**EDITORIAL COMMENTARY**

**Burkholderia pseudomallei** Tropism and the Melioidosis Road Map

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(See the major article by Owen et al., on pages 1761–70.)

Melioidosis, which is caused by the soil-dwelling gram-negative bacterium *Burkholderia pseudomallei*, is often called the great mimicker. Disease presentation can range from acute fulminant sepsis to a chronic tuberculosis–like illness [1]. Pneumonia is the most common presentation of melioidosis; however, infection can arise in virtually any organ. It is thought that up to 5% of patients have direct involvement of the central nervous system. Cerebral abscesses, brain stem encephalitis, encephalomyelitis, and meningitis are occasionally reported [2, 3]. In addition, a specific syndrome of meningoencephalitis with flaccid paraparesis or peripheral motor weakness occurs in 5% of cases in northern Australia [2, 3]. Although the majority of cases of melioidosis are considered to result from inoculation through skin scratches, it is postulated that severe weather events, such as tropical storms, are associated with a shift to inhalation of *B. pseudomallei* [4]. Disease severity may well be increased when inhalation rather than percutaneous inoculation is the mode of transmission, increasing fears about the use of *B. pseudomallei* as a potential biological weapon threat [1, 4]. Not surprisingly, increasing numbers of research groups around the world are studying this versatile pathogen. However, the uncertainty regarding the overall contributions of the mode of infection, the role of the natural barrier function of the mucosa and epithelium, the differences in disease presentation, and the question of how *B. pseudomallei* is spread through the body after infection reflect just how much remains to be studied about melioidosis. In this issue of the *Journal*, Owen et al. [5] provide us with new and exciting data about the early stages of melioidosis acquired via the respiratory route.

In their elegant study, the researchers inoculated BALB/c mice intranasally with lux-positive *B. pseudomallei*, which enabled them to monitor live infection by means of bioluminescence imaging. Bioluminescent bacteria, which were claimed to be stable through ~100 generations, were created by transforming *B. pseudomallei* with a plasmid that contained the lux bioluminescence operon from *Photorhabdus luminescens*. Mice were euthanized at ~2, ~24, ~48, and ~72 h after inoculation. Bioluminescence monitoring showed colonization and replication in the nasal cavity as early as 24 h after infection. More specifically, in searches for key areas of replication, the researchers excised the hard tissue of the soft palate to expose the nasal lymphoid tissue on the interior surface, and they found that the infection and replication seen in the nasal mucosa included nasal-associated lymphoid tissue (NALT). The demonstration of replication in NALT suggests a rapid tropism of *B. pseudomallei* for lymphoid tissue. Furthermore, coronal sections of the brain and nasal cavity were obtained and subsequently examined with immunofluorescence microscopy after incubation with olfactory marker proteins and *B. pseudomallei* antisera. This enabled the investigators to further demonstrate infection in both the respiratory epithelium and the olfactory epithelium. Interestingly, this included the associated nerve bundles that constitute monosynaptic pathways to the brain. *B. pseudomallei* was detected in the olfactory sensory axons and the olfactory bulb 48 h after infection. Of note, the olfactory epithelium and the brain were rapidly infected before bacteria were detected in the blood, underlining the hypothesis of Owen et al. [5] that the olfactory epithelium and nerve provide a direct pathway for *B. pseudomallei* to infect the brain, rather than via infection through hematogenous spread. This finding was underscored by additional experiments in which a capsule-deficient mutant of *B. pseudomallei*, known for its inability to survive in blood, was used. Indeed, this mutant invaded the blood very poorly but still robustly infected the nasal cavity and the brain. Taken together, the present data suggest that, after inhalation of *B. pseudomallei*, the olfactory nerve can be...
misused as a direct route into the brain, whereas infection of NALT provides a direct route for dissemination to other organs via the bloodstream and lymphatic system.

The mucosal immune system functions as a first line of physical and immunologic defense against invading pathogens [6]. NALT is an important inductive tissue for the generation of mucosal immunity through the inhalation of antigen in the respiratory tract [6]. The current finding that a pathogen can use NALT as a portal of entry is not new. Group A streptococci and avian influenza A (H5N1) virus are both known to have the ability to infect and replicate in NALT [7, 8]. Furthermore, as indicated by the authors, infection of the brain via olfactory epithelium has been previously described in association with, among other agents, herpes simplex virus and Streptococcus pneumoniae [9, 10]. However, in all cases, the exact mechanism involved remains to be elucidated. It has been suggested that so-called membranous cells in NALT transport invading bacteria across the epithelial layer in a manner similar to those in Peyer’s patches in the gut [7]. Owen et al. [5] have suggested that disruption of the olfactory epithelium could be mediated through a Toll-like receptor—(TLR) 2 directed inflammatory response [11]. Indeed, TLR3 mediates entry of West Nile virus into the brain, causing lethal encephalitis [12]. Furthermore, excessive activation of TLRs expressed on epithelial cells may lead to breakdown of the epithelial barrier [13]. Another possibility is that the actin-based motility of B. pseudomallei is involved in the intracellular movement in sensory axons or in glia [1, 14]. These are all interesting areas for further investigation. Moreover, the technique of bioluminescence bacterial imaging could be used in other fields of melioidosis research. For instance, a main outstanding question regarding melioidosis research is why certain patients develop acute suppurative parotitis, a symptom almost exclusively seen in Thai pediatric patients [1, 15]. Could this technique help in investigations of how B. pseudomallei travels to the parotid gland?

The present results are derived from experiments involving BALB/c mice. Although both BALB/c and C57BL/6 mice are excellent models in which to study acute septic melioidosis, the 50% lethal dose (LD₅₀) values for BALB/c mice are much lower. In this respect, it is interesting that, in a mouse model of lethal melioidosis in which C57BL/6 mice were intranasally inoculated with only 5 × 10² cfu, no bacterial growth in the brains of severely ill septic mice was observed after 48 h [16]. Certainly, immunologic reactions differ between BALB/c mice and C57BL/6 mice with melioidosis [17, 18]. Although the present data clearly suggest that the olfactory nerve is the route of entry into the brain, obvious caution should be taken to directly extrapolate this finding to cases of neurological melioidosis in humans. This caveat is underscored by the notion that NALT is the only organized mucosal lymphoid tissue in the murine upper respiratory tract, whereas, in humans, the presence of NALT is documented as a morphologically distinct structure that is seen in addition to the lymphoid structures of Waldeyer’s ring [19].

In conclusion, the present data suggest that, in the event of intranasal B. pseudomallei infection, the olfactory nerve serves as a direct route to the brain, whereas nasal lymphoid tissue acts as a potential portal of entry to systemic infection. This road map for murine melioidosis may well be paralleled in cases of neurological or systemic melioidosis in humans. Owen et al. [5] have not only provided more insights into the pathogenesis of melioidosis but have also raised new questions for future research. Last, the present findings may also benefit development of a melioidosis vaccine, because mucosal vaccination against bacterial respiratory infections might offer attractive advantages to conventional systemic vaccination, such as higher levels of antibodies and protection at the airway surface [1, 20].

References