PROJECT SUMMARY (See instructions):

Cerebral Malaria (CM) presents as malaria-infected patients with seizures and coma, and kills an estimated 575,000 people every year. While very little work has been done to date on the pathology of the disease, some autopsy studies have shown neuronal and vascular damage leading to brain swelling and inflammatory cell infiltration. There is a strong correlation between inflammatory cytokines and morbidity and mortality from this disease. Cerebral malaria has been modeled in rodents using highly virulent parasites, but we have discovered that even normally mild parasites can cause behavioral symptoms and vascular damage in a host with a single gene deficiency (IL-10-/-) leading to a stronger inflammatory response and significant mortality. Therefore, we will use a novel model of inflammation-induced cerebral malaria and study specific brain cell types affected including neuronal damage, astrocyte swelling and microglial activation. This constellation of brain pathology triggered by unregulated inflammation presents an opportunity to study mechanisms of neuro-immuno-pathology in a relevant human disease model. Inflammation-induced cerebral damage is of high significance to neuroscience as host inflammation has been shown to be central to the etiology of many brain insults. The complex interplay between activated astrocytes, vascular endothelium, microglia, neurons, and inflammatory cells have been implicated in many diseases of the human brain from stroke to seizures, and dementia to addiction and mechanisms of repair are likely to be conserved. In our preliminary data stemming from a unique collaboration started in response to a new initiative at UTMB to bring together neurobiologists with immunologists to study infectious diseases of the brain, we have determined that there are short-term and long-term behavioral symptoms, as well as vascular damage in the brain in this model. Therefore, we propose to investigate the inflammatory mechanisms driving neuronal damage during CM using a high-throughput and multi-parameter technique, flow cytometry, which is used primarily in immunology, to identify the particular areas of the brain and types of neurons affected by the inflammation, as well as the activation and damage caused by inflammation. Flow cytometry has been validated by several neurobiology studies, and markers have been identified. We are multi-plexing this approach using our 18-color flow cytometer. We have begun to identify the types of infiltrating leukocytes, and shown inflammatory monocytes as well as activated microglia and have established markers of neurons indicating a significant degree of inflammatory cell presence in the brain. We will extend this work by identifying the types of neurons as defined by a panel of specific neurotransmitter receptors. Using immunohistochemistry, we will determine whether the inflammatory cells remain in the vasculature, as the parasite solely infects red blood cells, or gain entry to the parenchyma. Then, we will use cutting-edge imaging automation methods for large volume acquisition with subcellular resolution and a variety of image processing algorithms to quantify co-localization of inflammation and vessel leakage with damaged neurons and activated microglia and astrocytes. This will be followed by novel clearing methods for three dimensional imaging, to quantify axonal, microglial and astrocyte damage and responses in three dimensions. In this way, we will establish critical parameters of neuronal damage in this important disease and provide new understanding, as immunologists, of the relationship between vascular and neuronal damage. This exciting collaboration represents an opportunity for cutting edge immunological techniques to be transferred to the neuroscience community for the study of the many diseases identified to date involving inflammation-induced neural damage.

RELEVANCE (See instructions):

Given the importance of CM as the predominant cause of death from malaria and that severe neuropathological events occur during disease (including in those that survive) but are poorly understood, study of the neuropathological events following this inflammation driven disease is extremely important and timely. This project will bring together experts in neurobiology, immunology, and imaging with availability of unique resources at UTMB in Behavioral Science and Neurobiology, as well as Immunology and Infectious diseases for multi-color flow cytometry and advanced microscopy allowing us to make a critical contribution essential to understanding of the neurological pathology of this devastating disease. Outcomes of this research could be expanded to understand how other inflammatory conditions impact the brain and we expect to transfer these techniques to the study of other diseases with lethal cerebral involvement, such as HIV, tuberculosis and emerging viral encephalopathies.