches are certainly promising. However, as already noted, F1-negative *Y. pestis* strains exist (38–42,87), and pathogenic *Yersinia* species express multiple LcrV variants, including some that may not confer cross-protective immunity (93). Thus, bioweapon engineers may circumvent vaccines based exclusively on F1 and LcrV. One solution could be the incorporation of additional antigens. Promising candidates include YpkA (117), YopD (117), YscF (118,119), YadC (120), and OppA (121).

Live attenuated plague vaccines

Vaccines based on live attenuated organisms provide the theoretical advantage of simultaneously priming immunity against many antigens, thereby reducing opportunities for circumvention by weapons engineers. Soon after the discovery of *Y. pestis*, Kolle and Otto (122) successfully vaccinated experimental rodents against plague by inoculating with relatively small quantities of live attenuated *Y. pestis* bacilli. Subsequently, Strong (123,124) reported that live attenuated vaccines protect humans from bubonic disease. Placebo-controlled clinical studies have not been reported, but experimental studies, supported by field observations, strongly suggest these vaccines protect humans against both bubonic and pneumonic plague (75,77,125). 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 (75,126–128). In addition, they frequently cause debilitating fever, malaise and lymphadenopathy in humans (76).

Safety concerns have limited enthusiasm for the development of live attenuated vaccines in the United States and Europe, where plague is uncommon and the risk of harm may outweigh the benefits of vaccination. However, live attenuated vaccines were administered to tens of millions of humans in Indonesia, Madagascar, and Vietnam, apparently without causing any deaths (125). Live attenuated vaccines also were studied extensively in the former Soviet Union (17,76,129), and the NIIEG line of pgm-negative strain EV 76 is still in

use today in Russia (17,130). 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' (128).

Several groups recently described attenuated *Y. pestis* strains with well-defined genetic modifications that may be useful as live vaccines. A strain with mutations in both the *pgm* and *pla* loci safely induces humoral responses in monkeys (128). Vaccination with strains harboring mutations in both the *pgm* and *lpxM* loci or only the *pcm* locus protects mice against subcutaneous challenge (130,131). Vaccination with a *yopH* mutant protects mice against both subcutaneous and pulmonary challenge (132), as does vaccination with strains engineered to constitutively produce LPS bearing hexa-acylated lipid A (66, STS, unpublished data).

How do vaccines protect against plague?

F1/LcrV-based vaccines protect mice and cynomolgus macaques but have failed, thus far, to adequately protect African green monkeys. Unless we develop reliable correlate assays for vaccine efficacy, it will be difficult to predict whether these vaccines, and others in development, will protect humans. Moreover, from a translational viewpoint, it is imperative that we develop correlate assays for protection, so that vaccines developed in animal models can be optimized and licensed in the absence of human efficacy trials. An understanding of how vaccines defend against pneumonic plague should aid the development of correlate assays for efficacy, while also informing the design of next-generation vaccines.

Antibody-mediated defense against plague

Passive transfer of specific antibody protects susceptible rodents against pneumonic plague (85,86,125,133,134). Given this documented efficacy of humoral immunity, pneumonic plague vaccine efforts have aimed, by and large, to prime high-titer antibody responses. However, prechallenge enzyme-linked immunosorbent assay (ELISA) titers do not correlate with protective efficacy in non-human primates vaccinated with F1/LcrV (134,135), and some vaccinated primates succumb to challenge despite possessing high-titer F1/LcrV-specific antibody (102,135). These observations, along with many supporting studies in rodents (125,136), strongly suggest that antibodies titers alone, at least as measured by standard ELISA, do not suffice in predicting the efficacy of pneumonic plague vaccines.

Early studies failed to demonstrate any appreciable bactericidal activity of plague immune serum and, rather, suggested that antibody-mediated defense against plague reflects a collaboration between humoral and cellular defense mechanisms (136–138). Indeed, Jawetz and Meyer (138) concluded 'the serum, plasma, or other body fluids of animals immune to plague infection are unable to destroy or lyse [*Y. pestis*] organisms *in vitro* and *in vivo* in the absence of phagocytic cells'. While those investigators did not quantify the extent to which phagocytes contribute to antibody-dependent protection *in vivo*, Straley and colleagues (139) recently reported that treating mice with neutrophil-depleting, Ly-6G-specific mAb 1A8 abrogates protection mediated by polyclonal anti-LcrV in a mouse model of septicemic plague. Likewise, we have found that treatment with neutrophil-depleting, Gr1-specific mAb RB6-8C5 abrogates serotherapy-mediated protection in a mouse model of pneumonic plague (STS, unpublished data). These *in vivo* studies strongly suggest that neutrophils contribute to antibody-mediated defense against pneumonic plague.

We also used the serotherapy model to investigate other mechanisms involved in antibody-mediated defense against pneumonic plague in mice. We found that genetic deficiency in the IFNγ receptor, TNFα, or of nitric oxide synthase 2 (NOS2) significantly impairs serotherapy-mediated protection (140). IFNγ and TNFα are known to upregulate phagocyte

expression of NOS2, thereby increasing production of antimicrobial nitric oxide (141). More recently, we strengthened and expanded these studies using experimental strategies that conditionally, rather than genetically, deplete cytokines. Specifically, we found that co-administration of antibodies that neutralize IFNγ and TNFα significantly impairs serotherapy-mediated protection (STS, unpublished data). These findings are supported by those of Williamson and colleagues (142), who observed that STAT4-deficient mice, which are impaired for production of IFNγ, generate robust antibody responses upon vaccination with F1/LcrV but, nonetheless, are poorly protected against *Y. pestis* challenge. Altogether, these studies suggest that antibody-mediated protection benefits from cytokine-mediated priming of phagocyte defense mechanisms.

Further studies are required to define precisely how antibodies defend against pneumonic plague. As already noted, prior studies concluded that convalescent serum has little bactericidal activity of its own. Moreover, it is widely appreciated that *Y. pestis* bacilli resist complement-mediated lysis (143). Since virulent F1-negative strains exist, most attention has focused on defining the mechanisms by which LcrV-specific antibodies confer protection. One possibility is that these antibodies counter LcrV-mediated suppression of neutrophil chemotaxis (49). This possibility is consistent with the above-mentioned studies demonstrating that LcrV antibody loses its capacity to limit bacterial growth in neutrophil-depleted mice (139). However, LcrV antibody has not yet been shown to impact neutrophil recruitment or migration *in vivo*, and our preliminary studies have thus far failed to demonstrate significant increases in the number of pulmonary neutrophils in mice challenged with *Y. pestis* and treated with protective serum or LcrV antibody.

Another possibility is that antibodies protect via opsonic mechanisms. Indeed, LcrV is expressed on the bacterial surface (144,145), and LcrV-specific antibodies help macrophages (139,146) and neutrophils (139) phagocytose *Y. pestis* bacilli *in vitro*. DynPort Vaccine Company LLC intends to use an opsonophagocytic assay to bridge animal and human studies as they manage the clinical development of F1/LcrV-based vaccines (100). However, they have yet to demonstrate that anti-LcrV-mediated opsonophagocytic mechanisms contribute significantly to defense against pneumonic plague or that opsonophagocytic assays provide robust correlates of vaccine-mediated protection.

Several groups have recently investigated whether the ability to neutralize *Yersiniae*-induced cytotoxicity might serve as a correlate assay for LcrV antibody-mediated protection. (134,135,147). Contact with *Y. pestis* bacilli can activate macrophage apoptosis *in vitro* through a Yop-dependent mechanism (146), and LcrV antibodies suppress both Yop translocation (63,139,144) and macrophage apoptosis (134,135,146,147). In combination with the opsonophagocytic mechanisms described above, these findings suggest that LcrV antibodies may promote phagocytosis in a manner that enables phagocytes to ingest *Y. pestis* bacilli, without themselves being killed in the process. USAMRIID researchers developed a quantitative, flow cytometry-based, cytotoxicity assay using the human HL60 cell line and a modified strain of *Y. pseudotuberculosis* that expresses the *Y. pestis* LcrV protein. The United Kingdom's defense department has reported a similar qualitative assay (134). Apparently *Y. pseudotuberculosis* was chosen for these studies, in part, because the enteric *Yersiniae* activate apoptosis much more effectively than *Y. pestis* (55,147). Regardless, LcrV-specific antibodies suppress cell death in these assays, and there is an association between suppression of cell death and survival when analyzing sera from F1/LcrV-vaccinated, aerosol challenged, non-human primates (135). These cytotoxicity assays thus provide a promising foundation for the development of correlate assays for protection mediated by LcrV antibodies. However, they may have little direct bearing on the actual mechanisms of antibody-mediated protection against pneumonic plague, since *Y. pestis* induces macrophage apoptosis weakly by comparison with other *Yersiniae* (55). Rather,

these recent findings suggest that cytolysis-blocking LcrV-specific antibodies antagonize some other, yet to be discerned, function(s) of LcrV that play important roles in *Y. pestis* virulence.

Acquired cellular defense against plague

Antibodies undoubtedly can contribute to defense against pneumonic plague. By comparison, relatively few studies have investigated roles for T cells and acquired cellular immunity. This neglect presumably reflects the initial success of studies of antibody-mediated defense and the methodological difficulties inherent to studies of cellular immunity. Nevertheless, even the early studies of Meyer and colleagues concluded 'the natural defense mechanism in plague is primarily cellular' (136) and 'cooperation of immune serum and immune cells is necessary for the efficient destruction of bacteria' (138). Pollitzer (1) echoed this sentiment in his 1954 monograph on plague, noting 'all available evidence tends to show that…in naturally plague-resistant rodents a principal role is played by cellular defense mechanisms'.

Cytokines

In 1977, Wong and Elberg (148) isolated spleen cells from immune mice, cultured the cells with *Y. pestis* bacilli, harvested supernatant, and then exposed naive phagocytes to the supernatant. They noted that the immune spleen cells produced soluble factors that protect phagocytes from cytolysis upon subsequent encounters with viable *Y. pestis*. Through depletion studies, they determined that T cells generated these soluble protective factors.

A number of studies now suggest that the factors described by Wong and Elberg likely include the cytokines IFNγ and TNFα. *In vitro*, *Y. pestis* replicates within naive macrophages (33,45,52), and within macrophages exposed to IFNγ after infection (56). However, pre-treatment with IFNγ and TNFα restricts intracellular replication (54). Presumably, this restriction reflects IFNγ and TNFα-mediated upregulation of phagocyte antimicrobial activities, such as the production of reactive oxygen and reactive nitrogen.

In vivo studies also indicate that IFNγ and TNFα play important roles during defense against plague. Nakajima and Brubaker (89) demonstrated that pre-injecting mice with IFNγ and TNFα protects against septicemic plague. As noted above, Williamson and colleagues (142) found that bubonic plague is poorly controlled by F1/LcrV vaccination in signal transducer and activator of transcription 4 (STAT4)-deficient mice, which are impaired for T-cell production of IFNγ, and we demonstrated that optimal antibody-mediated protection against pneumonic plague in mice requires host production of IFNγ, TNFα, and NOS2 (140). *Y. pestis* appears to actively counter these cellular defenses by producing factors that antagonize host production of reactive nitrogen (56) and by adaptively upregulating expression of factors that may reduce the antimicrobial impacts of reactive nitrogen (68).

Immune cell clusters at sites resolving, visceral *Y. pestis* infections

During the course of pneumonic plague, bacteria access the bloodstream and spread to the liver and spleen (21,29–31,42,149). Thus, effective resolution of pneumonic plague will likely require control of bacterial growth at both pulmonary and extra-pulmonary sites. *Y. pestis* bacilli evoke little notable inflammation as they grow within visceral tissues (5,20,25,31,66,74,150–152). However, Brubaker and colleagues (5,25,150) noted that active or passive immunization against LcrV allows for leukocyte recruitment and the appearance of discrete cell clusters within hepatic tissue. These cell clusters also form during hepatic clearance of *Y. pestis* strains lacking expression of certain Yops (67,151) or Pla (152) or engineered to produce more inflammatory LPS (66). Thus, the formation of immune cell clusters appears to be a general hallmark of resolving hepatic *Y. pestis* infections.

Granulomas and immune cell clusters are often observed during bacterial infections whose clearance benefits from T-cell-mediated, acquired cellular immunity (153). Published studies have yet to explicitly implicate T cells as critical mediators of the hepatic cell clusters that accompany resolving *Y. pestis* infections, but Brubaker and colleagues observed that injecting mice with IFNγ and TNFα prior to infecting with *Y. pestis* facilitates the formation of the hepatic cell clusters (89). Further research should be directed at establishing the functional importance of immune cell clusters during hepatic *Y. pestis* infection, as well as the mechanisms that regulate their formation. It also will be important to investigate whether analogous structures form in the lung during resolving, pulmonary *Y. pestis* infections.

T-cell-mediated defense against plague

Given observations that IFNγ and TNFα contribute to defense against plague (54,56,89,140,142), vaccines that pre-prime T cells with the capacity to produce IFNγ and TNFα upon re-encounter with *Y. pestis* should, theoretically, augment host defense mechanisms. However, a number of studies suggest that that *Yersiniae* virulence factors not only suppress innate immunity but also suppress T-cell responses, both by directly targeting T cells and by targeting the antigen-presenting cells that activate T cells. Particularly notable studies include demonstrations that the *Y. pestis* Yops target dendritic cells *in vivo* (43), that the *Y. pestis* YopJ protein suppresses dendritic cell migration *in vivo* (154), and that the *Y. enterocolitica* YopP protein (the equivalent of *Y. pestis* YopJ) suppresses the activation and expansion of antigen-specific $CD8^+$ T cells *in vivo* (155). While certainly suggestive, these prior studies did not specifically demonstrate suppression of T-cell responses by *Y. pestis*. Our recent studies (discussed below) indicate that the suppression of T-cell responses during *Y. pestis* infection, at most, is incomplete.

A likely explanation for the discrepancy between our findings and the prevailing dogma is that the dogma arose primarily from studies of the enteropathogenic *Yersiniae*. As noted above, a growing body of literature is now revealing significant differences between *Y. pestis* virulence mechanisms and those of the enteropathogenic *Yersiniae*. With regard to impacts on T-cell biology, it seems likely that these differences reflect the very different nature of these infections: the enteropathogens establish self-limiting chronic infections, whereas plague causes acute disease that rapidly kills susceptible hosts. The exceptionally rapid course of plague presumably alleviates selective pressure to evolve/maintain mechanisms that dampen naive T-cell responses, which typically require multiple days to develop. In other words, *Y. pestis* may not bother to counter T-cell responses, because such responses develop too slowly to combat plague.

A number of earlier observations inspired us to investigate potential roles for T cells during defense against pneumonic plague. First, vaccinating guinea pigs with live pgm-negative *Y. pestis* solidly protects against plague without eliciting significant protective antibody titers (125), and monkeys vaccinated with live attenuated *Y. pestis* ‘not infrequently survived challenge… with little antibody measurable’ (83). Given that live vaccines typically prime robust cellular immunity, these studies suggest that vaccine-primed, memory T cells may combat plague effectively, even if naive T cells have little opportunity to combat plague. Second, many studies suggest that T cells participate in defense against the enteric *Yersiniae*, even though these pathogens possess virulence mechanisms that dampen T-cell responses. For example, the passive transfer of specific T cells protects mice against lethal challenge with *Y. enterocolitica* (156–158). Third, Alonso and colleagues (159) demonstrated that the passive transfer of cells, but not sera, from *Y. enterocolitica* convalescent mice partially protects naive mice against subcutaneous *Y. pestis* challenge. Although the protective cell was not identified, this study certainly suggested that pre-primed, cross-reactive T cells can protect against plague. Wake and Sutoh (160) also

demonstrated that T cells contribute to vaccine-mediated protection against subcutaneous *Y. pestis* challenge, although they did not dissociate the cellular functions of T cells from their capacity to help B cells produce antibody.

We investigated whether vaccinating mice with live *Y. pestis* primes T cells that confer protection against pneumonic plague (161). To focus on T-cell-mediated protection, we vaccinated B-cell-deficient μMT mice, which cannot produce antibodies. To safely vaccinate the immunodeficient μMT mice, we combined vaccination with our post-exposure serotherapy protocol. Specifically, we vaccinated μMT mice with live pgm-negative *Y. pestis*, while preventing vaccine-induced mortality by administering a minimally protective dose of serotherapy 18 h later. We then waited 60 days before evaluating the capacity of these vaccinated μMT mice to resist a lethal intranasal challenge. We found that vaccination confers μMT mice with significant protection against mortality in this model of pneumonic plague. Subsequent studies of mice euthanized at day 3 post-challenge revealed that vaccination reduces bacterial burden in the lung, spleen, and liver and also increases the number of activated pulmonary T cells five to 10-fold (161). Consistent with protection mediated by acquired cellular immunity, depletion of IFNγ, TNFα, or both $CD4^+$ and $CD8^+$ T cells abrogates protection in this model (161). We conclude that vaccination with live *Y. pestis* primes T cells that protect mice against lethal pulmonary *Y. pestis* challenge.

Since the protocol described above employed B-cell-deficient mice that received small amounts of serotherapy, we were concerned about potential impacts of residual antibody. Control studies indicated that residual antibody, if present, was insufficient to confer protection on its own (161), and subsequent studies further demonstrated important, antibody-independent roles for T cells during defense against pneumonic plague. First, μMT mice are protected against lethal challenge if they are vaccinated (without serotherapy) using a highly attenuated strain of *Y. pestis* engineered to constitutively produce LPS with hexa-acylated lipid A (STS, unpublished data). Second, T cells isolated from vaccinated μMT mice confer significant protection when they are expanded in vitro and then transferred to naïve μMT mice (161). Finally, vaccinating and boosting wild type mice with live *Y. pestis* primes T cells that passively transfer protection to naive mice without any requirement for *in vitro* expansion (162). Interestingly, $CD8^+$ T cells alone provide significant protection in this transfer model, and the presence of $CD4^+$ T cells significantly enhances protection mediated by $CD8^+$ T cells, even though $CD4^+$ T cells alone fail to confer any measurable protection on their own (162). These findings indicate that the $CD4^+$ and $CD8^+$ T cells synergistically protect against pneumonic plague in this mouse model.

Most of our studies have employed pgm-negative *Y. pestis* as challenge (161,162). This use is noteworthy, because pgm-negative *Y. pestis* is approximately 10-fold less virulent than pgm-positive *Y. pestis* when administered via the intranasal route (unpublished observation) and because Pujol and colleagues (56) recently demonstrated that *ripA*, a gene within the pgm locus, suppresses production of antimicrobial nitric oxide by IFNγ-activated macrophages. Thus, pgm-deficient *Y. pestis* may be more sensitive to cell-mediated defense mechanisms than are pgm-positive *Y. pestis*. Nevertheless, vaccinating μMT mice with live *Y. pestis* under the cover of serotherapy confers protection against challenge with pgm-positive *Y. pestis* strain CO92, as evidenced by a significant delay in time to death ($p<0.002$ by Logrank test; M.A. Parent and S.T. Smiley, unpublished data). While less dramatic than the increased overall survival observed in our studies of pgm-negative strains, these preliminary studies indicate that T cells confer significant protection against both pgm-negative and pgm-positive strains. Notably, Elvin and Williamson's studies (142) of vaccinated STAT4-deficient mice also suggest that defense against fully virulent, pgm-positive *Y. pestis* requires host production of IFNγ.

Antigens recognized by T cells that protect against pneumonic plague

Very little is known about the antigens recognized by *Y. pestis*-specific T cells. In our recent studies of cellular defense against plague (161,162), we vaccinated mice using live *Y. pestis*, because replicating agents often prime robust cellular immune responses and because the use of the entire organism obviated the need to pre-identify protective antigens. However, technical challenges associated with the manufacture and storage of live vaccines, as well as safety concerns, temper enthusiasm for the development of live *Y. pestis* vaccines. Safer alternatives include recombinant subunit vaccines, DNA-based vaccines, and live vaccines employing safer and better-characterized vectors, such as vaccinia virus. To incorporate cellular immunity into such vaccines, it will be necessary to first identify specific *Y. pestis* antigens that prime protective T cells. Thus, identifying protective T-cell antigens is a critical step toward developing next-generation vaccines that elicit both humoral and cellular immunity. Identifying antigens recognized by protective T cells should also facilitate the development of sensitive assays for *Y. pestis*-specific T cells, thereby enabling mechanistic studies of how T cells defend against plague and how to optimally prime the most effective T cells. Ultimately, these antigen-specific assays may also facilitate development of robust correlate assays for vaccine efficacy.

Given that LcrV is a primary component of subunit vaccines under development, we began our efforts in this area by investigating whether LcrV-specific T cells can combat plague. Since vaccination with LcrV elicits protective antibodies and since B-cell production of high affinity antibodies typically requires help from class II-restricted $CD4^+$ T cells, it was predictable that $CD4^+$ T cells would recognize LcrV. Others had previously established that T cells from LcrV-vaccinated mice respond to LcrV *in vitro* (110,163,164). Using *in vitro* assays employing overlapping peptides as stimuli, we found that $CD4^+$ T cells from F1/V-vaccinated C57BL/6 mice recognize three discrete, I-Ab-restricted LcrV epitopes (165). Several other groups have since confirmed these epitopes and expanded these studies to other mouse strains and major histocompatibility complex (MHC) haplotypes (166–168).

Despite its utility as an antigen that elicits protective antibody, we believe there is no *a priori* reason to assume that LcrV is a useful antigen for priming protective cellular responses. Rather, it is critical to demonstrate formally that LcrV, or any other antigenic vaccine candidate, actually primes T cells that protect against plague. We have tried, repeatedly, to protect mice against plague by vaccinating with peptides containing the LcrV epitopes recognized by $CD4^+$ T cells. Our protocols prime IFNγ-producing, LcrV-specific T cells that confer protection against *Y. enterocolitica* (165), but they fail to protect against *Y. pestis*. We have even failed to demonstrate synergy between these primed LcrV-specific $CD4^+$ T cells and sub-protective doses of serotherapy (unpublished observations).

We now know that both $CD4^+$ and $CD8^+$ T cells contribute to protection and that *Y. pestis*-primed $CD4^+$ T cells do not suffice in conferring protection. An intriguing possibility is that LcrV-primed $CD4^+$ T cells, while not sufficient, might improve protection mediated by live *Y. pestis*-primed $CD8^+$ T cells. If true, then supplementing F1/V-based vaccines with an antigen that primes $CD8^+$ T cells might suffice in generating robust cellular immunity. However, we have not been able to demonstrate that combinations of LcrV-primed $CD4^+$ cells and *Y. pestis*-primed $CD8^+$ T cells confer protection. We tentatively conclude that incorporating robust cellular immunity into F1/V-based vaccines will likely require their supplementation with new antigens that prime both protective $CD4^+$ T cells and protective $CD8^+$ T cells.

We have begun to better define the *Y. pestis* antigens recognized by T cells that protect against pneumonic plague in mice. Specifically, we demonstrated that T cells isolated from mice vaccinated with live *Y. pestis* produce significantly greater quantities of IFNγ when

stimulated with antigen-presenting cells exposed to *Y. pestis*, as compared with antigen-presenting cells exposed to control bacteria, such as *E. coli* (162). Using that assay, we also find that *Y. pestis*-primed $CD4^+$ T cells and $CD8^+$ T cells each respond strongly to *Y. pestis* strains lacking the capacity to express F1, LcrV, and the plasmid-encoded Yops (162). These observations suggest that neither F1 nor LcrV are dominant antigens recognized by T cells primed by vaccination with live pgm-negative *Y. pestis*. We recently generated *Y. pestis*-specific T-cell clones and hybridomas, and now we are working to identify their cognate antigens.

Collaboration between humoral and cellular immunity

Acquired immunity is characterized by the expansion, differentiation, and persistence of antigen-specific B and T cells. While each of these cell types are pleiotropic, a primary function of B cells is to produce antibodies, thereby facilitating humoral defense, and a primary function of T cells is to produce phagocyte-activating cytokines, thereby facilitating cellular defense. Above, we discussed evidence that both humoral and cellular mechanisms participate in defense against pneumonic plague. The reader likely noted considerable overlap in these topics. Indeed, it seems clear that these two branches of acquired immunity cooperate and collaborate during defense against plague. We do not mean to imply that either humoral or cellular defense cannot suffice under certain conditions. Indeed, Green *et al.* (133) demonstrated that antibodies can protect T-cell-deficient mice, and we have demonstrated that T cells can protect antibody-deficient mice (161). Nevertheless, we consider it likely that vaccines that prime both humoral and cellular immunity will confer optimal defense against pneumonic plague.

Our laboratory's current working model is that antibodies help phagocytes internalize *Y. pestis* and suppress Yop-mediated disruption of innate host defense mechanisms, while activated T cells produce cytokines, like IFNγ and TNFα, that help phagocytes survive *Y. pestis* encounters and kill internalized bacilli. In suitably vaccinated animals, antibodies should slow disease progression and allow time for the activation, expansion and recruitment of memory T cells. In turn, these T cells should activate and amplify phagocyte defense mechanisms, thereby degrading intracellular niches for *Y. pestis* survival, while also encouraging the formation of protective granuloma-like structures.

The F1/LcrV-based vaccines under development stimulate antibody responses in mice, non-human primates, and humans. Serum from human vaccinees can passively protect mice against subcutaneous *Y. pestis* challenge (99). However, vaccines of this type only variably protect non-human primates against pneumonic challenge, and we do not yet know whether they will protect humans. Overall antibody titers, as measured by ELISA, are similar in protected and non-protected primates (135), but it is certainly possible that some primates fail to produce a subset of protective antibodies that recognize a particularly important LcrV epitope. Another possibility is that T-cell responses may be inadequate in some primates. Poor T-cell responses could result from a number of mechanisms, for example, by tolerance brought about by endogenous retroviruses or chronic exposure to related bacteria. Notably, our model does not require that an effective F1/LcrV-based vaccine must prime memory T cells. While it may be optimal to pre-prime such cells, it may suffice to pre-position antibodies that delay disease progression to an extent that allows time for the activation, expansion, and differentiation of naive T cells that recognize other *Y. pestis* antigens. Measurements of both F1/LcrV-specific and other *Y. pestis*-specific T-cell responses may help to determine whether differences in cellular immunity contribute to the variable efficacy of F1/LcrV-based vaccines in non-human primates.

When possible, supplementing F1/LcrV-based vaccines with antigens that prime protective T cells would seem prudent. Even then, subunit vaccines may fail to protect against cleverly weaponized strains: *Y. pestis* is amenable to genetic manipulation, so it is certainly conceivable that bioweapon engineers will circumvent subunit vaccines. Live attenuated *Y. pestis* vaccines should be much more difficult to circumvent. These vaccines appear to protect against pneumonic plague, probably by stimulating production of protective antibodies while also priming protective T cells. Historically, concerns about safety, not efficacy, have curbed the wide-scale use of live attenuated plague vaccines (77,126). Yet, they have been administered to tens of millions of humans, apparently without causing deaths (125), and they are still in use today in the former Soviet Union (17,130). We believe that research pertaining to the development of live attenuated vaccines should be strongly encouraged by funding agencies. Ideally, these vaccines must be stable, fully defined at the molecular level, and sufficiently attenuated to minimize vaccination sequelae in humans.

Given that humoral and cellular immunity often deploy complementary defense mechanisms, and given their clear potential for synergy, we believe that next generation plague vaccines, whether live attenuated or subunit-based, should strive to prime both humoral and cellular immunity. In addition, we believe that further research must be devoted to better defining precisely how vaccine-primed immunity combats pneumonic plague and to identifying *Y. pestis* virulence mechanisms that operate during pulmonary infections. These efforts will help to define robust correlates of protection that can serve as surrogates for efficacy in human clinical trials, while also providing valuable insight into immunity and bacterial pathogenesis. Indeed, the mouse model of pneumonic plague would seem to provide a remarkable opportunity to study host-pathogen responses in a setting where hormonal and cellular defense mechanisms collaborate to defeat one of the world's most deadly human pathogens.

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