

EDITORIAL



## Trojan horse L-selectin monocytes: A portal of *Burkholderia pseudomallei* entry into the brain

James A. St. John  <sup>a,b,c</sup>

<sup>a</sup>Clem Jones Centre for Neurobiology and Stem Cell Research, Griffith University, Queensland, Australia; <sup>b</sup>Eskitis Institute for Drug Discovery, Griffith University, Queensland, Australia; <sup>c</sup>Menzies Health Institute Queensland, Griffith University, Queensland, Australia

**ARTICLE HISTORY** Received 12 October 2016; Accepted 12 October 2016

**KEYWORDS** CD62L; central nervous system; leukocytes; L-selectin; melioidosis; neurological

Melioidosis is caused by the bacterium *Burkholderia pseudomallei* and infections can be severe with high mortality. It is estimated that around 165,000 cases of melioidosis occur each year with an annual death toll of some 89,000.<sup>1</sup> The bacterium is likely to be endemic to 77 countries and is most prevalent in south-east Asia and northern Australia. Infection with the bacterium does not always lead to melioidosis as many people can be seropositive without having reported symptoms<sup>2</sup> or in some cases melioidosis can arise after considerable latency even decades after exposure.<sup>2,3</sup> The bacterium is present in soil and water and can be inhaled through dust that may be raised during storms, or can enter the body via percutaneous inoculation. Infection is particularly prevalent during the wet season and can lead to septicemia and affect numerous different organs as it spreads haematogenously. Symptoms range from skin and nasal infections to systemic presentations with pneumonia and septic shock. In a minority of cases it can infect the brain where it causes neurological melioidosis and brainstem encephalitis—this form of the disease is particularly difficult to treat as it requires long term antibiotic administration. Neurological melioidosis presents with brainstem encephalitis, brain abscesses, fluctuating consciousness, brainstem signs, and there is a recognized syndrome of brainstem and spinal cord involvement. Neurological melioidosis leads to death in ~25 % of the cases despite treatment and can have very serious sequelae such as residual paralysis and ataxia.<sup>4</sup>

While invasion of the central nervous system has been shown in histological samples from deceased patients,<sup>4</sup> it is not clear how *Bp* invades the central nervous system as there are several potential pathways by which bacteria can enter the brain. Two direct

routes of entry into the brain which bypass the blood brain barrier are the olfactory nerve and the trigeminal nerve. Indeed, it has been shown in a Balb/C murine model that intranasal inoculation of *Burkholderia pseudomallei* can lead to rapid infection of the olfactory nerve after which the bacteria progress up into the olfactory bulbs that are situated within the central nervous system.<sup>5</sup> In contrast, in an outbred Quackenbush murine model, intranasal inoculation of *Burkholderia pseudomallei* led to rapid infection of branches of the trigeminal nerve after which the bacteria progressed directly to the brainstem and spinal cord within 48 hours after inoculation.<sup>6</sup> In these nerve infection routes, the bacterial infection resulted in the death of axons which created hollow nerve bundles through which the bacteria could travel.

In other regions of the brain, 2 cellular barriers which are the blood brain barrier and the blood cerebrospinal fluid barrier act to prevent invasion by bacteria from within the blood. Nevertheless, these barriers can be penetrated by some bacteria. Typical bacterial meningeal pathogens may potentially enter the cerebrospinal fluid by penetrating the blood brain barrier of cerebral microvessels and entering the extracellular fluid of the brain. A more direct entry into the cerebrospinal fluid may occur via penetration of the blood cerebrospinal fluid barrier which is formed by tight junctions between epithelial cells at the choroid plexus and between endothelial cells of the veins within the subarachnoid space.

To cross these cellular barriers to enter the central nervous system, bacteria can use several different methods: transcellular or paracellular penetration or the Trojan horse method by which infected leukocytes from the

**CONTACT** James A. St. John  [j.stjohn@griffith.edu.au](mailto:j.stjohn@griffith.edu.au)  Clem Jones Centre for Neurobiology and Stem Cell Research, Griffith University, 170 Kessels Road, Brisbane, Queensland 4111, Australia.

Comment on: Chen Y-S, et al. Involvement of L-selectin expression in *Burkholderia pseudomallei*-infected monocytes invading the brain during murine melioidosis. *Virulence* 2017; 8(6): 751-766; <https://doi.org/10.1080/21505594.2016.1232239>

© 2017 Taylor & Francis

blood circulation penetrate the endothelial or epithelial layer. Transcellular penetration occurs when bacteria adhere to endothelial or epithelial cells and are then translocated by pinocytosis or receptor-mediated mechanisms. Paracellular penetration can occur after disruption of the tight junctions between cells that form the blood brain barrier or cerebrospinal fluid barrier. The Trojan horse method can occur when bacteria are able to survive within circulating immune cells which are able to infiltrate into the central nervous system.

In this issue of *Virulence* Chen et al. 2016 showed that *Burkholderia pseudomallei* is able to use the Trojan horse method by which infected leukocytes infiltrate the central nervous system using L-selectin (CD62L) mediated migration across the cerebral endothelium. Previously in a hemogenous model of melioidosis meningitis, the same group has shown that *B. pseudomallei* established a primary focus of infection in the spleen, after which it migrated to the bone marrow and then spread to the brain.<sup>7</sup> In that study, *B. pseudomallei* was associated with CD11b+ cells within the bone marrow and spleen, and it was considered that these cells may serve as Trojan horses when the bacteria escaped the phagocytic machinery and survived within the cells. They had also demonstrated that the expression of L-selectin on *B. pseudomallei*-infected CD11b+ cells was required for the development of meningitis after adoptive transfer.<sup>7</sup> In the current paper they have now compared BALB/c mice with C57BL/6 mice and found that BALB/c mice were much more susceptible to neurological melioidosis than C57BL/6 mice. In particular, they found that brain-infiltrating leukocytes derived from Ly6C monocytes were elevated in infected BALB/c mice but not in infected C57BL/6 mice. By using adoptive transfer they found Ly6C monocytes that expressed L-selectin exacerbated the neurological infection, whereas Ly6C monocytes that lacked L-selectin had relatively reduced neurological infection. Importantly, it was noted that the infiltrating leukocyte Trojan horse entry was not the only entry as low but significant levels of neurological melioidosis continued to occur in the absence of L-selectin. Thus *Burkholderia pseudomallei* is likely to use more than one route of entry into the central nervous system. In addition to identifying the role of L-selectin in mediating neurological melioidosis, the authors have also identified the potential use of elevated L-selectin expression by leukocytes as a marker that could be used to aid the more rapid diagnosis of neurological melioidosis.

## Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

## Funding

This work was supported by funding from the Clem Jones Foundation and from the Perry Cross Spinal Research Foundation.

## ORCID

James A. St. John  <http://orcid.org/0000-0001-7536-3381>

## References

- [1] Limmathurotsakul D, Golding N, Dance DA, Messina JP, Pigott DM, Moyes CL, Rolim DB, Bertherat E, Day NP, Peacock SJ, et al. Predicted global distribution of *Burkholderia pseudomallei* and burden of melioidosis. *Nat Microbiol.* 2016; 1:15008; <https://doi.org/10.1038/nmicrobiol.2015.8>
- [2] Currie BJ. Melioidosis: evolving concepts in epidemiology, pathogenesis, and treatment. *Semin Respir Crit Care Med.* 2015; 36(1):111-25; PMID:25643275; <https://doi.org/10.1055/s-0034-1398389>
- [3] Ngaay V, Lemeshev Y, Sadkowski L, Crawford G. Cutaneous melioidosis in a man who was taken as a prisoner of war by the Japanese during World War II. *J Clin Microbiol.* 2005; 43(2):970-2; PMID:15695721; <https://doi.org/10.1128/JCM.43.2.970-972.2005>
- [4] Currie BJ, Fisher DA, Howard DM, and Burrow JN. Neurological melioidosis. *Acta Trop.* 2000; 74(2-3):145-51; PMID:10674643; [https://doi.org/10.1016/S0001-706X\(99\)00064-9](https://doi.org/10.1016/S0001-706X(99)00064-9)
- [5] St John JA, Ekberg JA, Dando SJ, Meedeniya AC, Horton RE, Batzloff M, Owen SJ, Holt S, Peak IR, Ulett GC, et al. *Burkholderia pseudomallei* penetrates the brain via destruction of the olfactory and trigeminal nerves: implications for the pathogenesis of neurological melioidosis. *MBio.* 2014; 5(2):e00025; PMID:24736221; <https://doi.org/10.1128/mBio.00025-14>
- [6] St John JA, Walkden H, Nazareth L, Beagley KW, Ulett GC, Batzloff MR, Beacham IR, and Ekberg JA. *Burkholderia pseudomallei* Rapidly Infects the Brain Stem and Spinal Cord via the Trigeminal Nerve after Intranasal Inoculation. *Infect Immun.* 2016; 84(9):2681-8; PMID:27382023; <https://doi.org/10.1128/IAI.00361-16>
- [7] Liu PJ, Chen YS, Lin HH, Ni WF, Hsieh TH, Chen HT, and Chen YL. Induction of mouse melioidosis with meningitis by CD11b+ phagocytic cells harboring intracellular *B. pseudomallei* as a Trojan horse. *PLoS Negl Trop Dis.* 2013; 7(8):e2363; PMID:23951382; <https://doi.org/10.1371/journal.pntd.0002363>