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MINI-REVIEW

Plague vaccines: new developments in an ongoing search

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Abstract

As the reality of pandemic threats challenges humanity, exemplified during the ongoing SARS-CoV-2 infections, the development of vaccines targeting these etiological agents of disease has become increasingly critical. Of paramount concern are novel and reemerging pathogens that could trigger such events, including the plague bacterium *Yersinia pestis*. *Y. pestis* is responsible for more human deaths than any other known pathogen and exists globally in endemic regions of the world, including the four corners region and Northern California in the USA. Recent cases have been scattered throughout the world, including China and the USA, with serious outbreaks in Madagascar during 2008, 2013–2014, and, most recently, 2017–2018. This review will focus on recent advances in plague vaccine development, a seemingly necessary endeavor, as there is no Food and Drug Administration–licensed vaccine available for human distribution in western nations, and that antibiotic-resistant strains are recovered clinically or intentionally developed. Progress and recent development involving subunit, live-attenuated, and nucleic acid–based plague vaccine candidates will be discussed in this review.

Key points

- *Plague vaccine development remains elusive yet critical.*
- *DNA, animal, and live-attenuated vaccine candidates gain traction.*

Keywords Live attenuated · DNA vaccines · Protein subunit · Humoral · Protection

Introduction

Of all *Yersinia* species (spp.), three are pathogenic to humans: *Y. enterocolitica*, *Y. pseudotuberculosis*, and *Y. pestis* (Rosenzweig et al. 2011; Rosenzweig and Chopra 2012). The two former species typically cause self-limiting gastroenteritis, often referred to as yersiniosis, although *Y. enterocolitica* is more commonly associated with the disease (Galindo et al. 2011). *Y. pestis*, by contrast, is a *bona fide* highly invasive human pathogen, the stuff [sic] of nightmares. Although only having evolutionarily diverged from *Y. pseudotuberculosis* some 1500–20,000 years ago (Achtman et al. 1999), *Y. pestis* causes three forms of human disease: bubonic (often promoting fulminant infection), septicemic, and pneumonic with high morbidity and mortality rates (approaching 100%) if left untreated (Titball and Leary 1998; Demeure et al. 2019a, b). More specifically, plague-induced mortality has claimed over 200 million human lives during the course of 3 major human pandemics ranging from 541 CE (Justinian plague) through the 1300s (Black Death plague) until today (Indo-China plague) (Rosenzweig et al. 2011; Sun 2016; Sun and Singh 2019; Williamson 2009). The cumulative, historical death-toll serves as a grim reminder of our extreme vulnerability. Raising global concerns, the most recent outbreak in Madagascar (2017–2018) resulted in 202 deaths (from 2348 cases, with ~76% of the cases being pneumonic) during a 3-month period (WHO Plague-Madgascar n.d.).

Genetically distinguishable from its two related gastrointestinal *Yersinia* spp., *Y. pestis* gained a subset of genes, enhancing survival in both flea and mouse/rat reservoirs, as well as lost subsets of its chromosome, including adhesin encoding genes used for gut epithelium attachment

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(Achtman et al. 1999; Demeure et al. 2019a, b). *Y. pestis* is typically transmitted via the bite of an arthropod vector, the flea, and persists in rodent populations. Unfortunately, humans occasionally interrupt the vector-rodent chain of transmission resulting in grave consequences (Greenfield et al. 2002; Rosenzweig et al. 2011; Rosenzweig and Chopra 2012).

Type three secretion system injectosome and type six secretion system

All three pathogenic yersiniae possess a 70-kb virulence plasmid that encodes a type three secretion system (T3SS), an evolutionarily repurposed flagellar, macromolecular complex/system (Abby and Rocha 2012). The 70-kb virulence plasmid, termed pCD1 in *Y. pestis*, piB1 in *Y. pseudotuberculosis*, or pYV in *Y. enterocolitica*, encodes the requisite machinery for the hyper-structure T3SS injectosome as well as its potent anti-host effector proteins/toxins (Cornelis et al. 1998). Multiple hyper-structures, including the T3SS, exist within yersiniae. Moreover, these hyper-structures or their components likely interact. For example, the RNA degradosome, a macromolecular hyper-structure involved in RNA decay and processing (Carpousis 2007), is believed to cooperate with the T3SS within yersiniae (Norris et al. 2012; Yang et al. 2008; Rosenzweig et al. 2005; Rosenzweig et al. 2007).

Twenty-seven *Yersinia* secretion proteins (Yscs) comprise the T3SS injectosome, and the substrates secreted through the Ysc needle conduit are termed *Yersinia* outer membrane proteins (Yops). There are 6 effector Yops, each exerting its own anti-host property, while the remaining Yops serve delivery-facilitating roles, including the low calcium response V (LcrV) antigen (Miletic et al. 2020; Demeure et al. 2019a, b; Grabowski et al. 2017; Trosky et al. 2008; Cornelis 2003). In addition to the T3SS, the type 2 secretion system (T2SS) of *Y. enterocolitica* has also been shown to support its virulence by promoting tissue invasion (von Tils et al. 2012).

Beyond the T2SS and the T3SS, a T6SS has been characterized in all three pathogenic yersiniae (Yang et al. 2018). The T6SS is evolutionarily derived from repurposed phage machinery, enabling bacteria to puncture target cells and subsequently deliver effector proteins (Zoued et al. 2014). *Y. pestis* was found to possess 5 T6SS encoding clusters of virulence-associated secretion genes (*vas*) (Andersson et al. 2017; Li et al. 2015). Interestingly, 3 of the 5 T6SS clusters were required for full virulence in murine models of infection (Ponnusamy et al. 2015). None of the T6SS antigens has yet been targeted as vaccine development candidates, although they represent potential candidates (Ponnusamy et al. 2015, Fitts et al., 2016, and Andersson et al. 2017).

Vaccine targets beyond the T3SS: other *Y. pestis* plasmids and their gene products

The plague pathogen is benefitted by having additional virulence factors extending beyond its T3SS injectosome. Although the 70-kb virulence plasmid is shared by all three pathogenic yersiniae, only *Y. pestis* possesses two additional plasmids: the 9.5-kb pPCP1 plasmid (pPla) and the 110-kb pMT1 plasmid (pFra). The pPCP1 plasmid encodes the plasminogen-activating protease (Pla), which promotes bacterial dissemination via disruptions in blot clot formation and complement cascade activation (Suomalainen et al. 2007). Additionally, a pPla plasmid addiction system encoding a bacteriocin, pesticin, and its immunity gene product, pesticin immunity protein, ensures both selection pressure on plasmid maintenance and an offensive strategy that kills bacterial neighbors lacking the immunity protein (Rosenzweig et al. 2011 and references therein).

The pMT1 plasmid encodes a highly immunogenic, anti-phagocytic capsular antigen Fraction 1, referred to as F1. Due to its ability to induce robust immune responses, the F1 antigen has been the subject of a large number of plague candidate vaccine development efforts (Williamson and Oyston 2013; Rosenzweig et al. 2011; Demeure et al. 2019a, b; Sun and Singh 2019). Additionally, the plasmid encodes the 61-kDa *Yersinia* murine toxin (Ymt) known to promote bacterial survival in the flea mid-gut, and purified Ymt has been shown to promote broad toxicity in mice, including decreased blood sugar levels and internal bleeding (Fan et al. 2016). Although Ymt is required for mid-gut colonization of *Y. pestis* in the flea 1–2 weeks following infection, Ymt is not required for early-phase transmission (3 days post-infection) from flea to mouse (Johnson et al. 2014). As plague vaccine development gains momentum and traction, new candidate vaccines (Tables 1 and 2) are being evaluated for their safety and efficacy with the hope of several achieving clinical trial status in the near future.

Subunit vaccine strategies

Major subunit plague vaccine candidates are utilizing F1 and LcrV antigens, prompting the development of recombinant F1-LcrV (rF1-V) vaccines (Rosenzweig et al. 2011). However, there have been shortcomings with rF1-V candidates necessitating modifications so as to elicit more robust cellular immune responses (Smiley 2008). Consequently, a CD137 ligand was included as an adjuvant together with alhydrogel in the rF1-V vaccine platform, and was demonstrated to induce enhanced cell-mediated immunity (Bowen et al. 2019). Unfortunately, enhanced cell-mediated immunity in male mice did not translate into protection when animals were primed and boosted with the rF1-V + alhydrogel + CD137 ligand vaccine candidate in a pneumonic plague

Table 1 *Y. pestis* protein subunit and DNA vaccine candidates

Vaccine	Animal model	Efficacy	Reference
Protein subunit			
rF1-V + CD137 ligand+ alhydrogel	Mouse	Enhanced CMI; no protection against pneumonic challenge	Bowen et al. 2019
Micro-vesicle (*Bacteroides* spp.) F1-V	Nonhuman primates	Robust IgA and IgG in blood and airways	Carvalho et al. 2019
Lyophilized F1 LcrV (stored 29 weeks at 40°C)	Balb/c mice	Protection against bubonic plague	Moore et al. 2018
F1-loaded microspheres [F1-Al(OH)3)]	Blab/c mice	100% protection against bubonic plague; robust IgG	Huang et al. 2014
F1 rV	Humans (18-55 year olds)	100% sero-conversion high IgG titers	Hu et al. 2018
Single-dose F1-V-loaded polyanhydride nanoparticle coupled with cyclic dinucleotides	Mouse	Short-term and long-term humoral immunity; protection against pneumonic plague	Wagner et al. 2019
F1-V + Myd88+ TLR4	Mouse	Protection against pneumonic plague	Dankmeyer et al. 2014
LcrV + F1 + *B. anthracis* PA and LF	Mouse	100% protection against pneumonic plague; 90% protection against anthrax toxin	Gallagher et al. 2019
OmpA, Ail, and Pla	Mouse	OmpA and Ail protected against bubonic plague; Pla protected against pneumonic plague	Erova et al. 2013
DNA			
LcrV-F1 and *B. anthracis* PA (electroporation system)	AJ mice	Balanced Th1/Th2 response; 100% protection against lethal plague and lethal *B. anthracis* spore challenge	Albrecht et al. 2012a
LcrV-F1 and truncated *B. anthracis* PA (gene gun delivery system)	AJ mice	Enhanced survival against pneumonic plague when boosted with a DNA vaccine encoding the *B. anthracis* PA	Albrecht et al. 2012b
LcrV DNA vaccine prime and LcrV protein subunit vaccine boost	Balb/c mice	High antibody titers of anti-LcrV antibodies	Li et al. 2014

model; only female mice were protected against pneumonic plague for unknown reasons (Bowen et al. 2019). In a novel approach, Carvalho et al. (2019) have employed micro-vesicles derived from recombinant commensal *Bacteroides* spp. expressing and producing plague F1 and LcrV antigens as a vaccine delivery platform. This system allowed for facile targeted delivery of plague antigen–charged micro-vesicles to both lung and gut mucosa of nonhuman primates resulting in robust IgG and IgA production in blood and airways, respectively (Carvalho et al. 2019).

Storage and ensuring integrity are of paramount concerns, particularly when shipping vaccines to various parts of the world where refrigeration may be unavailable. With that in mind, a dual subunit vaccine consisting of associated F1 and LcrV was lyophilized. Not only was the preparation stable for 29 weeks at 40°C, but subcutaneous administration and an orally delivered boost also protected immunized Balb/c mice from bubonic challenge (Moore et al. 2018). In a similar approach, F1-loaded microspheres were characterized for their antigen release kinetics in vitro as well as for their immunogenicity in a murine model. Not only was a robust anti-F1 IgG titer measured but also 100% protection in Balb/c mice was achieved following a prime-boost regimen (Huang et al. 2014).

Interestingly, in a Chinese human clinical study evaluating an F1 + rV vaccine candidate, not only was the vaccine well tolerated in 18–55-year-olds but also it generated significant antibody titers and 100% sero-conversion for F1 antibodies (Hu et al. 2018). Additionally, single doses of an F1-V-loaded polyanhydride nanoparticle vaccine, when coupled with cyclic dinucleotides capable of inducing interferon genes, induced both rapid short-term and long-term humoral immunity in mice that protected against pneumonic plague (Wagner et al. 2019). Eliminating a boost requirement makes this candidate an attractive option. In attempting to elucidate the F1-V vaccine-induced host signaling responses in mice, the myeloid differentiation primary response 88 protein (MyD88) and Toll-like receptor (TLR)-4, but not TLR-2, were required for optimal vaccine immune response as well as subsequent protection against pneumonic plague challenge (Dankmeyer et al. 2014).

In some instances, plague subunit vaccines have included proteins from other bacterial pathogens creating cocktail subunit vaccine candidates (Rosenzweig et al. 2011). A multiple

Table 2 *Y. pestis* recombinant, live-attenuated, and rodent vaccine candidates

Vaccine	Animal model	Efficacy	Reference
Recombinant			
Dual PA anthrax-LcrV-F1 plague nanoparticle T4 phage delivery system	Mice rats and rabbits	Complete protection against both lethal challenges of inhalation anthrax and pneumonic plague	Tao et al. 2018
Y. pseudotuberculosis expressing the *Y. pestis* F1 antigen	Mice	Protection against both bubonic and pneumonic challenge, and serum transfer to naïve mice protected against bubonic challenge; protection against challenge with F1 variant	Demeure et al. 2017, 2019a, b
Oral *Y. pseudotuberculosis* Δ*yopK* Δ*yopJ* Δ*asd* + *Y. pestis* fusion protein (truncated YopE$_{1-138}$-LcrV	Mouse	Conferred 80% and 90% survival against bubonic and pneumonic challenge; strong humoral and CMI responses; protection against lethal *Y. enterocolitica* and *Y. pseudotuberculosis* challenge	Singh et al. 2019
Y. pestis KIM Δ*yopJ* overexpressing the *Y. enterocolitica* YopP	OF1 mice	Protection against pneumonic and bubonic challenge and against *Y. enterocolitica* challenge and *Francisella tularensis* challenge	Zauberman et al. 2013
Lactobacillus plantarum expressing LvrV-F1 and Tobacco Mosaic Virus (TMV) expressing LcrV-F1	Mouse	TMV LcrV-F1 provided 100% protection against a pneumonic plague challenge; *L. plantarum* LcrV-F1 conferred only partial protection	Arnaboldi et al. 2016
Salmonella expressing LcrV, F1 and pesticin receptor (Psn)	Mouse	Oral immunization, conferred 100% protection against both bubonic and pneumonic plague	Sananpala et al. 2016
Francisella tularensis Δ*capB* + F1-LcrV and a multiple-gene-deleted *Listeria monocytogenes* vaccine strain, + F1-LcrV	Mouse	Following a prime-boost schedule with both platforms, mice were protected against pneumonic plague challenge	Jia et al. 2018
Live-attenuated vaccines			
EV vaccine efficacy measure	Humans (Kazakhstan)	Highest level of protective anti-F1 serum antibodies was observed 4 months following vaccination with significant reduced antibody titers at both 8 and 12 months	Sagiyev et al. 2019
EV plague vaccine strain	Humans	Robust cell-mediated responses to Pla protease in immunized humans for up to 1 year following vaccination	Feodorova et al. 2018
Y. pestis subspecies: altaica 1-2948/3, 1-3749, and 1/3480	Mouse	Elicit strong cell-mediated responses	Balakhonov et al. 2017
Y. pestis EV vaccine strain and the microtus 201 (avirulent in humans) strain	Rhesus macaques (intravenous (i.v.) infection model)	The microtus strain infected monkey lungs and led to 100% mortality in 10^{10} i.v.-challenged animals; none of the EV-challenged animals died at that same dose	Tian et al. 2014
Rodent			
Sylvatic plague vaccine (SPV), a virally vectored bait system vaccine	Wild prairie dogs	Capture of unique prairie dogs on vaccine-treated fields was significantly higher in each of the 2 years tested on 29 paired plots of land in 7 Western US states years tested	Rocke et al. 2017
SPV	Wild prairie dogs	Bait uptake of the SPV vaccine, during a 3-year study, was as high as 70% over 58 plots of land; heavier animals exhibited increased bait uptake; baiting later in the growing season influenced bait uptake	Abbott et al. 2018
SPV	Wild prairie dogs	In two of the three plots evaluated, both pesticide dusting and oral SPV improved prairie dog survival	Trip et al. 2017
SPV	Wild prairie dogs and non-target rodents	70% of the bait-based vaccine was consumed by non-target rodents over a 3-year period in which no effects were observed	Bron et al. 2018
LMA and LMP live-attenuated vaccines	Mice and rats	100% efficacy during bubonic and pneumonic plague (short- and ling-term), generate robust humoral and cell-mediated immune responses	Tiner et al. 2015a, b, 2016; Van Lier et al. 2014, 2015

antigen fusion protein consisting of the *Y. pestis* LcrV and F1 antigens, as well as the *Bacillus anthracis* protective antigen and lethal factor, was used to immunize and boost mice prior to *Y. pestis* and anthrax toxin challenges. Encouragingly, immunized mice were completely protected (i.e., 100%) against subsequent *Y. pestis* challenge and 90% protected against anthrax toxin (Gallagher et al. 2019). Some efforts have cast a wider net and are interrogating other antigens as potential vaccine candidates. The attachment invasion locus (Ail/OmpX), outer membrane protein A (OmpA), and Pla are three such candidates. In mice, OmpA and Ail vaccines were protective against bubonic challenge with an F1^{-} *Y. pestis* variant while the Pla candidate vaccine was protective against pneumonic plague (Erova et al. 2013).

DNA vaccines

Oligonucleotide vaccine platforms offer several advantages and have been gaining some traction over the years. Criticism has emphasized poor immunogenicity; however, the early success of the Pfizer and Moderna mRNA SARS-CoV-2 vaccines may renew interest in oligonucleotide plague vaccine development. Some plague DNA vaccine development has been highlighted in the literature (Rosenzweig et al. 2011; Verma and Tuteja 2016 and references therein); however, there have been a paucity of current advances.

In a report, plasmid constructs encoding two codon-optimized plague antigens, F1 and LcrV, together with the protective antigen (PA) from *B. anthracis*, were used to develop DNA vaccine candidates; constructs were delivered to mice using an electroporation-based system following a prime-boost schedule. Not only were the pVAX constructs encoding F1 and LcrV 100% protective in A/J inbred mice following plague challenge but also the pVAX construct encoding PA conferred 100% protection against a lethal *B. anthracis* spore challenge (Albrecht et al. 2012). Most importantly, the aforementioned DNA vaccine candidates promoted a balanced Th1/Th2 response as evidenced by elevated levels of both interferon-γ and interleukin-4 (Albrecht et al. 2012a). Such a response profile is certainly more desirable than the Th2-skewed response profiles of many protein subunit vaccines. Similarly, a gene gun delivery of a DNA vaccine encoding a fusion of *B. anthracis* truncated lethal factor protein and *Y. pestis* LcrV or F1 enhanced survival of mice against pneumonic plague when boosted with a DNA vaccine encoding the *B. anthracis* PA (Albrecht et al. 2012b). Interestingly, DNA vaccines coupled with subunit boost may work synergistically to acquire the greatest protection. More specially, following immunization with a DNA vaccine encoding LcrV and subsequent protein LcrV subunit vaccine boost, high titers of anti-LcrV antibodies were measured in Balb/c mice (Li et al. 2014; Wang et al. 2004).

Recombinant vaccines

Y. pestis and *B. anthracis* are the two most likely candidate bacterial pathogens that could be weaponized for bio-warfare (Rosenzweig et al. 2011). Therefore, some approaches combine the two, or derivatives thereof, into a single vaccine platform. Previously, a combined vaccine was protective in both a mouse and rabbit model of bubonic plague and cutaneous anthrax (Ren et al. 2009). More recently, a dual anthrax-plague nanoparticle that employed a T4 phage delivery system was evaluated for efficacy. The anthrax-protective antigen and the plague F1 and LcrV antigens were fused to phage T4 outer capsid proteins. Encouragingly, the vaccine conferred complete protection against both lethal challenges of inhalation anthrax and pneumonic plague in mice, rats, and rabbits (Tao et al. 2018).

In another approach involving the use of recombinant *Y. pseudotuberculosis* expressing the *Y. pestis* F1 antigen (referred to as the VTnF1 vaccine candidate; Derbise et al. 2015), protection against bubonic and pneumonic challenge was observed in both inbred and outbred murine models. Furthermore, serum transfer to naïve mice demonstrated protection against bubonic challenge (Demeure et al. 2019a, b). Following a single oral dose of VTnF1, mice were protected against bubonic and pneumonic plague including challenge with a *Y. pestis* variant devoid of the F1 antigen (Demeure et al. 2017). This is a very attractive feature as *Y. pestis* strains have been found to be lacking F1 in nature and are as virulent as encapsulated strains.

On account of the absence of F1 in some *Y. pestis* strains, several pipeline vaccine candidates have pivoted away from F1. For example, an attenuated *Y. pseudotuberculosis* Δ*yopK* Δ*yopJ* Δ*asd* triple mutant, unable to produce the translocator YopK and effector YopJ proteins, ectopically expressed a *Y. pestis* fusion protein composed of a truncated YopE$_{1\text{-}138}$-LcrV. The candidate vaccine conferred 80% and 90% survival following bubonic and pneumonic challenge, respectively, in mice having received a single-dose oral immunization. Strong humoral and cell-mediated responses were also observed with protection against lethal *Y. enterocolitica* and *Y. pseudotuberculosis* challenge (Singh et al. 2019). Still other *Y. pestis* attenuated strains are being evaluated as potential recombinant vaccine candidates, including the KIM strain. More specifically, the KIM background strain was used to generate a Δ*yopJ* mutant overexpressing the *Y. enterocolitica* YopP; the recombinant *Y. pestis* vaccine candidate was able to protect Oncins France 1 (OF1), albino, outbred mice against pneumonic and bubonic challenge via host interferon-γ involvement. Surprisingly, the vaccine candidate was also able to protect against subsequent *Y. enterocolitica* challenge. Interestingly, after infecting mice with a subcutaneous dose of 1×10^4 cfu of the recombinant *Y. pestis* KIM strain, ~70% of the mice were protected against subsequent intranasal challenge with either 500 or 5000 cfu of *Francisella tularensis*, an unrelated organism (Zauberman et al. 2013). This peripheral benefit is due to the vaccine candidate eliciting cross-protective antibodies against *F. tularensis* (Zauberman et al. 2013).

Some recent recombinant platforms have involved organisms other than yersiniae. A recombinant *Lactobacillus plantarum* and a recombinant Tobacco Mosaic Virus

(TMV), both expressing LcrV-F1, were employed in a mouse immunization study. Only TMV expressing LcrV and F1 protected 100% of pneumonic plague–challenged mice, while the *L. plantarum* LcrV-F1 expressing recombinant conferred only partial protection as measured by mouse mortality (Arnaboldi et al. 2016). Another strong candidate is a well-characterized *Salmonella* recombinant vaccine that was engineered to express 3 plague antigens: a truncated LcrV, F1, and pesticin receptor (Psn). Following oral immunization using the aforementioned vaccine candidate, mice were 100% protected against both bubonic and pneumonic plague (Sananpala et al. 2016).

Additionally, a live vaccine strain for *Francisella tularensis* devoid of its *capB* gene and an attenuated multiple-gene-deleted *Listeria monocytogenes* vaccine strain, both expressing F1-V plague recombinant protective antigens, were evaluated. Following a prime-boost schedule with both platforms, mice were protected against pneumonic plague challenge (Jia et al. 2018). With fear of potential reversion, a low-probable reality when considering a live-attenuated *Y. pestis* vaccine or a recombinant *Y. pseudotuberculosis*, *Salmonella* Typhimurium, *F. tularensis*, or *L. monocytogenes* vaccine may offer a potentially safer alternative without compromising efficacy.

Adenovirus vector vaccines

While some strains of *Y. pestis* lack the F1 capsular antigen, evidence has shown that these strains can be fully virulent (Sha et al. 2011; Quenee et al. 2008). In addition, divergence of LcrV variants presents issues for efficacy of F1-V vaccines. Therefore, efforts have been focused on discovering combinations of immunogenic antigens that will provide protection against these strains. Vaccination of mice with YscF, a T3SS needle structure protein, showed increased protection against intravenous (via the retro-orbital sinus) challenge with the KIM5 strain (Matson et al. 2005). Based on this evidence, a trivalent vaccine utilizing an adenovirus vector has shown promise. Sha et al. (2016) employed a replication-defective human type-5 adenovirus (Ad5) vector to construct a recombinant YFV fusion gene vaccine encompassing *ycsF*, *caf1*, and *lcrV*. Impressively, one intranasal dose of the trivalent rAD5-YFV vaccine combined with an intramuscular prime-boost of recombinant fusion protein rYFV provided up to 100% protection in murine and nonhuman primate (NHP) models when challenged with a high aerosol dose of CO92. Furthermore, histopathological studies revealed vaccinated NHPs showed no signs of lesions in various organ tissues (Sha et al. 2016).

The World Health Organization (WHO) has since released a target product profile for plague vaccines that includes recommendations for needle-free vaccines in 2 or fewer doses (WHO Workshop 2018). In response to this recommendation, the rAd5-YFV vaccine was further evaluated using 1 or 2 intranasal doses without the rYFV prime-boost strategy. It was shown that 2 doses provided 100% protection in both pneumonic and bubonic plague models, as well as an induction of humoral, mucosal, and cell-mediated immunity (Kilgore et al. 2021). Importantly, the vaccine was equally (100%) protective in mice when challenge occurred with either the parental *Y. pestis* CO92 strain or its F1-negative variant (Kilgore et al., 2021). However, most humans likely possess pre-existing antibodies against Ad5, so immunogenicity of Ad5-vectored vaccines could be low as a result. Both Sha et al. (2016) and Kilgore et al. (2021) demonstrated that intranasal administration of the rAd5-YFV vaccine has the potential to bypass pre-existing antibodies to Ad5 vectors. Furthermore, the Ad5 vector, when compared to other adenovirus-vectored vaccines, elicits minimal proinflammatory responses (Teigler et al. 2012). As with the most recent SARS-CoV-2 vaccines distributed by Johnson & Johnson and AstraZeneca, adenovirus-vectored vaccines can be successfully implemented against high-consequence pathogens.

Live-attenuated vaccines

Until the recent advent of attenuated *Y. pestis* genetically mutated strains, the Western world has generally frowned upon the utilization of a live-attenuated plague vaccine for widespread distribution (Sun et al. 2011; Wang et al. 2013). Although the live-attenuated plague vaccine EV76, created in 1936 in the Former Soviet Union, is still used by some former Soviet countries, the vaccine is not utilized in the USA, Europe, or Canada due to its strong reactogenicity (Titball and Williamson 2004). Even so, millions of people have received the live-attenuated EV76 vaccine in the past 80 years with minimal and ephemeral side effects (Feodorova et al. 2014).

In fact, a protocol was developed to rapidly assess live-attenuated EV vaccine candidates in bubonic plague infection models of both mice and guinea pigs (Feodorova et al. 2016). Furthermore, to evaluate plague EV vaccine efficacy more specifically, Sagiyev et al. (2019) measured anti-F1 antibody titers to determine the undefined period of protection and make future vaccine dosing schedule recommendations in Kazakhstan. The highest level of protective anti-F1 serum antibodies was observed 4 months following vaccination with significant reductions in antibody titers at both 8 and 12 months following vaccination. As a result, recommendations were made to vaccinate approximately 4 months ahead of spring when rodent populations become active in Kazakhstan (Sagiyev et al. 2019). To further evaluate the EV plague vaccine strain, the cell-mediated responses to Pla protease were measured in immunized humans and found to be robust up to 1 year post-vaccination (Feodorova et al. 2018).

Several other seemingly useful live-attenuated vaccine candidates with varying plasmid compositions have also emerged. *Y. pestis* subspecies altaica 1-2948/3, 1-3749, and 1/3480 were able to elicit strong cell-mediated responses in a mouse model of infection (Balakhonov et al. 2017). In that same vein, a direct comparison of the virulence of the *Y. pestis* EV vaccine strain to the microtus 201 (avirulent in humans) strain in an intravenous (i.v.) rhesus macaque infection model revealed that both strains were well tolerated in NHPs at high doses. However, the microtus strain infected the lungs and led to 100% mortality in 10^{10} i.v.-challenged animals; none of the EV-challenged animals died at that same dose (Tian et al. 2014). The side effects associated with the EV strain in humans as well as the ability of the pigmentation locus (pgm) minus strains of *Y. pestis* to cause a fatal disease in patients with hemochromatosis suggest that the EV mutant strain could be further attenuated through specific gene knockouts to generate live-attenuated plague vaccine candidates, as has been recently reported (Tiner et al. 2015A&B; Tiner et al. 2016; van Lier et al. 2014).

Another target for live-attenuated vaccine candidates has been LPS and its derivatives. In *Y. enterocolitica*, the acetyltransferase, encoded by the *msbB* gene, was upregulated at 21°C, relative to the mammalian temperature of 37°C, leading predominantly to a hexa-acylated lipid A as compared to predominantly tetra-acylated lipid A at 37°C. Furthermore, lipid A acylation status was shown to directly affect virulence-associated gene expression levels as well as sensitivity to polymyxin B (Pérez-Gutiérrez et al. 2010). Additionally, when the *Escherichia coli*–derived acyltransferase LpxL was expressed in *Y. pestis* at 37°C, an atypical hexa-acylated lipid A was observed, thereby promoting dendritic cell migration, which was mollified by *Y. pestis* disruption of TLR-4-induction of IL-12 (Robinson et al. 2008). Finally, by using humanized mice expressing TLR-4, *Y. pestis* was shown to use its thermal regulation of lipid A acylation states to evade recognition by human TLR-4 (Hajjar et al. 2012). In that vein, our group characterized the role of chromosomally encoded Braun lipoprotein (Lpp) and the MsbB acetyltransferase in the virulence of *Y. pestis* CO92. While Lpp activates TLR-2, MsbB catalyzes the addition of lauric acid to the lipid A moiety of LPS, thus increasing its biological potency by activating TLR-4 (Sha et al. 2013).

We demonstrated that combinatorial mutants (e.g., Δ*lpp*Δ*msbB*/*ail* [LMA] and Δ*lpp*Δ*msbB*Δ*pla* [LMP]) were more significantly attenuated (100% animal survival) than single or double mutants, provided long-term protective immunity (cell-mediated and humoral) in rodents, and, thus, could provide a platform for developing an efficacious live-attenuated plague vaccine(s) (Tiner et al. 2015A&B; Tiner et al. 2016; van Lier et al. 2014; van Lier et al. 2015). The vaccine strains cleared from animals within 24 h with no histopathological lesions in various organs during immunization or after challenge of immunized animals (Tier et al. 2015B, Tiner et al. 2016; van Lier et al. 2014). These vaccine candidates provide protection against both bubonic and pneumonic plague. Our vaccine strains have the following advantages: (1) rationally designed with complete deletion of three genes, (2) are stable with no risk for reversion because of the deletion of three genes located on different DNA regions; the mutants have been sequenced with no secondary mutations, (3) *lpp* and *msbB* deletions greatly reduce host reactogenicity relative to EV76 vaccine, (4) LMA/LMP mutants generate immune responses to thousands of *Yp* antigens, thus would provide cross-protection against different *Yp* biovars/strains, (5) the mutants are excluded from the CDC select agent list, and (6) fulfill the target product profile provided by the WHO.

Rodent vaccinations

Another approach to tackling the plague threat is to directly vaccinate rodent zoonotic reservoirs. The major goal of such efforts is conservation of delicate ecosystem balances by protecting rodents susceptible to plague-induced mortality (Salkeld 2017; Roth 2019). Furthermore, by such control, plague infection spillover into human populations as collateral damage can also be prevented (Richgels et al. 2016). Despite the obvious challenges, including poor vaccine uptake and unintended targets, several groups have taken this approach.

In an ambitious 2-year study, 29 paired plots of land seeded with either a sylvatic plague vaccine (SPV), a virally vectored bait system vaccine, or a placebo were compared in 7 Western states in the USA. The capture of unique prairie dogs on vaccine-treated fields was significantly higher in each of the years tested, suggesting that an SPV can protect prairie dogs from sylvatic plague (Rocke et al. 2017). In a separate 3-year study, bait uptake of the SPV vaccine was as high as 70% over 58 plots of land. Interestingly, heavier animals exhibited increased bait uptake, and baiting later in the growing season influenced bait uptake (Abbott et al. 2018). In parallel, Tripp et al. (2017) compared the effectiveness of dusting prairie dog burrows with an insecticide to baiting with an oral SPV. In two of the three plots evaluated, pesticide dusting and oral SPV improved prairie dog survival and suggested a method by which the species can be protected from collapse due to plague-induced mortality (Tripp et al. 2017). Additionally, Bron et al. (2018) sought to evaluate the impact of a SPV on non-target rodents over a 3-year period. Although targeting protection in prairie dogs, 70% of the bait-based vaccine was consumed by non-target rodents in which no effects were observed (Bron et al. 2018).

Future considerations

Generally, it is believed that live-attenuated vaccines confer the highest degree of protection, generate robust humoral and cell-mediated responses, and typically result in long-term or life-long immunity (Slifka and Amanna 2014; US Department of Health and Human Services n.d.). However, the challenge associated with live-attenuated vaccines is the threat of a low-frequency reversion event restoring full virulence, which can now be circumvented by the development of new generation designer vaccines.

Interestingly, some unintended pathologies could be rooted in host genetics rather than reversion of the attenuated *Y. pestis* strain. In one such example, a researcher died following exposure to a laboratory nonpigmented (*pgm*$^-$), attenuated mutant UC91309 *Y. pestis* strain (unable to produce the yersiniabactin siderophore required for iron acquisition). The patient was found to have been compromised by the inherited genetic malady hemochromatosis, resulting in increased iron within tissues (Frank et al. 2011). In fact, increased iron presence within host tissues was shown to complement *Y. pestis* *pgm*$^-$ attenuated strains in a murine model, and vaccination using a subunit vaccine was sufficient to achieve protection upon challenge (Quenee et al. 2012). Furthermore, in a *Y. pestis* EV76 murine vaccine study, mobilized iron-regulating factors supported vaccine efficacy. More specifically, hemopexin (a host heme binding protein) and transferrin (a host iron-binding protein) demonstrated anti-bacterial properties in the serum of EV76-immunized mice shortly following the immunization (Zauberman et al. 2017). Based on those findings, patients should be pre-screened, and subunit vaccines should be offered, if available, to avoid host factors complementing live-attenuated vaccine strains.

Subunit vaccine efforts tend to favor F1, LcrV, or combinations thereof; however, screening for novel candidates is ongoing. In one such effort, over 4000 *Y. pestis* proteins were screened computationally and experimentally for the ability to elicit a cell-mediated response by CD8$^+$ T cells. Ultimately, 178 unique CD8$^+$ T cell epitopes, derived from 113 *Y. pestis* proteins, were identified and could be exploited as novel antigens in subunit vaccine development (Zvi et al. 2017). Additionally, novel clustered regularly interspaced short palindromic repeat interference (CRISPRi) techniques allow reversible characterization of virulence potential of these novel gene candidates (Wang et al. 2019), which could be exploited as future vaccine targets.

In the event of a widespread bio-attack, attention has centered on post-exposure antibiotic treatment. However, as mentioned earlier, drug-resistant strains are naturally occurring; moreover, *Y. pestis* could be engineered as a multiple-drug-resistant weapon (Rosenzweig et al. 2011). As a result, a mouse model was employed to evaluate the efficacy of a combined post-exposure vaccination followed by antibiotic treatment with a second-line chemotherapeutic. More specifically, a live-attenuated EV76 immunization was paired with a lethal pneumonic challenge and subsequent antibiotic treatment (with a second-line chemotherapeutic); the vaccine and antibiotic treatment worked synergistically to arrest disease progression and lessen morbidity (Zauberman et al. 2019).

With regard to *Y. pestis*, the reemerging plague pathogen that could become weaponized; novel approaches and creative strategies need to be applied and translated into viable prophylactic and post-exposure treatments. A vaccine that is well tolerated with high efficacy and has the ability to promote cell-mediated and humoral responses is the plague research community's top priority. While some progress has been made on live-attenuated candidates, subunit vaccines still appear to be the favored approach in filling the plague vaccine void. The COVID-19 pandemic prompted an unprecedentedly short turn around on two approved mRNA vaccine deliverables, and two adenovirus-based vaccines. With renewed awareness of the seriousness global pandemics can pose to human health and economic stability, perhaps approval of a novel plague vaccine is adequately motivated. Importantly, a combinatorial approach of Ad5-YFV vaccine followed by a booster of live-attenuated LMA or LMP mutant or vice versa could be highly advantageous for preventative and reactive scenarios, as well as more effective possibly due to differing mechanisms of protection provided by these two vaccines. Furthermore, both vaccines can be administered by the intranasal route, avoiding the use of needles. Such studies are being conducted in our laboratory.

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Declarations

The authors declare no competing interests.

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