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Review article

Tularemia vaccine: past, present and future

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Abstract

Francisella tularensis is a Gram negative intracellular pathogen that causes the highly debilitating or fatal disease tularemia. *F. tularensis* can infect a wide range of animals and can be transmitted to humans in a variety of ways, the most common being by the bite of an infected insect or arthropod vector. The attenuated *F. tularensis* live vaccine strain (LVS) has been used previously under investigational new drug status to vaccinate at-risk individuals. However the history of the strain and lack of knowledge regarding the basis of attenuation has so far prevented its licensing. Therefore the focus of current research is on producing a new vaccine against tularemia that would be suitable for licensing.

Francisella tularensis

Francisella tularensis is a small Gram negative bacterium that is able to infect a wide range of animal species. It was originally isolated in 1911 from ground squirrels in Tulare County, California (McCoy and Chapin 1912), but it is now known to be endemic across the Northern hemisphere. There are two species in the *Francisella* genus based on 16S rDNA sequencing and fatty acid composition; *F. tularensis* and *Francisella philomiragia* (Hollis et al. 1989). There are currently four recognised subspecies of *F. tularensis* (Table 1). Microarray analysis revealed limited genetic variation within *F. tularensis*, but it was possible to differentiate subspecies *tularensis* and *holarctica* (Broekhuijsen et al. 2003), the two most commonly isolated subspecies. *F. tularensis* subspecies *tularensis* is the most virulent of the four subspecies and is found primarily in North America, whereas *F. tularensis* subspecies *holarctica* is less virulent and is found mainly in Europe and Asia. *F. tularensis* subspecies *mediasiatica* is isolated in central Asia and *F. tularensis* subspecies *novicida* primarily in North America. However, recently a *novicida*-like organism was isolated in Australia indicating a wider range for *Francisella* than had been previously thought. (Whipp 2003). Subspecies *mediasiatica* and *novicida* rarely cause disease in humans. The highly virulent *F. tularensis* subspecies *tularensis* strains have infectious doses for humans of less than 10 cfu (Table 1), making this one of the most highly infectious bacterial pathogens known.

Tularemia has been isolated from several parts of the Northern Hemisphere but has rarely been found in the Southern Hemisphere. The disease has been regularly reported in the US, Sweden, Finland, Czech Republic, Slovakia, Russia, Kazakhstan, Uzbekistan and Japan. Typical average rates of infection in the US and Sweden are around 125 and 90 cases per year respectively. *F. tularensis* is probably maintained in the environment by mammals such as rabbits, hares and rodents,

Table 1. Geographical distribution and virulence of *F. tularensis* subspecies.

Subspecies	Primary geographic distribution	Human LD50 (cfu)[a]	Mice LD50 (cfu)[a]
tularensis	North America	< 10 Gurycova (1998)	< 10 Eigelsbach and McGann (1984)
holarctica	Europe, former Soviet Union, Asia, Japan and North America.	$< 10^3$ Eigelsbach and McGann (1984)	< 1 Eigelsbach and McGann (1984)
mediasiatica	Central Asia	NR	NR
novicida	North America and recently Australia	$> 10^3$ Eigelsbach and McGann (1984)	$< 10^3$ Eigelsbach and McGann (1984)

[a] All doses were given subcutaneously.
NR – Not reported.

although its environmental niche is not known. Hunters, walkers, farmers and veterinarians in endemic zoonotic areas are at the greatest risk of contracting tularemia due to contact with infected wild animals. Transmission to humans usually results from the bite of an insect or arthropod vector, such as biting flies, ticks and mosquitos, that has recently fed on an infected animal. This results in ulceroglandular tularemia, which has a mortality rate of less than 3 % without treatment (Evans et al. 1985). Infection can also occur through inhalation of infectious aerosols and following ingestion of contaminated food and water. The symptoms and severity of this disease vary depending on the route of transmission, the infecting dose and the *Francisella* subspecies involved. Pneumonic tularemia and typhoidal tularemia are the most severe, with mortality rates of 30–60% (Gill and Cunha 1997). However, even in non-fatal cases of tularemia, the infection is severely debilitating for extensive periods.

The live vaccine strain

Live attenuated strains were developed prior to the Second World War in the former Soviet Union. This was achieved by either repeatedly sub-culturing a virulent strain of *F. tularensis* subspecies *holarctica* on media containing antiserum or by drying the organisms (Khatenever 1943). Several strains were identified as attenuated including strains 15 and 155, which were transferred from the Institute of Epidemiology and Microbiology (Gamaleia Institute), Russia, to the US Army Medical Research Institute of Infectious Diseases (Tigertt 1962). From these a suitably attenuated strain was isolated, tested for safety and efficacy and subsequently designated *F. tularensis* Live Vaccine Strain (LVS) (Eigelsbach and Downs 1961).

Although various routes of delivery have been evaluated, including oral (Hornick et al. 1966) and aerogenic (Hornick and Eigelsbach 1966) immunization, the LVS vaccine is routinely delivered by scarification. Retrospective studies on the efficacy of the LVS vaccine based on laboratory acquired infections have shown that it affords good, but not complete, protection against typhoidal tularemia, leading to a dramatic decrease in cases. However the incidence of ulceroglandular tularemia is not reduced in vaccinated individuals, although there appears to be a reduction in the severity of the clinical symptoms (Sandstrom 1994). Previous publications have reviewed the human immune response following infection with virulent *F. tularensis* or vaccination with LVS (Tarnvik 1989; Sandstrom 1994), and the response to LVS infection in mice (Elkins et al. 2003). Intra-dermal inoculation with 10^5 cfu LVS was able to protect mice against subsequent intra-dermal challenge with over 50 LD_{50} of a fully virulent subspecies *tularensis* strain (Shen et al. 2004). However, the immunised mice remained susceptible to challenge by the aerosol route.

The LVS vaccine was assigned Investigational New Drug status by the US Food and Drug Administration (FDA) in the early 1960s. Thus, LVS has only been used to vaccinate at-risk personnel. However, the LVS vaccine remains unlicensed due to several significant problems with the vaccine. The first drawback with the strain is that the basis of attenuation and protection is not known. Secondly, the LVS strain retains virulence for mice. The median lethal dose varies from 10^7 cfu when delivered subcutaneously to less than

10 cfu when given intraperitoneally (Ellis et al. 2002). Also, when cultured, two phenotypic variants occur: one variant is immunogenic and protective in the mouse whereas the other variant is not (Eigelsbach and Downs 1961). The phase variation was shown to be due to alteration in both O-antigen and lipid A (Cowley et al. 1996). Therefore, either further work needs to be undertaken in order to license the LVS vaccine, or a new vaccine needs to be developed.

Approaches to developing a new vaccine

Early vaccine studies evaluated the efficacy of whole killed cells as a crude tularemia vaccine (Coriell et al. 1948; Kadull et al. 1950). Studies in both humans and non-human primates demonstrated a low level of protection, indicating that a sub-unit approach may be feasible if protective antigens could be identified. In addition, transfer of immune sera from either LVS immunised individuals or convalescent cases could also be used to treat tularemia (Foshay et al. 1942; Foshay 1946). However, the effectiveness of the LVS vaccine, coupled with problems with reactogenicity of whole cell vaccines (Foshay 1950), meant that for a long time little effort was expended on identifying suitable antigens for a sub-unit vaccine.

The only antigen to have been shown to induce a protective immune response against *F. tularensis* is lipopolysaccharide (LPS). LPS is probably the antigen responsible for the protection observed with the whole cell vaccines. However, protection is only observed against *holarctica* strains, such as LVS and a virulent strain 108 (Fulop et al. 1995; Fulop et al. 2001; Conlan 2002): protection against highly virulent *tularensis* strains, however, appears to require a cellular immune response (Tarnvik 1989). Thus LPS would not be a suitable candidate for a sub-unit vaccine on its own. Therefore there has been a search for proteins expressed by *F. tularensis* to enhance the protection induced by a sub-unit vaccine. Heat shock proteins (Hsps) have attracted significant attention as protective antigens against a range of diseases caused by bacterial pathogens. Hsp60 has been shown to be up-regulated by strain LVS under conditions designed to mimic those inside macrophages (Ericsson et al. 1994). Mice immunized with purified Hsp60 isolated from *F. tularensis* showed some protection against a subsequent challenge with subspecies *holarctica* strains LVS and HN63. However, protection appeared to be due to trace amounts of LPS, which were too low to be detected by using the *Limulus* amoebocyte lysate assay (Hartley et al. 2004).

Ideally, the antigens would stimulate both cellular and antibody responses. Several proteins have been identified which are able to induce the proliferation of T cells isolated from LVS vacinees or convalescent individuals (Sandstrom et al. 1987; Surcel et al. 1989; Sjostedt et al. 1990). The role of these proteins in infection is not known. Of the six proteins identified, one polypeptide of 17 kDa was evaluated as a sub-unit in the mouse. Although it was able to induce an immune response, it was not able to induce protection in the murine model of tularemia (Sjostedt et al. 1992). The FopA protein was also evaluated after it was found to be recognised by sera taken from convalescent tularemia patients (Bevanger et al. 1989). Again, this protein was immunogenic but not protective in the mouse model (Fulop, Manchee, and Titball 1995). It may be that in order to stimulate a suitable immune response, a protein antigen would have to be delivered with an appropriate adjuvant or using a vector system that would allow induction of a cellular immune response, for example using a DNA vaccine.

The LVS vaccine has shown that an attenuated mutant can induce a protective immune response against virulent strains of *F. tularensis*. Defined allelic replacement mutants in a variety of other bacterial species have been used to produce vaccine strains capable of limited replication in the host. They are thus safe and highly immunogenic. In many cases, the genes targeted are in biosynthetic pathways, such as purine or aromatic amino acid biosynthetic pathways. The genes for these pathways are all present in *F. tularensis*, and the organism can grow in media lacking purines or tyrosine respectively, showing the pathways to be fully functional (Karlsson et al. 2000). However, the main problem associated with developing such defined attenuated strains has been the lack of genetic tools to manipulate the organism. However, a method for producing defined isogenic allelic replacement mutants has been published recently (Lauriano et al. 2003), opening the way to the production of attenuated vaccines.

The identification of both potential sub-units and attenuation targets will be greatly helped by

the availability of genome sequence data. A virulent subspecies *tularensis* strain, SchuS4, and the LVS strain have been sequenced recently. These genomes are available at http://artedi.ebc.uu.se/Projects/*Francisella* and http://bbrp.llnl.gov/bbrp/html/microbe.html respectively. Analysis of preliminary sequence data failed to identify traditional virulence factors, such as toxins, which would be good targets for vaccine development (Karlsson et al. 2000; Prior et al. 2001). Searching the genome data did, however, lead to the identification of type IV pili (Gil et al. 2004). These are adhesins with roles in adhesion, twitching motility and biofilm formation in a range of pathogens. However, their role in *F. tularensis* is not known: they may be important in virulence or in the environment.

Conclusions

F. tularensis subspecies *tularensis* is one of the most infectious bacterial pathogens, with a high associated mortality rate. For these reasons, the bacterium was developed as a weapon during the 20th Century by several nations. Due the possibility that such a weapon may be used by the unscrupulous, and also to protect at-risk personnel in endemic areas, an effective vaccine is required. The LVS vaccine, although unlicensed, shows such a vaccine should be achievable, whether by developing a defined attenuated strain to replace LVS or by identifying a protective sub-unit.

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