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Nutritional virulence of *Francisella tularensis*

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One of the most fundamental aspects of infectious diseases is microbial acquisition of nutrients *in vivo*, which impacts virulence and antibiotic treatment. Therefore, it is not surprising that part of the innate host defense against microbial infection is to limit access of various invading pathogens to sources of host nutrients (Abu Kwaik and Bumann, 2013; Eisenreich et al., 2013). Despite this host nutritional restriction, there has been a long held presumption that the host cell cytosol is a rich haven of plentiful nutrients sufficient to power proliferation of various intracellular pathogens. However, recent studies on the two intravacuolar pathogens *Anaplasma phagocytophilum* (Niu et al., 2012), *Legionella pneumophila* (Price et al., 2011) and the cytosolic pathogen *Francisella tularensis* (Steele et al., 2013) have clearly shown that the levels of amino acids in the host cell cytosol are not sufficient to meet the tremendous demands for carbon, energy, and nitrogen to power the robust intracellular proliferation of these pathogens (Abu Kwaik and Bumann, 2013). Therefore, these intracellular pathogens have evolved with efficient strategies to boost the levels of host amino acids to meet their demands for high levels of carbon and energy sources (Abu Kwaik and Bumann, 2013). There is an emerging paradigm of specific microbial strategies that directly trigger the host cell to boost the cellular levels of essential microbial nutrients, and this paradigm has been designated as nutritional virulence strategies (Abu Kwaik and Bumann, 2013). This opinion article is focused on nutritional virulence of *F. tularensis*.

Upon entry into mammalian and arthropod-derived cells, the vacuole harboring *F. tularensis* matures into an acidified late endosome-like phagosome followed by rapid bacterial egress into the cytosol within 30 min of bacterial entry (Akimana and Kwaik, 2011; Asare and Abu Kwaik, 2011). Amino acids are major sources of carbon and energy for *F. tularensis* but the levels of amino acids in the host cell cytosol are not sufficient to meet the pathogen metabolic needs for the robust intracellular proliferation. Although the mammalian cell cytosol is presumed to have plentiful levels of amino acids, the cells are auxotrophic for His, Lys, Met, Phe, Trp, Leu, Ile,Val, and Thr, while Cys is considered semi-essential and is the most limiting amino acid in mammals (Price et al., 2013). *F. tularensis* is auxotrophic for six amino acids that include His, placeLys, Met, Cys, Arg, and Tyr. Therefore, mammalian cells have limited capacity to provide 4 of the 6 amino acids essential for *F. tularensis*, and this synchronization in amino acid auxotrophy could have played a factor in nutritional and metabolic evolution as well as adaptation of *F. tularensis* to the intracellular environment of mammalian cells (Price et al., 2013). Similar to *L. pneumophila, F. tularensis* is auxotrophic for Cys and requires high levels of this amino acids, which is metabolized into pyruvate that feeds the TCA cycle as the major metabolic pathway for generation of energy and some amino acids. To boost the cellular levels of Cys in the host cell cytosol, *F. tularensis* exploits host glutathione (GSH), which is a non-ribosomal tri-peptide (L-γ-L-glutamyl-L-Cysteinyl-glycine) and is the most abundant source of Cys in the host cytosol (Alkhuder et al., 2009; Meibom and Charbit, 2010). The γ-glutamyl transpeptidase (Ggt) enzyme of *F. tularensis* cleaves GSH to liberate Cys (Alkhuder et al., 2009; Meibom and Charbit, 2010). The *ggt* mutant of *F. tularensis* exhibits a severe intracellular growth defect but is rescued upon supplementation of Cys (Alkhuder et al., 2009).

In addition to requirements to boost cellular levels of Cys, *F. tularensis* signals the host cell to boost levels of all amino acids by triggering the host macroautophagy degradation machinery, which is required for optimal intracellular bacterial growth (Steele et al., 2013). Inhibition of the host macroautophagy diminishes intracellular growth of *F. tularensis*, but the defect is rescued upon supplementation of excess amino acids or pyruvate (Steele et al., 2013). The macroautophagy degradation pathway triggered by *F. tularensis* is non-canonical, since it is independent of the ATG5 autophagy pathway. Thus, in addition to degradation of the host GSH, an additional nutritional virulence strategy for *F. tularensis* is to trigger a major host degradation machinery to boost the cellular levels of amino acids, and both nutritional virulence strategies are required for intracellular proliferation and manifestation of tularemia.

Availability of excess amino acids in the host cell cytosol is of no benefit to the pathogen if they are not imported by efficient bacterial transporters. The *Francisella* genome encodes numerous amino acids transporters and predicted secondary carriers that include the major facilitator superfamily (MFS), such as several putative orthologous of PhtA, which import threonine (Meibom and Charbit, 2010). A novel amino acid transporter, designated AnsP, belongs to the MFS secondary transporters has been recently characterized in *F. tularensis* (Gesbert et al., 2013). The AnsP MFS transporter imports Asn, which is a non-essential

amino acid for *F. tularensis* or mammalian cells. The AnsP transporter is not required for growth of *F. tularensis in vitro* or for bacterial egress from the vacuole into the cytosol of mammalian macrophages. However, AnsP is essential for proliferation of *F. tularensis* in mammalian macrophages and for manifestation of tularemia in the mouse model (Gesbert et al., 2013). Defect of the *ansP* mutant in intracellular growth is overcome upon supplementation of the cells with Asn or Asn-containing dipeptides, or by a combination of Asp and ammonium (Gesbert et al., 2013). In addition, the *ansP* mutant of *F. tularensis* is attenuated in the mouse model of tularemia but the attenuation is reversed upon injection of Asn. Thus, the non-essential amino acid Asn is important for intracellular proliferation of *F. tularensis* in the macrophage cytosol. It is most likely that there are other amino acids transporters in *F. tularensis* (Gesbert et al., 2013) and many of them would be required to import the respective amino acids needed for intracellular proliferation of *F. tularensis*. Therefore, in addition to microbial nutritional virulence strategies, microbial transporters of nutrients are potential targets for therapy against tularemia (Abu Kwaik, 2013).

It is possible that some intra-vacuolar pathogens may not be able to obtain sufficient amino acids from the host cell due to inefficient import of amino acids across the pathogen-containing vacuolar membrane. Therefore, triggering the host cell to elevate the cellular levels of amino acids would enhance their import across the pathogen-containing vacuolar membrane. However, it is very clear from the studies on *F. tularensis* that resides and proliferate in the host cell cytosol that it requires triggering host autophagic degradation as a source of amino acids. This clearly documents that the host cell cytosol does not have sufficient levels of amino acids to support intracellular proliferation of many intra-vacuolar and cytosolic pathogens. The need to raise the cellular levels of amino acids by some pathogens is likely more crucial for intracellular pathogens with shorter generation times in the host cell that does not have sufficient levels to meet the high metabolic needs of the pathogen for additional sources of carbon and energy. The intra-vacuolar *L. pneumophila* (Price et al., 2011) and the cytosolic *F. tularensis* (Alkhuder et al., 2009; Meibom and Charbit, 2010; Steele et al., 2013) are classic examples for such group of pathogens. It is most likely that many other intracellular pathogens trigger various host degradation machinery, intercept host biosynthetic pathways, or other essential sources of carbon and energy as major sources of amino acids needed for robust build up of bacterial biomass during intracellular replication.

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