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Tularemia vaccines

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Abstract *Francisella tularensis* is the causative agent of the potentially lethal disease tularemia. Due to a low infectious dose and ease of airborne transmission, *Francisella* is classified as a category A biological agent. Despite the possible risk to public health, there is no safe and fully licensed vaccine. A potential vaccine candidate, an attenuated live vaccine strain, does not fulfil the criteria for general use. In this review, we will summarize existing and new candidates for live attenuated and subunit vaccines.

Introduction—*Francisella tularensis*

Francisella tularensis (*F. tularensis*) is a non-motile, gram-negative, facultative intracellular pathogen that is the etiological agent of the potentially lethal disease tularemia in both humans and animals. This species is considered a biological weapon and classified as a category A bioterrorism agent by the US Centers for Disease Control and Prevention (Khan et al. 2000) due to its high infectivity, potential airborne transmission and ability to cause severe disease. During the Cold War, *F. tularensis* belonged to the group of agents produced and stockpiled by the former Soviet Union and the USA (reviewed in Dennis et al. 2001). In 1970, the World Health Organization committee categorized *F. tularensis* as a biological threat and estimated that the dispersal of 50 kg of its aerosolized virulent form over an urban area with five million inhabitants would result in 250,000 incapacitating causalities and 19,000 deaths (World Health Organization 1970). Today's major concerns are the misuse of *F. tularensis* during possible terrorist attacks.

F. tularensis belongs to the class *γ-Proteobacteria*, family *Francisellaceae* and genus *Francisella* (Forsman et al. 1994; World Health Organization 2007). The species *F. tularensis* is divided into three subspecies: *tularensis*, *holarctica* and *mediasiatica*, which vary in their pathogenicity and geographic distribution (Oyston 2008). *F. tularensis* subsp. *tularensis* (classified as type A) is found predominantly in North America and consists of two different genetic sub-populations, AI and AII (Johansson et al. 2004), which are characterized by the extreme virulence, as less than 10 bacteria can lead to lethal disease (reviewed in Tärnvik and Berglund 2003). *F. tularensis* subsp. *holarctica* (type B) occurs primarily in the Northern Hemisphere and causes a milder form of tularemia. *F. tularensis* subsp. *mediasiatica* was detected in Central Asia, and its virulence resembles the *holarctica* subspecies. The species *novicida*, isolated in North America and Australia, is rarely responsible for human tularemia (reviewed in Pechous et al. 2009).

Natural hosts for *F. tularensis* include lagomorphs, rodents, carnivores, ungulates, marsupials, amphibians, birds, fish and invertebrates (Mörner 1992). However, despite the wide distribution of *Francisella* in numerous wildlife species, its primary reservoirs remain unknown. Natural infection can be transmitted to humans through arthropod vectors, such as ticks, flies or mosquitoes, or by direct contact during handling of infected animals, drinking of contaminated water or inhaling of aerosols (Mörner 1992; Tärnvik and Berglund 2003).

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Tularemia

Tularemia is an acute febrile disease whose severity depends on the route of infection and virulence of the strain (Ellis et al. 2002). The incubation period normally ranges from 3 to 5 days; however, the period may be extended to up to 21 days. In the early phase of infection, tularemia is frequently misdiagnosed because disease symptoms resemble flu symptoms, such as high fever, body aches, and swollen lymph nodes. The most common form of tularemia is ulceroglandular tularemia, which is usually spread through vector-borne transmission (reviewed by Tärnvik and Berglund 2003). A painless ulcer develops at the site of inoculation followed by enlargement of regional lymph nodes (Ohara et al. 1991). The infrequent clinical form, oculoglandular tularemia, occurs after direct contact of the eye with the bacteria. Oropharyngeal tularemia, which is accompanied by stomatitis and pharyngitis, results from contaminated food or water intake (reviewed by Tärnvik and Berglund 2003; World Health Organization 2007). The most severe form is respiratory tularemia, which is caused by the inhalation of aerosolized *F. tularensis* subsp. *tularensis*. The mortality rate for respiratory tularemia ranges from 30 to 60 % without effective antibiotic therapy (reviewed by Tärnvik and Berglund 2003). Treatment successfully resolves infection when administered in the early phase of infection. Antibiotics of choice are aminoglycosides, tetracyclines, chloramphenicol and quinolones (reviewed by Dennis et al. 2001).

The human immune response to tularemia was described in naturally infected patients or live vaccine strain (LVS) vaccinated volunteers (Koskela and Herva 1982; Koskela and Salminen 1985; Surcel et al. 1991; Sjöstedt et al. 1992a; Poquet et al. 1998; Ericsson et al. 1994). Considering that *F. tularensis* is an intracellular pathogen, it was thought that a cell-mediated immune response is required to clear infection. $CD4^+$ and $CD8^+$ T cells are detectable 2 weeks post-infection, as well as proinflammatory cytokines, including interferon-γ (IFN-γ), TNF-α and interleukin 2 (IL-2) (Koskela and Herva 1982; Surcel et al. 1991; Sjöstedt et al. 1992a). The cell-mediated response is long-lasting and even inducible 30 years after the onset of disease (Ericsson et al. 2001). In naturally infected individuals, phosphoantigen-directed $V\gamma 9/V\delta 2$ T cells arise during the first week after infection (Poquet et al. 1998). Human peripheral blood cells show increased expression of IFN-γ-regulated genes within 2–3 days post-infection (Andersson et al. 2006). In respect to humoral immunity, the production of specific IgM, IgA and IgG antibodies reaches its highest levels at 1–2 months and persists 0.5 to 11 years post-infection (Koskela and Salminen 1985). Recent studies in the murine model of tularemia showed that both components of adaptive immunity are critical for the induction of full protection against tularemia (Lavine et al. 2007; Cole et al. 2011; Kubelkova et al. 2012).

The current problem in tularemia prophylaxis is the lack of a vaccine. The only available prophylactic tool is LVS, which is not intended for public use due to its attenuation background. It is therefore vital to develop a new vaccine that will be safe and effective in inducing protective long-lasting immune response against respiratory challenge with the most virulent strain of *F. tularensis*. Currently, *F. tularensis* vaccine development has focused on developing live attenuated (Table 1) and subunit vaccines (Table 2).

Killed whole-cell vaccines

Killed whole-cell vaccines are composed of non-infectious modified bacterial suspensions. The earliest tularemia vaccine was developed using acetone extraction or phenolization by Foshay et al. (1942). The vaccine protected non-human primates against challenge with 740 CFU of *F. tularensis* subsp. *tularensis* Schu S4 (Schu S4); however, it caused symptoms of disease (Coriell et al. 1948) and was not efficient to protect against highly virulent strains in animal models (Foshay et al. 1942; Pechous et al. 2009).

Recent studies showed that protection induced by killed whole-cell vaccines are enhanced through the use of boosters and adjuvants. Eyles et al. (2008) found that intramuscular (i.m.)-delivered, inactivated LVS vaccination in conjunction with immune-stimulating complexes and immunostimulatory CpG oligonucleotides had a protective effect against aerosol challenge with *F. tularensis* subsp. *holarctica*, but not against low-dose aerosol challenge with Schu S4. Baron and co-workers (2007) determined that inactivated LVS administered via the intranasal (i.n.) route protected mice against i.n. infection with LVS, although only in combination with IL-12 administration.

Live attenuated vaccines

The first anti-*Francisella* live attenuated vaccine was generated from *F. tularensis* subsp. *holarctica*, which was isolated in the former Soviet Union (Tigertt 1962). A sample of the vaccine was provided to the US where multiple passages of the strain led to the preparation of LVS (Eigelsbach and Downs 1961). The results obtained from vaccine trials in humans showed that LVS induced protective immunity against a low-dose aerosol challenge with Schu S4 (McCrumb 1961). However, LVS has not been officially licensed by the Food and Drug Administration as a human vaccine due to an unknown mechanism of attenuation, the instability of colony phenotype and the partial virulence after vaccination via the aerosol route (Hornick and Eigelsbach 1966; Hartley et al. 2006; Petrosino et al. 2006).

Table 1 *Francisella* live attenuated vaccines

Gene	Species or subspecies	Vaccination dose (route)	Boost	Challenge dose and strain (route)	Survival in mice	Reference
guaA *guaB*	LVS	2.2×10^7 CFU (i.p.) 3.6×10^7 CFU (i.p.)	No	2.8×10^5 CFU of LVS (i.p.)	100 % in BALB/c	(Santiago et al. 2009)
purMCD	LVS	5×10^6 CFU (i.p.)	No	5×10^3 CFU LVS (i.p.)	100 % in BALB/c	(Pechous et al. 2006)
	LVS Schu S4	10^6 CFU (i.n.) 10^4 CFU (i.n.)	10^6 CFU (i.n.) 10^6 CFU (i.n.)	100 CFU Schu S4 (i.n.)	100 % in BALB/c 71 % in BALB/c	(Pechous et al. 2008)
capB	LVS	1×10^5 CFU (i.n.)	No	$10 \times LD_{50}$ of Schu S4 (aerosol)	100 % in BALB/c	(Jia et al. 2010)
FTT_1103	Schu S4	1.3×10^8 CFU (i.n.) 2.6×10^8 CFU (i.n.)	No	37 CFU of Schu S4 (i.n.) 68 CFU of Schu S4 (i.n.)	100 % in C57BL/6 50 % in C57BL/6	(Qin et al. 2009)
		1×10^8 CFU (i.n.)		95 CFU of Schu S4 (i.n.)	75 % in BALB/c	
clpB	Schu S4	10^7 CFU (i.d.)	No	105 CFU of Schu S4 (i.n.)	100 % in BALB/c	(Golovliov et al. 2013)
iglB	*F. novicida*	10^6 CFU (i.n.) 10^3 CFU (orally)	No	3×10^4 CFU of LVS (i.n.) 25 CFU of Schu S4 (i.n.)	100 % in BALB/c 67 % in C57BL/6	(Cong et al. 2009)
iglC	Schu S4	10^6 CFU (i.d.) 10^7 CFU (i.d.)	No	500 CFU of FSC033 (i.d.) 10 CFU of FSC033 (aerosol)	0 % in BALB/c	(Twine et al. 2005)
iglH	FSC200	3×10^7 CFU (s.c.)	No	3×10^2 CFU of FSC200 (s.c.)	100 % in BALB/c	(Straskova et al. 2012)
mglA	*F. novicida*	1×10^5 CFU (aerosol)	No	6×10^2 CFU of *F. novicida* (aerosol)	0 % in BALB/c	(West et al. 2008)
wbtA	LVS	10^5 CFU (i. p.)	No	10^4 CFU of LVS (i.p.)	100 % in BALB/c	(Raynaud et al. 2007)
wbtI	LVS	10^5 CFU (i.d.) 5×10^4 CFU (i.p.)	10^5 CFU (i.d.) 5×10^4 CFU (i.p.)	$25 \times LD_{50}$ of LVS (i.p.) $250 \times LD_{50}$ of LVS (i.p.)	100 % in BALB/c 60 % in BALB/c	(Li et al. 2007)
wzy	LVS	1.5×10^7 CFU (i.n.) 3.5×10^6 CFU (i.n.)	2.4×10^7 CFU (i.n.) 3×10^7 and 3.2×10^7 CFU (i.n.)	1.2×10^5 CFU of LVS (i.n.) 8 CFU of Schu S4 (i.n.)	100 % in BALB/cByJ 84 % in BALB/cByJ	(Kim et al. 2012)
acpA *acpB* *acpC* *hapA*	*F. novicida*	10^6 CFU (i.n.)	No	10^6 CFU of *F. novicida* (i.n.)	100 % in BALB/c	(Mohapatra et al. 2008)
sodB	LVS	5.27×10^3 CFU (i.n.) 5.16×10^2 CFU (i.n.)	No 1.21×10^3 CFU (i.n.)	14 CFU of Schu S4 (i.n.) 10^3 CFU of Schu S4 (i.n.)	40 % in C57BL/6 42 % in C57BL/6	(Bakshi et al. 2008)
FTT_1676	Schu S4	50 CFU (i.d.)	No	50 CFU of Schu S4 (i.d.) 10 CFU of Schu S4 (i.n.)	100 % in BALB/c	(Rockx-Brouwer et al. 2012)
FTT_0369c	Schu S4	50 CFU (i.d.)	No	50 CFU of Schu S4 (i.d.)	100 % in BALB/c	
				10 CFU of Schu S4 (i.n.)	90 % in BALB/c	

Table 2 *Francisella* subunit vaccines

Antigen	Name	Adjuvant	Delivery system	Vaccination dose (route)	Boost	Challenge dose and strain (route)	Survival in mice	Reference
LPS	Lipopolysaccharide	No	No	100 ng (i.d.)	No	10^4 of LVS (i.p.)	100 % in BABL/cByJ	(Dreisbach et al. 2000)
				50 µg (i.p.)	No 1×10^6 CFU of LVS (i.p.)	100 CFU of SchuS4 (i.p.)	0 % in BALB/c 80 % in BALB/c	(Fulop et al. 2001)
DnaK	Chaperone protein	GPI-0100	No	20 µg of DnaK, together with 10 µg of Tul4 (i.n.)	20 µg of DnaK, together with 10 µg of Tul4 (i.n.)—twice	1.5×10^6 CFU of LVS (i.n.)	85.7 % in C57BL/6	(Ashtekar et al. 2012)
Tul4	Membrane protein	No	Ad-opt	1×10^5 PFU of Ad-opt-Tul4 (i.m.)	1×10^5 PFU of Ad-opt-Tul4 (i.m.) - twice	210 CFU of LVS (i.p.)	60 % in BALB/c	(Kaur et al. 2012)
IglC	Intracellular growth locus, subunit C	No	*rLm*	1×10^6 CFU of *rLm*/IglC (i.d.)	1×10^6 CFU of *rLm*/IglC (i.d.)	4400 CFU of LVS (i.n.)	87.5 % in BALB/c	(Jia et al. 2009)
				1×10^7 CFU of *rLm*/IglC (i.d.)	1×10^7 CFU of *rLm*/IglC (i.d.)	$1 \times LD_{100}$ of SchuS4 (aerosol)	100 % in BALB/c	
FopA	Outer membrane protein	$Al(OH)_3$ IL-12	Liposomes	10 µg of liposomal rFopA (i.p.)	20 µg of liposomal rFopA—twice	$1 \times LD_{50}$ of LVS (i.n.)	100 % survival in C57BL/6	(Hickey et al. 2011)

Currently, live attenuated vaccines are prepared from live organisms and take into account the balance between attenuation and immunogenicity. Namely, over-attenuation could lead to the loss of partial bacterial virulence and an insufficient protective immune response. These types of vaccines are constructed by deleting genes involved in metabolic and virulence pathways, which are also necessary for *F. tularensis* intracellular replication and in vivo survival.

Mutations in genes involved in *Francisella* metabolic pathways

Screening for genes involved in purine biosynthetic pathways in the Schu S4 strain revealed novel candidates for live attenuated vaccines (Prior et al. 2001). *F. novicida* mutants *ΔpurA*, *ΔpurCD* and *ΔpurM* were attenuated in mice; protection against challenge with a homologous wild-type strain was not observed (Tempel et al. 2006; Quarry et al. 2007). Mutant *ΔpurF* protected mice against intraperitoneal (i.p.) challenge with *F. novicida*, but not against the virulent Schu S4 strain (Quarry et al. 2007). In another study, an attenuated LVS mutant lacking the purine biosynthetic locus *ΔpurMCD* protected against LVS lethal challenge (Pechous et al. 2006); however, a single dose of this vaccine did not demonstrate a protective effect against i.n. or intradermal (i.d.) Schu S4 infection. In contrast, i.n. immunization with the Schu S4 mutant *ΔpurMCD* protected against i.n. challenge with a parental strain; however, the challenge's outcome was influenced by the side effects of immunization (Pechous et al. 2008). Targeted deletion of the genes *guaA* and *guaB* in LVS leads to the attenuation in mice and to the stimulation of a protective immune response to i.p. challenge with a lethal dose of the parental strain (Santiago et al. 2009). However, Schu S4 mutants were not able to protect against the wild-type strain (Santiago et al. 2015).

The capsule synthesis gene (*capB)* encodes an ATP-dependent ligase that is involved in capsule polysaccharide biosynthesis (Larsson et al. 2005). An LVS mutant with a targeted deletion in *capB* is significantly attenuated in mice, and its protective effect against i.n. challenge with a dose 10-fold greater than the LD_{50} of Schu S4 was 100 % (Jia et al. 2010). Jia and colleagues prepared a vaccine regimen from a highly attenuated LVS mutant, *ΔcapB*, which served as a primary immunogen, and *rLm/*iglC, which is an attenuated recombinant *Listeria monocytogenes* expressing *F. tularensis* protein IglC, which was used as a booster. Mice vaccinated with *ΔcapB* and *rLm*/iglC exhibited prolonged survival and mean time to death with a challenge dose 10 times the LD_{50} of aerosolized Schu S4 compared to immunization by *ΔcapB* or parental strain alone. The use of a booster also invoked increased T cell immunity and enhanced IFN-γ secretion (Jia et al. 2013).

Mutations in genes from the *Francisella* pathogenicity island

The *Francisella* pathogenicity island (FPI) is a ~30 kb region of the *Francisella* genome (Nano et al. 2004) that is duplicated in all *F. tularensis* subspecies except *F. novicida* (Larsson et al. 2009). The FPI contains the IglABCD operon, as well as pdpABC, that is essential for virulence. The majority of FPI proteins forms the type VI-like secretion system and is required for phagosomal escape following intracellular replication (Nano et al. 2004; Straskova et al. 2012).

In LVS, deletion of the *iglC* gene, which encodes a 23 kDa intracellular growth locus protein, led to an intracellular macrophage growth defect and attenuation in mice (Golovliov et al. 2003). The same mutation in *F. novicida* provided protection against i.n. infection with the parental strain. These effects were mediated by induction of Th1-type cytokine and antibody response (Pammit et al. 2006). In contrary, *ΔiglC* of Schu S4 origin did not protect mice against aerosol exposure to type A *F. tularensis* (Twine et al. 2005). Cong and colleagues (2009) prepared the Δ*iglB* mutant of *F. novicida* U112, which protected mice against pulmonary challenge with the virulent Schu S4 strain. In a recent study, the *F. novicida* mutant Δ*iglB*, which expresses the D1 domain of FljB flagellin from *Salmonella typhimurium (S. typhimurium)* and is a potent Toll-like receptor 5 (TLR5) agonist, was constructed. Oral vaccination with the construct protected rats against pulmonary challenge with Schu S4 (Cunningham et al. 2014). In addition, the deletion of another FPI gene, *iglH*, in the FSC200 strain established an attenuated phenotype that protected mice against subcutaneous challenge with a fully virulent, homologous wild-type strain (Straskova et al. 2012); thus, these studies underline the potential of FPI genes in the development of live attenuated vaccines.

To survive, *Francisella* reacts to stimuli from its surroundings and, in response, regulates virulence factor production. Several factors were identified to regulate FPI gene expression and include the following: FevR, MglA, PmrA and SspA (Charity et al. 2007; Mohapatra et al. 2007). Deletion of either the *mglA* or *pmrA* genes in *F. novicida* led to the attenuation of virulence in mice (Lauriano et al. 2004; Mohapatra et al. 2007), although only the *ΔpmrA* mutant protected against challenge with the parental strain but not the Schu S4 strain (Mohapatra et al. 2007).

Mutations in various *Francisella* genes

Lipopolysaccharide (LPS), which consists of lipid A, core oligosaccharide and O-polysaccharide (O-PS) are the outer membrane components of a majority of gram-negative bacteria. In case of *Francisella*, these components are designed to support the pathogenic behaviour of bacteria (Okan and Kasper 2013). Due to the unusual tetraacylated structure of lipid A (Vinogradov et al. 2002), *Francisella* is able to evade detection by TLR4 (Dueñas et al. 2006). Attempts to mutate the LVS genes *wbtA* and *wbtI*, which are responsible for biosynthesis of O-PS, led to loss of O-PS and the attenuation of the strain's virulence in mice. Moreover, mutants were able to protect against i.p. challenge with the parental strain (Raynaud et al. 2007; Li et al. 2007). Consistent with previous studies, the deletion of the gene *wzy*, which encodes O-PS polymerase, in LVS caused the strain to be highly attenuated in mice and demonstrated the protective effect of i.n. vaccination against i.n. challenge with the parental strain and virulent strain Schu S4 (Kim et al. 2012).

Acid phosphatases are the enzymes required for hydrolysis of phosphomonoesters, and they are the major virulence factors because of their connection to intracellular survival through repression of the oxidative burst in phagosomes (Reilly et al. 1996). Mohapatra and colleagues (2008) observed that the *F. novicida* quadrupole mutant, which lacks genes *acpA*, *acpB*, *acpC* and *hapA*, showed impaired phosphatase activity, phagosomal escape and intracellular survival in vitro and in mice, and its attenuated phenotype provided protection against *F. novicida* challenge.

Another bacterial protein involved in elimination of reactive oxygen intermediates is iron superoxide dismutase, which is encoded by the gene *sodB*. An LVS mutant, *sodB*, led to a significant attenuation of virulence in mice (Bakshi et al. 2006) and provided greater protection when compared to LVS administration after i.n. challenge with a lethal dose of Schu S4 (Bakshi et al. 2008).

The role of KatG is to catalyze bactericidal molecules, including H_2O_2 and $ONOO^-$. Intracellular growth of LVS or the Schu S4 mutant Δ*katG* was not affected, although mutants showed enhanced susceptibility to H_2O_2 during in vitro analysis. The results from the i.d. immunization study demonstrated attenuation of the LVS mutant Δ*katG* compared to the homologous wild-type strain. However, no differences were detected between the Schu S4 mutant Δ*katG* and corresponding wild-type strain (Lindgren et al. 2007).

The type IV pili (Tfp) are multifunctional, flexible adhesive fibres expressed in many gram-negative bacteria (Chakraborty et al. 2008). Genome analysis of *Francisella* revealed genes required for the expression of Tfp system (Larsson et al. 2005). The pilin PilA is considered a critical virulence factor for the type B strain in the mouse model, as its deletion results in attenuation and an inability of bacteria to spread from the original site of infection (Forslund et al. 2006). Consistent with this finding, the mutation of other Tfp components, such as PilF, PilT, PilE5 and PilE6 in the LVS strain, led to the virulence attenuation (Chakraborty et al. 2008; Ark and Mann 2011). Moreover, Forslund et al. (2010) observed that mice infected with in-frame deletion mutants of the genes *pilA*, *pilC* and *pilQ* in Schu S4 strain experienced a moderately delayed time to death.

In gram-negative bacteria, the formation of disulphide bonds in many proteins (including virulence factors) depends on the DsbA protein (Senitkova et al. 2011). A mutant lacking the gene *FTT_1103*, which encodes a *dsbA* homologue in Schu S4, was unable to escape phagosomes. The strain was attenuated in mice and showed a protective effect against i.n. Schu S4 challenge in BALB/c or C57BL/6 mice (Qin et al. 2009). Similar results were obtained for BALB/c mice infected with the *ΔdsbA* mutant on the FSC200 background (Straskova et al. 2015).

Intradermal immunization with the deletion mutant of chaperone ClpB in Schu S4 protected BALB/c mice against respiratory challenge with a homologous wild-type strain (Twine et al. 2012). Moreover, Golovliov et al. (2013) observed that a Schu S4 mutant exhibited an enhanced protective effect when compared to a mutant in *F. tularensis* subsp. *holarctica* FSC200 (FSC200).

During the *Francisella* intracellular life cycle, the expression of *FTT_1676* and *FTT_0369c* genes is upregulated (Wehrly et al. 2009). The genes encode a glycosylated membrane lipoprotein (Balonova et al. 2012) and the Sel1-family tetratricopeptide repeat-containing protein, respectively. Inactivation of both genes led to attenuation in mice (Wehrly et al. 2009). Rockx-Brouwer et al. found that i.d. inoculation with a low concentration of both mutants were protective against i.n. or i.d. challenge with Schu S4. However, the degree of protection correlated with the replication ability of mutants in the host (Rockx-Brouwer et al. 2012). In a recent study, *F. novicida* lacking an orthologue of *FTT_1676*, transposon mutant *FTN_0109*, displayed impaired intracellular growth. Authors observed a complete protective effect against pulmonary infection with 10 LD_{50} of LVS in BALB/c mice, whereas intratracheal challenge with 25 LD_{50} of Schu S4 provided partial protection of Fisher 344 rats against the same dose of LVS (Cunningham et al. 2015).

Subunit vaccines

Subunit vaccines are considered a safe vaccine because of their composition, which consists of synthesized or isolated microbial antigens. In the case of *Francisella*, there are several bacterial structures that are considered potential subunit vaccines. One bacterial structure is *Francisella* LPS, which was able to induce some degree of protective immune response. Intradermal treatment of mice with LPS isolated from LVS provided protection against lethal challenge with a homologous strain (Dreisbach et al. 2000), but not against Schu S4 (Fulop et al. 2001). LPS purified from Schu S4 was able to extend the time to death in mice, but it was not able to protect against challenge with the parental strain (Prior et al. 2003). The failure of LPS to evoke a fully protective immune response probably results from its inability to stimulate robust cell-mediated immunity. In theory, the poor protection ability of LPS may be improved by adjuvant systems that induce T cell immunity. Richard et al. explored the immunogenic properties of synthetic nanoparticles prepared from catanionic surfactant vesicles that were activated by the incorporation of *Francisella* components. Adjuvant-associated LPS from LVS was used as a vaccine. Treated mice were protected against i.p. challenge with LVS yet remained vulnerable to i.n. infection with Schu S4. Authors enhanced effectiveness by incorporating components from LVS or Schu S4 whole bacterial lysates. However, they reached only partial protection against i.n. challenge with Schu S4 (Richard et al. 2014).

The weak proinflammatory nature of LPS turned our attention to *Francisella* immunogenic proteins. The partial protective effect against lethal respiratory challenge with LVS in mice was induced by *Francisella* heat shock protein DnaK and surface lipoprotein Tul4 with co-administration of GPI-0100 i.n. as an adjuvant (Ashtekar et al. 2012). Recently, Banik et al. prepared a multivalent subunit vaccine by using tobacco mosaic virus as delivery system in combination with *Francisella* proteins, Tul4, DnaK and OmpA. Treated mice were protected against lethal LVS infection (Banik et al. 2015). Tul4 served as a basis for a subunit vaccine constructed from a replication-incompetent adenovirus carrying a codon-optimized gene for its expression, Ad-opt/Tul4. As a result, 60 % of mice were protected against i.p. infection with LVS following an i.m. immunization with a construct and two boosters (Kaur et al. 2012). In another report, authors vaccinated using a construct from an attenuated *Δasd Δcya Δcrp S. typhimurium* mutant carrying Tul4. The construct provided partial protection against intravenous challenge with LVS (Sjöstedt et al. 1992b). Similar results were obtained by Golovliov et al. (1995) who tested Tul4 in combination with immunostimulating complexes.

Jia et al. used an approach which employed attenuated *rLm* as a delivery vehicle that stably expressed various *Francisella* proteins, including AcpA, Bfr, DnaK, GroEL, KatG, Pld or IglC. However, only i.d. immunization with IglC-producing *rLm* evoked sufficient protection against i.n. lethal challenge with LVS or aerosolized Schu S4 (Jia et al. 2009).

Another candidate for a potential subunit vaccine, outer membrane protein A (FopA), was utilized as a recombinant protein incorporated into liposomes. Immunized mice showed a specific antibody response, and they were protected against a lethal i.n. and i.d. challenge with LVS, but not with the type A strain. Passively transferred FopA-immune serum to the naive mice protected against LVS infection (Hickey et al. 2011).

Conclusions

A tularemia vaccine has to fulfil various criteria; it must be safe and should be able to induce complete long-lasting

protective immunity in individuals of all ages and with diverse levels of immunocompetence. The vaccine should protect against respiratory tularemia invoked not only by the most virulent type A strain Schu S4 but also by other less virulent strains. Despite intensive research in this area, there are still serious hurdles that impede the significant progress in tularemia vaccine development. Currently, LVS strain represent the most extensively studied vaccine candidate; however, as it was already mentioned, it does not provide sufficient protection against respiratory infection with *Francisella* type A strains and the molecular basis of its attenuation has not been clarified, as well. On the other side, a plethora of new promising candidates for live attenuated vaccines with defined gene deletion and good protective efficacy against type A strains have been prepared. Nevertheless, their experimental and clinical testing is in its infancy. Additionally, they usually exhibit high variability in their protective effects that is associated with the selection of the vaccination strain, dose and route of administration. The selection of proper animal model represents another weak point in a vaccine development. Mice are generally used for vaccination studies, but they are more sensitive to primary pulmonary infection with *Francisella tularensis* than humans; therefore, their usage for evaluation of basic mechanisms of *Francisella* pathogenesis and immune response to this microbe is not sufficient. It is necessary to combine several animal models in order to confirm the potential benefit of experimental vaccine for humans. Last but not the least, the mechanism of vaccine-elicited immune response has not been elucidated in a sufficient way up to now. This knowledge is a prerequisite for the identification of reliable correlates of post-vaccination protection.

Although live attenuated vaccines show promising protective effects, current trends in prophylaxis development, due to safety reasons, favour subunit vaccines rather than the live attenuated strains. Because *Francisella* is an intracellular pathogen, a *Francisella* subunit vaccine needs to induce cell-based response. However, the identification of T cell specific epitopes is not trivial. One of the most promising approaches is whole genome immunoinformatic analysis, which detects immunogenic *Francisella* peptides that bind to MHCI (Rotem et al. 2014; Zvi et al. 2011). Alternatively, a protein array-based approach can identify epitopes from MHCII complexes from various serological targets (Valentino et al. 2011). It is expected that these new “omics” approaches can provide novel peptides epitopes for the development of the effective subunit vaccines.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

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