Published in final edited form as:

Future Microbiol. 2008 October ; 3: 503–506. doi:10.2217/17460913.3.5.503.

Effective host response to *Francisella tularensis* requires functional mast cells

Thomas J Cremer and

Molecular, Cellular & Developmental Biology Program, Department of Internal Medicine, The Ohio State University, Columbus, OH 43210, USA

Susheela Tridandapani, PhD[†]

Molecular, Cellular & Developmental Biology Program, Department of Internal Medicine, The Ohio State University, Columbus, OH 43210, USA

Division of Pulmonary & Critical Care, Department of Internal Medicine, Room 415, HLRI, 473 West, 12th Ave, The Ohio State University, Columbus, OH 43210, USA, Tel.: +1 614 247 6768; Fax: +1 614 247 8106; tridandapani.2@osu.edu

Abstract

Evaluation of: Ketavarapu JM, Rodriguez AR, Yu J *et al.*: Mast cells inhibit intramacrophage *Francisella tularensis* replication via contact and secreted products including IL-4. *Proc. Natl Acad. Sci. USA* 105(27), 9313–9318 (2008). The intracellular pathogen *Francisella tularensis* is a highly infectious organism that infects cells of the immune system. Mast cells have been known for their role in anaphylaxis, although they are also important for their ability to aid in the defense against pathogens. The report by Ketavarapu *et al.* has demonstrated that mast cells function to limit the replication of *F. tularensis* live vaccine strain within macrophages *in vitro* as well as *in vivo*. It was determined that IL-4 is one secreted mediator of this effect thus highlights a previously unknown mechanism of host defense against *F. tularensis*.

Keywords

Francisella tularensis; IL-4; macrophages; mast cells

The following article will review the recent publication by Ketavarapu *et al.* [1]. *Francisella tularensis* is a Gram-negative intracellular pathogen and is the causative agent of the disease tularemia. Due to the highly infective nature of this pathogen and low dose required for lethality, it has been classified as a category A select agent by the CDC because of the potential use as a biological weapon. *F. tularensis*, subspecies *Tularensis*, known as the ‘type A’ strain is highly pathogenic to humans, while the subspecies *holarctica*, or the ‘type B’ strain, poses a lesser threat of lethality. A live vaccine strain (LVS) derived from the type B strain of *F. tularensis* has been in existence for several decades and is commonly utilized in a mouse model of the host response to this pathogen [2–5].

[†]Author for correspondence. Division of Pulmonary & Critical Care, Department of Internal Medicine, Room 415, HLRI, 473 West, 12th Ave, The Ohio State University, Columbus, OH 43210, USA, Tel.: +1 614 247 6768; Fax: +1 614 247 8106; tridandapani.2@osu.edu.

Financial & competing interests disclosure: The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received, pending or royalties. No writing assistance was utilized in the production of this manuscript.

F. tularensis is found to primarily infect phagocytic cells of the immune system. While initially contained within a phagosome the bacterium has the ability to escape and subsequently replicate within the favorable environment of the cytosol. Thus, studying the interaction of *F. tularensis* in macrophages, monocytes and neutrophils is of interest [6–10]. Additionally, the findings of Ketavarapu *et al.* demonstrate an important and previously unrecognized contribution to host defense by mast cells. Previous studies have shown that the proinflammatory cytokine response is largely dependent on TLR2 and thus is also MyD88-dependent [11–12]. Additionally, subsequent downstream signaling through the MAPK and PI3K/Akt pathway has been shown to be critical to the host defense [13–15]. IFN- γ and TNF- α , among others, have been identified as important secreted molecules that aid in the defense against this pathogen [16–19]. This paper identifies a role for IL-4 in limiting the growth of *F. tularensis* within the host.

The authors of this study examined immune cell infiltration of the lungs and cervical lymph nodes in mice that had been intranasally challenged with *F. tularensis* LVS. It was found that there was an early infiltration of mast cells within the lung at 24 h post-infection, though no infiltration of macrophages, dendritic cells or neutrophils at that same time. To understand the function of these mast cells during *F. tularensis* infection, a co-culture system of bone marrow-derived macrophages and mast cells was devised. Macrophages or mast cells alone were permissive to replication of *F. tularensis* LVS 24 h post-infection; however, co-culture of the two cell types limited replication over the same period of time. While the uptake of bacteria is lower in mast cells than in macrophages, in the co-culture system it was shown that the inhibition of intracellular replication was not due to overall decreased uptake in the co-culture system.

Upon further examination, it was determined that direct contact between mast cells and macrophages was not required for the growth-limiting effect. Additionally, it was found that filtered, conditioned media from infected mast cells was able to limit growth of *F. tularensis* LVS in macrophages, thus indicating that a soluble secreted factor of mast cell origin was responsible. Mast cell-derived IL-4 was found to be a candidate because, while abundantly produced by infected mast cells, it was nearly undetectable in macrophages. In addition, it was found that IL-4 production by mast cells was enhanced in the presence of macrophages, thus demonstrating an important interaction between the two cell types.

Treating macrophages or co-cultures of macrophages and mast cells with recombinant IL-4 limited bacterial survival, although mast cells alone showed no clear differences. Addition of IL-4-neutralizing antibodies was sufficient to reverse the effect. Furthermore, the effect of limited bacterial replication was lost in co-cultures of wild-type macrophages and mast cells from IL-4-deficient mice. In survival studies, a greater percentage of IL-4 receptor-deficient mice were found to succumb to *F. tularensis* LVS challenge than wild-type mice. They also displayed increased bacterial burden in the lung and spleen after 3 days of infection compared with the wild-type.

In order to understand the complete contribution of mast cells, *in vivo* studies were conducted with wild-type or mast cell-deficient mice that were challenged with *F. tularensis* LVS. As expected, the mast cell-deficient mice showed lower rates of survival and increased bacterial load in the lung and spleen when compared with the wild-type. Interestingly, when mast cells derived from wild-type or IL-4-deficient mice were adoptively transferred to mast cell-deficient mice, there was roughly equal protection conferred, which indicates that mast cell protection against *Francisella* is not exclusively mediated through IL-4. However, treatment with disodium cromoglycate to stabilize mast cell membranes led to increased bacterial load within the lung and indicated that secreted factors from mast cells were involved to some degree.

Conclusion & future perspective

Identifying protective mechanisms utilized by the host against *F. tularensis* is of interest for understanding its pathogenesis, as well as for the development of novel therapeutics. In the reviewed study, it was established that IL-4 functions to limit bacterial growth within macrophages and that mast cells contribute to this effect through the secretion of IL-4, as well as through IL-4-independent mechanisms. Finding that mast cells infiltrate the lungs early during *F. tularensis* infection and that they function to promote defense against this pathogen provides insight into the importance of this cell type. It appears that mast cells and IL-4 function to limit *F. tularensis* LVS proliferation within macrophages, although determining whether the effect of limiting intracellular growth also extends to other cell types such as dendritic cells or neutrophils would be of interest. Furthermore, it should be examined if there are differences of effects elicited by the more virulent type A strain of *F. tularensis* in comparison with *F. tularensis* LVS.

Previous *in vivo* studies conducted in mice infected with *F. tularensis* LVS found higher bacterial loads when treated with neutralizing antibodies to IL-4, yet there was no clear effect on survival and thus they did not provide a conclusive explanation as to its function [19]. However, from the data presented in the current study, it appears convincing that IL-4 does function to limit intramacrophage growth from treatment with recombinant IL-4, and this effect is reversed by neutralizing IL-4 *in vitro*. Furthermore, IL-4-deficient mice display decreased survival upon bacterial challenge. Given these results, it can be concluded that IL-4 is favorable for the host in combating *F. tularensis* infection.

While the *in vitro* data show that IL-4 limits bacterial growth, it appears that it is not the exclusive mediator of the protective effect derived from mast cells. The fact that IL-4-deficient mast cells provided equal protection to wild-type when transferred to a mast cell-deficient animal does appear to conflict with the data showing that IL-4 receptor-deficient animals have increased susceptibility to *in vivo* challenge. However, it is likely that additional secreted factors and contact-dependent events may contribute to the effect and that alternative sources of IL-4 may exist in this model [20–22]. Identifying those additional mechanisms would be of interest to determine whether there are indeed multiple mechanisms serving redundant functions. In summary, the report by Ketavarapu *et al.* shows that mast cells perform a critical function in the host defense against *F. tularensis* by limiting intracellular replication, furthering the understanding of mast cell influence on the immune response.

Executive summary

Objectives of this study

- To identify infiltrating immune cells within the lung and cervical lymph nodes after intranasal challenge with *Francisella tularensis*.
- To determine the function of mast cells during the host response to infection.
- To clarify the role of IL-4 in response to *F. tularensis* infection.
- To examine the mechanisms utilized by mast cells to mediate their effects.

Methods

- C57BL/6, BABL/c and WBB6F₁ mice were challenged with *F. tularensis* live vaccine strain (LVS) *in vivo*.
- Flow cytometry was used to monitor immune cell populations in the lung and cervical lymph node.

- Co-cultures of bone marrow-derived mast cells and macrophages were infected with *F. tularensis* LVS and IL-4 levels were measured.
- Colony-forming unit assays were conducted to determine bacterial burden.

IL-4 is secreted by mast cells & limits intramacrophage growth

- Mast cells secrete IL-4 in response to *F. tularensis*.
- Macrophages treated with recombinant IL-4 permit limited growth of *F. tularensis* LVS.
- Neutralizing antibodies reverse the effect of recombinant or co-culture-produced IL-4.

Mast cells limit bacterial load through multiple mechanisms

- Mast cell-deficient mice displayed increased bacterial load and decreased survival.
- Transfer of mast cells from wild-type or IL-4-deficient mice recovered survival.
- Stabilization of mast cell membranes by disodium cromoglycate resulted in a modest increase of bacterial numbers *in vivo*.

Conclusion

- Mast cells limit the growth of *F. tularensis* within macrophages and *in vivo* through multiple mechanisms, including the release of IL-4.

Bibliography

1. Ketavarapu JM, Rodriguez AR, Yu J, et al. Mast cells inhibit intramacrophage *Francisella tularensis* replication via contact and secreted products including IL-4. *Proc Natl Acad Sci USA* 2008;105(27):9313–9318. [PubMed: 18591675]
2. Dennis DT, Inglesby TV, Henderson DA, et al. Tularemia as a biological weapon: medical and public health management. *JAMA* 2001;285:2763–2773. [PubMed: 11386933]
3. Ellis J, Oyston PC, Green M, Titball RW. Tularemia. *Clin Microbiol Rev* 2002;15(4):631–646. [PubMed: 12364373]
4. McLendon MK, Apicella MA, Allen LA. *Francisella tularensis*: taxonomy, genetics, and immunopathogenesis of a potential agent of biowarfare. *Annu Rev Microbiol* 2006;60:167–185. [PubMed: 16704343]
5. Pechous RD, McCarthy TR, Mohapatra NP, et al. *Francisella tularensis* Schu S4 purine auxotroph is highly attenuated in mice but offers limited protection against homologous intranasal challenge. *PLoS One* 2008;3(6):e2487. [PubMed: 18575611]
6. Balagopal A, MacFarlane AS, Mohapatra N, Soni S, Gunn JS, Schlesinger LS. Characterization of the receptor-ligand pathways important for entry and survival of *Francisella tularensis* in human macrophages. *Infect Immun* 2006;74(9):5114–5125. [PubMed: 16926403]
7. Para KV, Butchar JP, Rajaram MV, Cremer TJ, Tridandapani S. The tyrosine kinase Syk promotes phagocytosis of *Francisella* through the activation of ERK. *Mol Immunol* 2008;45(10):3012–3021. [PubMed: 18295889]
8. Gavrilin MA, Bouakl IJ, Knatz NL, et al. Internalization and phagosome escape required for *Francisella* to induce human monocyte IL-1 β processing and release. *Proc Natl Acad Sci USA* 2006;103(1):141–146. [PubMed: 16373510]
9. Henry T, Brotcke A, Weiss DS, Thompson LJ, Monack DM. Type 1 interferon signaling is required for activation of the inflammasome during *Francisella* infection. *J Exp Med* 2007;204(5):987–994. [PubMed: 17452523]

10. Conlan JW, Kuolee R, Shen H, Webb A. Different host defenses are required to protect mice from primary systemic vs pulmonary infection with the facultative intracellular bacterial pathogen, *Francisella tularensis* LVS. *Microb Pathog* 2002;32:127–132. [PubMed: 11855943]
11. Malik M, Bakshi CS, Sahay B, Shah A, Lotz SA, Sellati TJ. Toll-like receptor 2 is required for control of pulmonary infection with *Francisella tularensis*. *Infect Immun* 2006;74(6):3657–3662. [PubMed: 16714598]
12. Collazo CM, Sher A, Meierovics AI, Elkins KL. Myeloid differentiation factor-88 (MyD88) is essential for control of primary *in vivo* *Francisella tularensis* LVS infection, but not for control of intra-macrophage bacterial replication. *Microbes Infect* 2006;8:779–790. [PubMed: 16513388]
13. Telepnev M, Golovliov I, Sjostedt A. *Francisella tularensis* LVS initially activates but subsequently down-regulates intracellular signaling and cytokine secretion in mouse monocytic and human peripheral blood mononuclear cells. *Microb Pathog* 2005;38(5–6):239–247. [PubMed: 15925273]
14. Parsa KV, Ganesan LP, Rajaram MV, et al. Macrophage pro-inflammatory response to *Francisella novicida* infection is regulated by SHIP. *PLoS Pathog* 2006;2(7):e71. [PubMed: 16848641]
15. Rajaram MV, Ganesan LP, Parsa KV, Butchar JP, Gunn JS, Tridandapani S. Akt/Protein kinase B modulates macrophage inflammatory response to *Francisella* infection and confers a survival advantage in mice. *J Immunol* 2006;177(9):6317–6324. [PubMed: 17056562]
16. Anthony LS, Ghadirian E, Nestel FP, Kongshavn PA. The requirement for γ -interferon in resistance of mice to experimental tularemia. *Microb Pathog* 1989;7:421–428. [PubMed: 2516219]
17. Santic M, Molmeret M, Abu Kwaik Y. Modulation of biogenesis of the *Francisella tularensis* subsp. *novicida*-containing phagosome in quiescent human macrophages and its maturation into a phagolysosome upon activation by IFN- γ . *Cell Microbiol* 2005;7(7):957–967. [PubMed: 15953028]
18. Parsa KV, Butchar JP, Rajaram MV, et al. *Francisella* gains a survival advantage within mononuclear phagocytes by suppressing the host IFN γ response. *Mol Immunol* 2008;45(12):3428–3437. [PubMed: 18514317]
19. Leiby DA, Fortier AH, Crawford RM, Schreiber RD, Nacy CA. *In vivo* modulation of the murine immune response to *Francisella tularensis* LVS by administration of anticytokine antibodies. *Infect Immun* 1992;60(1):84–89. [PubMed: 1729199]
20. Malaviya R, Ikeda T, Ross E, Abraham SN. Mast cell modulation of neutrophil influx and bacterial clearance at sites of infection through TNF- α . *Nature* 1996;381(6577):21–22. [PubMed: 8609979]
21. Galli SJ, Nakae S, Tsai M. Mast cells in the development of adaptive immune responses. *Nat Immunol* 2005;6(2):135–142. [PubMed: 15662442]
22. Malaviya R, Abraham SN. Mast cell modulation of immune responses to bacteria. *Immunol Rev* 2001;179:16–24. [PubMed: 11292019]