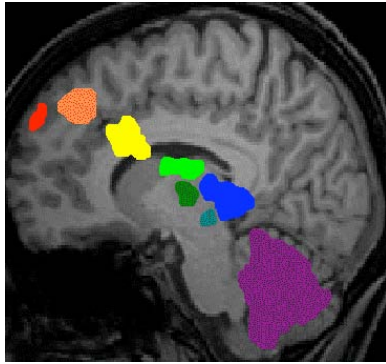


JOINT NEUROSCIENCE, RCM I & COBRE SEMINAR



Specialized Neuroscience Research Program
Asia-Pacific Institute of Tropical Medicine and Infectious Diseases
Pacific Center for Emerging Infectious Diseases Research

Taking *Tryps* Across the Human Blood-Brain Barrier

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Wednesday, January 28, 2009 at 12:30 p.m.
John A. Burns School of Medicine, Kaka'ako
Medical Education Building, Room 301
For further information, call 692-1668

This joint seminar is supported by grants from the National Institute of Neurological Disorders and Stroke (U54NS056883) and the National Center for Research Resources RCM I (G12RR/AI/03061) and COBRE (P20RR018727)

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Research and Professional Experience

* The characteristic neurological symptoms of Human African trypanosomiasis (HAT; also called sleeping sickness) and Lyme neuroborreliosis in man are due to the penetration of the CNS by the protozoan parasite *Trypanosoma brucei gambiense* (or *T. b. rhodesiense*) and the spirochete *Borrelia burgdorferi*, respectively. Until recently, how these pathogens cross the Blood-Brain Barrier [BBB] remained an unresolved issue. At Hopkins, I have initiated and continue to do research to identify the mechanisms these pathogens use to enter the human CNS via the blood-brain barrier. Using an in vitro model for the human BBB we have found that a) *B. burgdorferi* and bloodstream forms of *T. brucei* are able to cross the brain microvascular endothelial cells that comprise the BBB model; b) these pathogens appear to bind to human BMEC close to intercellular junctions; c) during the transmigration process by these pathogens, transient changes in monolayer integrity occur, and d) this process involves pathogen (i.e. trypanosome cysteine proteases) and/or host proteases (i.e. matrix metalloproteases/plasminogen) and host cell calcium signaling pathways.

* I am also interested in the development of better HAT diagnostics based on two new recently introduced technologies. The first, Loop-Mediated Isothermal Amplification (or LAMP) is a recently introduced alternative to PCR for the detection of HAT that is based on *Bst* DNA polymerase and, compared to normal PCR, has advantages of reaction simplicity and detection sensitivity (J. Clin. Microbiol. 41:5517-24). Because LAMP requires 4 primers that identify 6 specific sites on the target DNA molecule, LAMP is also highly specific. Furthermore, since LAMP does not require complicated thermal cycling steps; a simple water bath (63-65°C) is sufficient to amplify the target DNA to detectable levels (>10 µg of target DNA within 30 to 60 min). A useful by-product of the reaction is the copious white precipitate of magnesium pyrophosphate, which allows easy and rapid visual confirmation that the target DNA was amplified by LAMP. It is important also to note that *Bst* DNA polymerase used in LAMP is not affected by blood-derived tissue components that can inactivate *Taq* DNA polymerase used in PCR. In the second procedure, using controlled site-specific chemistry, Ian Burbulis and colleagues (Nat. Methods 2:31 - 37) have been able to covalently tag a 'DNA barcode' to a single site on a protein, they call a "tadpole". Because the DNA barcode contains a region for PCR amplification, this affords a range of ultra-sensitive analyses should have an important potential application for pathogen detection, disease diagnosis and environmental monitoring. Alone, the above two procedures are clearly very powerful. However, we are currently looking into the possibility of using the *Bst* DNA polymerase procedure of 'LAMP' to replace the PCR of 'tadpole', to create even more powerful technique methods for not only HAT, but other tropical diseases (see Nat Meth 2:635, 2005).

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