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Editorial

Editorial: *Yersinia pestis* survives in neutrophils and sends a PS to macrophages: bon appétit!

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The Gram-negative bacteria *Yersinia pestis* is the causative agent of plague, a fatal disease that has killed millions of people in three major pandemics. During the third pandemic, *Y. pestis* spread worldwide and became established as an important zoonosis in new locations, including North America [1]. The most common type of plague in humans is the bubonic form, in which bacteria are transmitted through bites of pathogen-infected fleas. The bacteria also can be transmitted from human to human by inhalation of respiratory droplets, causing pneumonic plague [1]. Pathogenesis of *Y. pestis* has a characteristic biphasic pattern that has been best characterized in the murine pneumonic model of plague. At the initial stages of infection, bacteria replicate at the site of infection with little inflammation. By 48 h postinfection, however, the host innate-immune response results in a highly inflammatory state associated with cytokine production and tissue damage [2].

Y. pestis produces an array of virulence factors to counteract the host immune response. For example, a 70-kb virulence plasmid (pCD1) encodes a type III secretion system and its effector proteins (termed Yops) that can inhibit phagocytosis and suppress proinflammatory cytokine production [3]. *Y. pestis* also produces a capsule composed of a F1 protein that helps to inhibit phagocytosis [1]. Likely as a result of these factors, bacteria are more often found extracellularly within the tissues of an infected host at the later, highly inflammatory stages of infection. However, these virulence factors are maximally expressed only after cultivation at 37°C and not at an ambient temperature (e.g., 26°C) [1]. Therefore, the challenge for *Y. pestis* is to survive and suppress the host immune response during the initial phase of the infection when the bacteria have not been acclimated to the mammalian host temperature of 37°C.

Y. pestis is considered to be a facultative intracellular bacterium, as various in vitro studies have demonstrated that *Y. pestis* can survive and replicate within macrophage phagosomes [2]. Detection of *Y. pestis* in the in vivo host environment at the early stages of infection has proven to be technically challenging. Nevertheless, several in vivo studies have observed *Y. pestis* inside macrophages and polymorphonuclear leukocytes (or neutrophils) [2]. Most recently, Shannon et al. [4] revealed that neutrophils are recruited to the site of infection soon after the initial intradermal inoculation of mice and that *Y. pestis* mainly associates with neutrophils, as well as macrophages. In a murine intranasal infection model, the *Y. pestis* type III secretion system was found to target alveolar macrophages and neutrophils for Yop injection, suggesting close association of *Y. pestis* and these host immune cells [5]. These observations have led to the hypothesis that *Y. pestis* escapes the initial encounter with the components of innate-immune response by surviving within host macrophages, before the bacteria replicate more robustly in the extracellular environment [2].

Although past in vivo studies have consistently indicated that neutrophils are recruited to the initial site of infection and that *Y. pestis* associates with neutrophils and macrophages, the current model [2] has focused on macrophages as a permissive site for bacterial replication during the early stages of infection. This is because previous in vitro studies have suggested that the majority of *Y. pestis* bacteria engulfed by neutrophils is killed, whereas *Y. pestis* can survive and replicate within macrophage phagosomes [2, 6]. However, most of the previous studies examined the survival of *Y. pestis* inside neutrophils only for a short period of time (up to ~6 h) postinfection. Therefore, it has remained unclear whether any of the bacteria ingested by neutrophils can survive and replicate intracellularly for the long term. In the study published in the current *Journal of Leukocyte Biology*, Spinner et al. [7] show that a small portion of *Y. pestis* engulfed by human neutrophils survives and replicates, raising the possibility that neutrophils could be used as a temporary niche or transport vehicle for *Y. pestis*.

Abbreviations: IL-1ra=IL-1R antagonist, PS=phosphatidyl serine

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With the use of live cell confocal imaging, electron microscopy, and CFU assays, Spinner et al. [7] observed the fate of *Y. pestis* KIM strains (with or without the virulence plasmid pCD1) after incubation with human-derived neutrophils. The authors demonstrate that the majority of bacteria is phagocytosed rapidly by neutrophils and subsequently killed, but a small percentage survives and replicates within neutrophils after 22 h of incubation. *Y. pestis* was found inside phagosomes of the neutrophils, and its localization and survival were pCD1-independent. These experiments did not use continuous gentamicin treatment to prevent replication of extracellular bacteria. Consequently, future studies will need to rule out definitively the possibility that replication of *Y. pestis* is occurring after escape from neutrophils. The environment inside neutrophils appears to be less permissive to *Y. pestis* than macrophages, as studies done in macrophages do not show the majority of the bacteria in phagosomes being degraded [2].

The authors next show that after 12 h of incubation, *Y. pestis*-containing neutrophils present PS, a marker of an early apoptosis, on their surfaces. The kinetics of PS presentation does not seem to be altered by *Y. pestis* infection compared with uninfected neutrophils, in contrast to some pathogens that can alter the lifespan of neutrophils upon invasion [8]. This PS presentation allows human macrophages to recognize neutrophils that are undergoing apoptosis and clear them in a process called efferocytosis. Indeed Spinner et al. [7] observed that some of the PS-presenting neutrophils containing *Y. pestis* are taken up by the macrophages by efferocytosis. This provides an alternative route of entry for *Y. pestis* into macrophages, a preferred site of replication (**Fig. 1**).

The efficiency of efferocytosis, as well as the fate of bacteria taken up by macrophages via efferocytosis, is less clear. Live imaging of *Y. pestis*-containing neutrophils inside macrophages suggests that some bacteria survive and replicate after neutrophils are degraded, whereas other bacteria appear to be killed. Careful quantification is needed to determine what percentage of the bacteria can survive after efferocytosis.

Spinner et al. [7] also presents data showing that macrophages incubated with

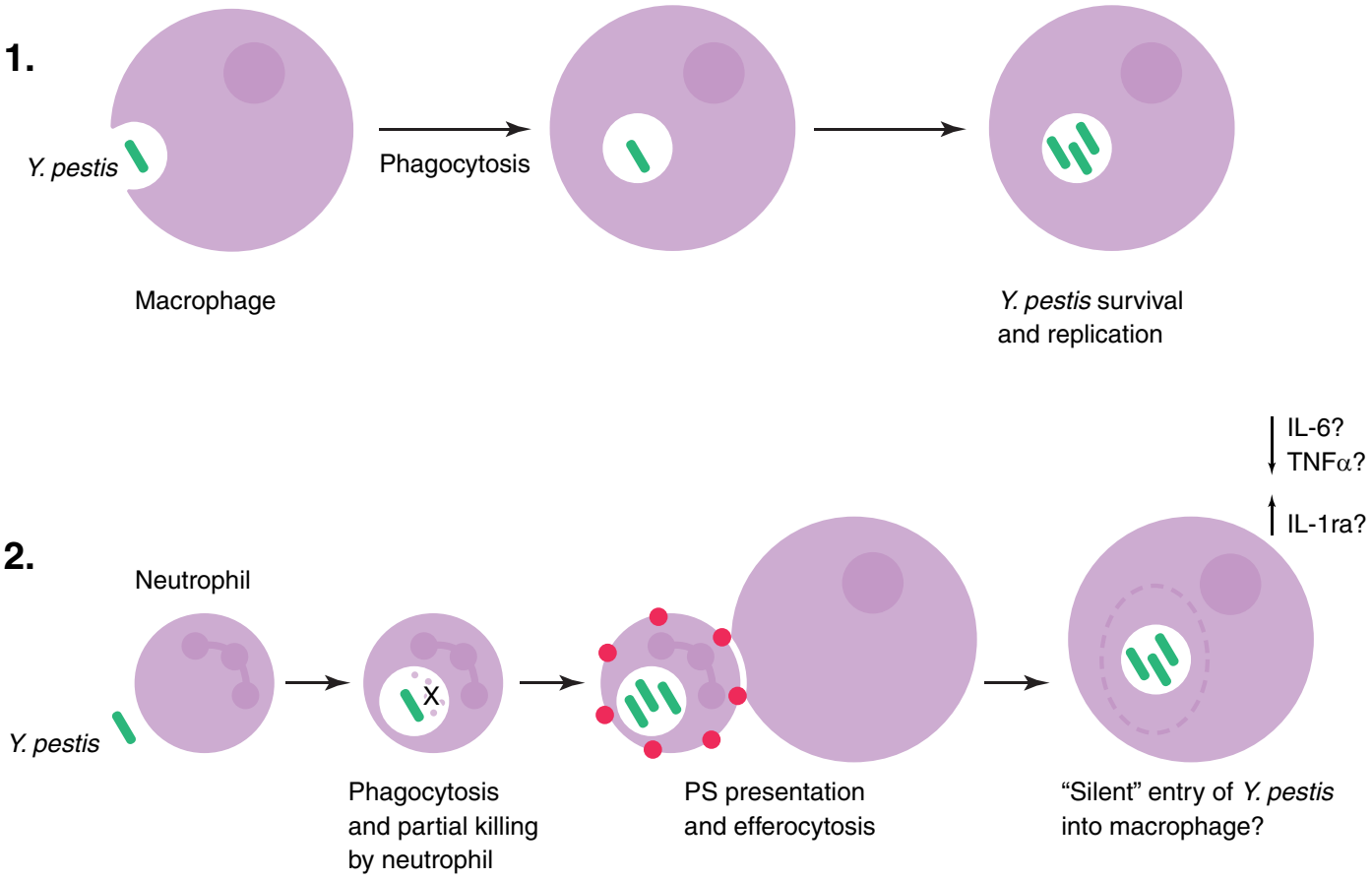

Figure 1. Possible routes of entry for *Y. pestis* into macrophages. 1. In a classical scenario, *Y. pestis* is taken up directly by macrophages, a preferred host cell type, through phagocytosis. Intracellular *Y. pestis* survives and replicates within macrophage phagosomes. 2. In an alternative scenario proposed by Spinner et al. [7], *Y. pestis* is first taken up by neutrophils. The majority of intracellular *Y. pestis* is degraded, but a small percentage survives and replicates. Infected neutrophils present PS (a marker of early apoptosis) on their surfaces and are subsequently ingested by macrophages via efferocytosis. This latter scenario may provide *Y. pestis* with a less inflammatory route of entry into macrophages, resulting in decreased proinflammatory signaling (i.e., decreased IL-6 and TNF-α and increased IL-1ra secretion).

Y. pestis-infected neutrophils secrete decreased levels of proinflammatory cytokines TNF-α and IL-6, while increasing production of IL-1ra, as compared with macrophages infected directly with *Y. pestis*. Based on these results, the authors suggest that entry of *Y. pestis* into macrophages via efferocytosis is less inflammatory than direct entry via phagocytosis. However, caution has to be made in interpreting these data, because as the authors acknowledge, the number of bacteria in contact with macrophages could differ greatly among these conditions. In addition, the increased IL-1ra production could be an indirect consequence of decreased IL-6 secretion in macrophages incubated with *Y. pestis*-containing neutrophils. Taken together, it remains to be seen whether the entry of *Y. pestis* into macrophages via efferocytosis is truly an effective route for the bacteria to survive without activating the host immune cells. Further studies will likely provide additional insights into the biology of the pathogen-neutrophil interaction, as well as the interaction of neutrophils with other host immune cells.

The molecular mechanism by which *Y. pestis* survives inside neutrophils is largely unknown. It appears that some factors required for the survival of *Y. pestis* within macrophages also play an important role in its survival inside neutrophils. For example, a global transcriptional regulator PhoP has been shown to promote bacterial resistance to killing by both macrophages and neutrophils [2, 9]. PhoP promotes intracellular survival by increasing bacterial resistance to antimicrobial peptides through modifications of LPS and by counteracting magnesium limitation in the intraphagosomal environment. However, macrophages and neutrophils differ significantly in the steps leading to phagosomal maturation. Whereas macrophage phagosomes slowly mature into highly acidic phagolysosomes through interactions with components of the endosomal/lysosomal pathway, neutrophil phagosomes seem to fuse with a large number of preformed granules, resulting in a massive oxidative burst and acquisition of various other antimicrobial components [10]. The acidity of the neutrophil phagosome remains close to neutral for a prolonged period [10]. Therefore, it is likely that *Y. pestis* uses intracellular survival strategies unique to neutrophils. *Y. pestis* can prevent phagosomal acidification within murine macrophages, which has been suggested as a strategy for *Y. pestis* to survive inside this cell type [11], but such a mechanism may not be required for survival inside neutrophils. A number of other pathogenic bacteria are known to prevent oxidative bursts, and *Y. pestis* type III secretion system effector Yops can also counteract oxidative bursts in human neutrophils [6]. However, the type III secretion system does not seem to alter the fate of *Y. pestis* within human neutrophils [6].

Based on the findings in this study, Spinner et al. [7] propose the "Trojan horse" hypothesis for *Y. pestis*, where neutrophils serve as a temporary niche for *Y. pestis* and provide an alternative, less inflammatory (or "silent") route of entry into macrophages via efferocytosis (Fig. 1). The hypothesis is attractive, considering that neutrophils are the major cell type to associate with this pathogen and that *Y. pestis* needs to survive and disseminate without causing much initial inflammation. The Trojan horse mechanism has been implicated in the dissemination of other pathogens, such as *Leishimania* [7]. However, it is not known whether these events actually take place during *Y. pestis* infection in vivo.

Whether neutrophils play a critical role in *Y. pestis* dissemination as a Trojan horse is an intriguing question that awaits further investigation. In a recent study, Shannon et al. [4] found that whereas neutrophils are the major cell type to associate with *Y. pestis* at the site of infection, neutrophil depletion does not affect dissemination of the bacteria in a murine intradermal infection model. Pechous et al. [5] also found that neutrophil depletion had no statistically significant effect on bacterial burdens in the lung at 48 h postinfection or on mean time to death in a murine intranasal infection model, although less inflammation was observed in the lungs of neutrophil-depleted mice. These results imply that neutrophils are not essential for dissemination of *Y. pestis*. However, the interpretation of these results is complicated, as neutrophils (and other immune cells) are likely to have roles in protection of the host and dissemination of pathogens. It should also be noted that the results obtained in murine models might not always be applicable to humans, as murine and human neutrophils might have distinct functionalities. Clearly, *Y. pestis* is able to survive within multiple cell types as well as in the extracellular environment and thus, is likely to disseminate using multiple strategies. Defining in vivo contributions of different immune cell types in dissemination as well as defense against *Y. pestis* poses a great technical challenge, but such information should greatly advance our understanding of *Y. pestis* pathogenesis and perhaps vector-borne infectious diseases in general.

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