Francisella tularensis: A Red-blooded Pathogen

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(See the article by Horzempa et al, on pages 51–59.)

Francisella tularensis subspecies *tularensis*, when inhaled is one of the deadliest of all human pathogens; mortality rates of 30%–60% were reported in the pre-antibiotic era [1]. Moreover, the inhaled minimum infectious dose is ≤15 colony forming units (CFU) [2]. On inhalation by mice, subsp *tularensis* suppresses pulmonary immune responses, allowing it to replicate uncontrolled in the lungs before disseminating to lymph nodes, liver, and spleen [3]. The fact that *F. tularensis* elicits few overt signs of pulmonary involvement in humans during the early stages of respiratory infection [2, 4] suggests that it is also immunosuppressive for humans. The systemic infection, rather than the initial pulmonary infection, is thought to be mainly responsible for morbidity and mortality. For these reasons, subsp *tularensis* was exploited as an aerosolizable biologic weapon by the United States, the former USSR, and other countries. The United States reportedly had a 450 kg stockpile of dry-powder *F. tularensis* when it scrapped its bioweapons program in 1973, and it is easy to envisage that the Soviet program produced substantially more, because it continued clandestinely for another 20 years [5]. According to estimates from the Centers for Disease Control and Prevention and the World Health Organization, aerosol release of 50 kg of *F. tularensis* over a large metropolitan area under ideal atmospheric conditions could cause 250,000 casualties, including 19,000 deaths [5]. In nature, subsp *tularensis* exists only in the United States and Canada and causes <100 cases of tularemia per year. Moreover, most cases are caused by systemic inoculation of the pathogen into the skin by various hematophagous insects, including ticks, deer flies, and mosquitoes [6]. The human infectious dose via this entry portal is also on the order of 10 CFU [7] but results in a substantially lower mortality rate (5%) than infection that enters through the lungs. Rabbits, squirrels, and hares, among other animals, are thought to be the reservoirs for vector-borne human tularemia. Recent work has shown, however, that mosquito larvae can acquire *F. tularensis* directly from contaminated water [8], but it is not obvious whether these bacteria survive through metamorphosis.

Except for its weaponization, *F. tularensis* subsp *tularensis* remained little more than a medical curiosity during modern times until the anthrax attacks committed via the US mail in 2001 initiated a massive surge in federal spending on biodefense research. As an indicator, at the time of the anthrax event, I held the only National Institutes of Health grant to study the pathogenesis of subsp *tularensis*. At that time, a few groups in Canada, Sweden, the United Kingdom, and the United States formed the vanguard of *F. tularensis* pathogenesis research in the West. Consequently, progress was relatively slow, especially when compared with research on other facultative intracellular bacteria. Nevertheless, with research in mouse models of infection, much was learned and relearned about *F. tularensis* infection and immunity, including the ability of the pathogen to multiply to large numbers within infected cells without lysing them [9]. It was also demonstrated that macrophages activated by the cytokines interferon-γ and tumor necrosis factor were critical for protective immunity and could be provided by various populations of T cells [10]. In vitro experiments support the idea that cell-mediated immunity is also critical for protection in humans [11].

During the past decade, the National Institutes of Health have fueled an explosion of new tularemia research that has significantly accelerated our understanding of *F. tularensis* pathogenesis. This expanded work has revealed many surprises about this pathogen's interactions with its hosts, perhaps none more surprising than the findings by Horzempa et al [12], reported in the current issue of the *Journal*, that *F. tularensis* is able to parasitize

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erythrocytes. Because *F. tularensis* can replicate to large numbers inside macrophages, these cells had been assumed to be the blood source of the pathogen for insect vectors. Recently, however, others showed that some bacteria can exist extracellularly in the blood of experimental mice, especially when massive septicemia occurs during the terminal stage of lethal infection [13]. Now, Horzempa et al [12] add another potential blood source of the pathogen: erythrocytes. Prompted by the knowledge that *Plasmodium* species, and some other protozoal as well as bacterial pathogens, can invade erythrocytes, Horzempa et al [12] determined whether this property was also expressed by *F. tularensis*. They convincingly showed that a clinical subsp *tularensis* isolate introduced into the lungs of mice can infect their erythrocytes in vivo. They also showed that the same pathogenic strain can invade human erythrocytes in vitro and can persist therein for $\geq$72 h. Given the long lifespan of erythrocytes in vivo, *F. tularensis* could potentially persist in this environment for several months.

Horzempa et al [12] also showed that erythrocytes protected *F. tularensis* from antibiotics. Relapse after inadequate antibiotic treatment is not uncommon with *F. tularensis* infection [14, 15], and the authors suggest that the ability of parasitized erythrocytes to shield the pathogen from antimicrobials might contribute to this phenomenon. They also showed that infected erythrocytes could transmit active infection to naive mice. Because iron concentration is known to influence virulence gene expression in *F. tularensis* [16, 17], the authors speculate that virulence could be modulated by the high hemoglobin content in erythrocytes. Interestingly in this regard, their results showed that mice inoculated intravenously with infected erythrocytes died earlier than mice challenged with free bacteria. Horzempa et al [12] found that replacing normal human serum with heat-inactivated serum in their in vitro assay reduced but did not eliminate the ability of *F. tularensis* to invade erythrocytes. This finding implies that invasion is partly dependent on the erythrocyte complement receptor.

Unlike the nucleated cells that *F. tularensis* invades, erythrocytes do not support pathogen replication. Thus, they are probably not a major contributor to the pathogenesis of mammalian tularemia. On the other hand, infected erythrocytes could serve as an important part of the pathogen reservoir for vector-borne infection. In this regard, although the total number of mouse erythrocytes infected is very small, and each erythrocyte contained only a single bacterium, enough of them to initiate human disease could be ingested from such a reservoir as a blood meal by several insects commonly associated with the dissemination of *F. tularensis* infection. For this purpose, the failure to replicate within erythrocytes could be advantageous, because bacterial replication could damage erythrocytes, leading to their removal from the circulation by the fixed macrophages of the reticuloendothelial system.

Like many research surprises, the finding that *F. tularensis* can invade erythrocytes opens a veritable treasure chest of potential follow-up research. Areas for research include defining the precise mechanism of pathogen uptake by erythrocytes, for example, by identifying the bacterial virulence factors necessary for the invasive process and demonstrating whether they are the same as those involved in invasion of other cell types. In particular, the existence of mutants that are unable to parasitize erythrocytes but are otherwise indistinguishable from the wild type would answer key questions about the importance of this mechanism to pathogenesis and transmission of infection. Many *F. tularensis* mutants have already been generated and could be screened for such a purpose [18]. It would also be interesting to determine whether infected erythrocytes home to the same tissue sites as free bacteria and whether they trigger similar innate immune responses and pathology. However, the heyday of biodefense research spending appears to be waning fast, and it remains to be seen how much this will crimp future research on *F. tularensis* infection and immunity. Regardless, it is highly probable that *F. tularensis* has several more tricks that contribute to its extreme pathogenicity.

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