Y. pestis is the causative agent of plague, a rapidly progressing and frequently lethal flea-borne disease. The bacteria colonize the flea midgut and are transmitted to mammals after forming an infectious biofilm that lodges in the proventriculus of the flea (1). Once deposited in the dermal layer of the mammalian host, the bacteria will migrate to the draining lymph node, establish a replicative niche, and eventually spread systemically, causing multiorgan failure and death of the host. Y. pestis can invade epithelial cells and survive and replicate in macrophages, but tissue damage and disease are primarily caused by its rapid extracellular growth and toxicity to host cells (2, 3).

The infection is believed to progress as an initial anti-inflammatory response mediated at least in part by the type III secretion system (T3SS) (4). When grown at low temperature and in the flea, the T3SS is poorly expressed, requiring 37°C for maximal transcriptional induction. The molecular mechanism and timing of how this transition occurs in vivo have not been well characterized, and it appears likely the initial interactions with host cells would proceed with bacteria vulnerable to immune activation. Furthermore, Y. pestis lipopolysaccharide (LPS) is immunostimulatory at low temperature and must undergo a biosynthetic change at 37°C that provides stealth and attenuation of Toll-like receptor signaling such that inflammation can be controlled. The question is, since these virulence factors are thermally regulated, what happens in the early stage during the transition from stimulatory to less stimulatory life cycles?

Following adherence of bacteria to host immune cells, the type III secretion system delivers effector proteins, collectively known as Yops, to the host cell cytosol. In vivo, Y. pestis preferentially targets phagocytic cells for injection of Yops, thus preventing their activation (5, 6). Y. pestis is nonmotile but invasive due to the activity of an extracellular broad-spectrum protease (plasminogen activator [Pla]) whose cleavage of fibrin and plasminogen enhances adhesion and promotes growth in tissues (7). There is little information available on the mechanism or kinetics of bacterial dissemination from the skin to the lymph node during the early stage of infection, and no surface proteins have yet been described that function as homing receptors which Yersiniae could use to traffic to the lymph node. Prevailing models for bacterial trafficking to the primary lymph node involve intracellular transport via the lymphatic system or extracellular vascular dissemination.

In their article, Shannon et al. (8) visualize host-pathogen interactions shortly after infection to identify possible host cell vehicles that might transport Y. pestis to the lymph node in a murine intradermal model of bubonic plague. Infection of transgenic mice expressing fluorescently labeled neutrophils (Ly5-GFP, where GFP is green fluorescent protein) or dendritic cells (CD11c-YFP, where YFP is yellow fluorescent protein), with Y. pestis strains constitutively expressing dsRed, allowed the investigators to visualize the early stage of infection by intravital microscopy. Their provocative findings suggest that interactions between bacteria and neutrophils dominate the early stage and may contribute to systemic circulation of the infection.

During an inflammatory response initiated by tissue injury as well as recognition of Y. pestis pathogen-associated molecular patterns (PAMPs), neutrophils and monocytes are recruited from the peripheral blood (9). Neutrophils are by far the largest cell population and quickly migrate to the infection, where they are believed to mediate bacterial clearance. Inflammatory monocytes also enter infected tissue from the blood, where they mature to carry out macrophage or dendritic cell functions in host defense, including bacteriocidal activity, tissue repair, and antigen presentation (10). In addition, dendritic cells routinely traffic to the lymph node to display antigen to B and T cells and have previously been shown to
shuttle pathogens from the skin to the lymph node through the lymphatic system or the vasculature. During \textit{Y. pestis} infection, Shannon et al. (8) observed robust and active recruitment of LysM-expressing cells to the infected area, indicating neutrophils and/or inflammatory macrophages are recruited shortly after the initiation of the infection. Furthermore, bacteria appeared to colocalize with these cells and subsequently mobilized and migrated away from the plane of vision. Strikingly, neutrophil recruitment and apparent phagocytosis of bacteria occurred even when the T3SS was present.

In agreement with previous observations, Shannon et al. (8) reported that phagocytosis of T3SS$^{+}$ \textit{Y. pestis} does not activate neutrophils, with no upregulation of the activation marker CD11b (11). The T3SS is believed to be largely inactive when bacteria are in the phagolysosome, and whether this is due to an environment that downregulates expression of the T3SS or because the translocation pore cannot assemble across the phagosomal membrane is not known (12). It is therefore likely that these neutrophils were not activated because of a soluble anti-inflammatory signal induced by the activity of the T3SS on other cells rather than a direct effect of Yop injection by intracellular bacteria. This interpretation is consistent with the lung model, where T3SS$^{+}$ \textit{Y. pestis} establishes an anti-inflammatory state that is permissive for growth of avirulent T3SS$^{-}$ bacteria (4).

\textit{Y. pestis} infects multiple tissues, with continuous bacterial growth at the inoculation site, as well as seeding of primary and secondary immune tissues followed by rapid bacterial growth in these sites. As bacteria continue to grow in the inoculation site and lymph node and as both sites become diseased, neutrophils are recruited from the circulation. Although the data are consistent with a model whereby neutrophils with lowered activation and recruited from the blood also express the LysM promoter and there-


to the primary lymph node may be more limited at a low challenge dose. Although questions remain about the mechanism(s) whereby bacteria transit to the lymph node, it is clear that LysM-expressing cells mobilize following phagocytosis of Gram-negative bacteria. Do intracellular \textit{Y. pestis} bacteria survive in neutrophils and later escape to further the infection? Will these neutrophils reenter circulation and migrate to the primary lymph node? And how does flea saliva influence the response in the dermis? With the further development of the sensitivity of intravital microscopy and the availability of genetically engineered mice expressing cell-specific markers, these questions will soon be answered. This exciting technology has the ability to address fundamental questions of trafficking where tissue architecture influences subsequent host-pathogen interactions that may be critical to the development of disease or immunity and have been missed by standard methods of analyzing homogenized tissues.

**REFERENCES**


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