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SCIENCE DIRECT®
Microbes and Infection 8 (2006) 561–567
Microbes and Infection
www.elsevier.com/locate/micinf

Review

Intracellular survival mechanisms of *Francisella tularensis*, a stealth pathogen

Anders Sjöstedt *

Department of Clinical Microbiology, Clinical Bacteriology, Umeå University, 90185 Umeå, Sweden

Available online 15 September 2005

Abstract

Research on the highly virulent and contagious, facultative intracellular bacterium *Francisella tularensis* has come into the limelight recently, but still little is known regarding its virulence mechanisms. This review summarizes recent studies on its intramacrophage survival mechanisms, some of which appear to be novel.

Keywords: Francisella tularensis; Intracellular survival; Virulence mechanisms

1. Introduction: the bacterium *Francisella tularensis* and the disease tularemia

F. tularensis is a highly virulent and contagious, facultative intracellular bacterium. It causes the zoonotic disease tularemia in a large number of mammals. Rabbits, hares and small rodents play an important role in its natural life cycle and through intermediate vectors such as flies, mosquitoes, and ticks, it infects mammals [1]. Several pieces of evidence indicate that *F. tularensis* may have a yet not identified reservoir in nature separate from mammals and arthropods. Amoebae have been suggested to be one potential reservoir. There are four subspecies of *F. tularensis* subsp. *tularensis* and *holarctica*, subsp. *mediasiatica*, and subsp. *novicida* [2]. The former two subspecies are clinically important. Subspecies *tularensis* is confined to North America, whereas subspecies *holarctica* is found in many countries of the Northern Hemisphere. Subspecies *novicida* has a strong association with water but is also a rare, opportunistic human pathogen [1]. Also outbreaks caused by strains of subsp. *holarctica* show a strong predilection for lakes and rivers. Thus, the natural reservoir for several of the subspecies seems to be related to water. Strains of subsp. *tularensis* and *holarctica* are contagious since the infectious dose even in humans is very low, at most 10 CFU, and they can infect via several routes and give rise to various clinical forms such as respiratory, ulceroglandular, or oropharyngeal tularemia [1]. There is, however, a distinct subspecies-dependent difference in virulence insofar as strains of subsp. *tularensis* cause a very aggressive form of human disease with a mortality as high as 30% if untreated, whereas *holarctica* strains give rise to a non-fatal disease [1].

Several attenuated *F. tularensis* strains were developed in the 1950's and the most successful candidate was the so called live vaccine strain, *F. tularensis* LVS, which has been given to tens of thousands of individuals and affords good albeit not complete protection against laboratory-acquired forms of tularemia (reviewed in [3]). The strain was originally developed by attenuation of a Russian strain of the subsp. *holarctica* but subsequently transferred to the US and there produced as a vaccine.

2. Little is known regarding virulence mechanisms of *F. tularensis* but there has been some recent progress

At present, essentially nothing is known that explains the high virulence of subsp. *tularensis*, however, the genome sequence of the prototypic strain of the subspecies, SCHU S4 has been published recently [4]. The 1892 kb genome contains 1804 genes. A few notable features were revealed from the genome sequence; a set of genes encoding type IV pili, a type II secretion system, a surface polysaccharide, a putative poly-D-glutamic acid capsule, an iron acquisition systems, and a conspicuously high proportion, > 10%, of genes con-

* Tel.: +46 90 785 1120; fax: +46 90 785 2225.
E-mail address: Anders.Sjostedt@climi.umu.se (A. Sjöstedt).

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doi:10.1016/j.micinf.2005.08.001

Table 1
Summary of genes described as essential for the intracellular replication of *F. tularensis* LVS or subspecies *novicida*

Gene designation	Gene similarity	Comment (reference)
PdpA	No similarity in GenBank	Located in genomic island [8]
PdpD	No similarity in GenBank	Located in genomic island [8]
IglA	No similarity in GenBank	Located in genomic island [12]
IglC	No similarity in GenBank	Located in genomic island [5,7]
MglA	Shows similarity to SspA of *E. coli*	Regulator of genes present in genomic island [5,11]
MglB	Shows similarity to SspB of *E. coli*	Regulator of genes present in genomic island [11]
Glutamine phosphoribosyl-pyrophosphate amidotransferase	Similar to phosphoribosyltransferases of several bacteria	Participates in purine biosynthesis [12]
Alanine racemase	Similar to alanine racemase from *Bacillus cereus*	Involved in peptidoglycan biosynthesis [12]
ClpB protease	Similar to ClpB protein from *Coxiella burnetii*	Heat shock-inducible protein that forms multicomponent protease in *E. coli* [12]

taining insertions/deletions or substitution mutations. The latter is also reflected in a considerable proportion of disrupted metabolic pathways, explaining the fastidious nutritional requirements of the bacterium. Although there is evidence for a secretion system in the genome, no secreted bacterial products have been identified. Moreover, there is no evidence for any exotoxin production by *F. tularensis*.

The genome sequence did not reveal any other obvious explanations for the high virulence of the bacterium. One notable feature is the presence of a duplicated 33.9 kb region containing 25 genes lacking homologs in other characterized bacterial species. A variable GC content, between 26.6% and 31.2% vs. 33.2% for the whole genome, and lack of bilateral flanking elements makes the origin of the duplicated region unclear. There is accumulating evidence that genes of this genomic island are essential for intracellular survival even for low virulent strains of *F. tularensis*, such as *F. tularensis* LVS and strains of subsp. *novicida* [5–8]. When genes of the region have been inactivated, for example the *iglC* gene, the mutants do not escape from the phagosome and do not multiply intracellularly as the wild type strains do [9,10]. Besides the *igl* genes, mutants of subspecies *novicida* defective in expression of the *pdpD* and *pdpA* genes, both of which are located in the genomic island, are markedly attenuated in their replication in mouse bone marrow-derived macrophages (BMM) [8]. The phenotypes of the mutants of the genome island have summarized in Table 1.

Many of the genes of the genomic island appear to be regulated by a global regulator, MglA [6]. MglA shows similarity to the SspA protein of *Escherichia coli*, which functions as transcriptional regulator during nutritional stress [11]. Both MglA and MglB mutants of *F. tularensis* subsp. *novicida* are defective for intracellular growth in macrophages [11].

The genes of the region do not explain the high virulence of subspecies *tularensis* or *holarctica* but rather appear to be essential for *F. tularensis* to survive intracellularly. It should be noted that the mechanisms executed by the *igl* operon and regulated by MglA are critical in other experimental models as well, since the Δ*iglC* and Δ*mglA* strains of subsp. *novicida* are markedly attenuated in an amoeba model [6] and the Δ*iglC* mutant of *F. tularensis* LVS is avirulent in mice [7]. A few other genes, summarized in Table 1, have been identified that contribute to intracellular macrophage growth since inactivation of *F. tularensis* genes encoding homologues of glutamine phosphoribosyl-pyrophosphate amidotransferase, alanine racemase, and the heat shock-inducible ClpB protease resulted in attenuated phenotypes in vitro [12]. Altogether, the genome information suggests that virulence mechanisms different from those described in other intracellular bacteria are operative in *F. tularensis*.

3. *F. tularensis*—a facultative intracellular bacterium

F. tularensis is a bacterium capable of infecting and multiplying in a wide variety of phagocytic cells such as human, rat, rabbit, guinea pig, and mouse monocytic cells as well as non-phagocytic cells such as hepatic cells, fibroblasts, tick epithelial cells, endothelial cells, and HeLa cells (reviewed in [13]). There is virtually no available data on the receptors involved in its uptake and if the bacterium engages specific TLRs.

3.1. Mechanisms controlling the F. tularensis *infection in murine macrophages*

Most modern studies on *F. tularensis* have employed various forms of monocytic cells of human or mouse origin. Resident or elicited macrophages from mice, rats and guinea pigs, bone marrow-derived mouse macrophages, mouse macrophages cell lines, such as J774, human peripheral blood mononuclear cells support the growth of *F. tularensis* [14–19]. Generally, virulent strains and LVS proliferate efficiently intracellularly for 24–48 h in non-activated cells. Notably, bacterial replication does not start until 6–12 h after invasion [20]. IFN-γ has been shown to be crucial to activate the macrophages and execute control of the infection [14,15,18,19,21,22]. In most cell types, the IFN-γ-mediated effect is partially reversed in the absence of iNOS, by NO inhibitors, or by neutralizing anti-TNF antibodies [21,23], the exception being murine alveolar macrophages [19]. In the latter case, there was no effect of inhibitors of NO or of reactive oxygen metabolites. In a recent study, employing mouse peritoneal exudate cells (PEC), it was observed that iNOS-gene-deficient PEC showed compromised killing of LVS whereas