The Role of Pattern Recognition Receptors in Glial Responses to Viral Challenge

**Background** - Herpes Simplex Virus (HSV) infection is relatively common throughout the general population. It has been shown that 58% of the population has HSV (Xu et al, 2006). However, this virus can be associated with encephalitis. This disease associated with neuroinflammation is caused by the movement of the virus from the site of infection through either the trigeminal or olfactory nerve into the central nervous system. The implications of this infection are severe and can lead to neurological deficits and in many cases, to death. Mortality rates for encephalitis patients range from 40-70% (Furr and Marriott, 2012). Studying the inflammatory responses elicited by viral infection are the key to understanding and limiting encephalitis.

Prior research has determined that the cytosolic proteins RIG-I and DAI act as novel intracellular pattern recognition receptors (PRRs) for viral RNA and DNA, respectively (Furr and Marriott, 2012). PRRs are present in glial cells, of the CNS. Glial cells are non-neuronal components of the CNS which act to protect the nervous system. Although it has been determined that PRRs respond in the presence of viral nucleic acids, it is still unclear if the responses triggered by these sensors serve to protect the host or exacerbate inflammatory neurological damage of resident cells in the CNS. Further experimental research will help identify if these PRRs act to produce
antiviral cytokines which help limit viral replication. The overall objective of this and future research, is to limit viral replication while limiting neuronal cell death. In the future, this research may be used to limit encephalitis. It is predicted that intracellular viral sensors, RIG-I and/or DAI, are essential for the production of protective antiviral mediated production that can limit viral replication in neuronal cells. Prior research has revealed that DAI-mediated responses increase overall levels of neuronal cell death (Furr et al, 2011). However, the role of DAI has not been determined following RIG-I knockdown. Such experiments would help identify if RIG-I-mediated responses are different than those of DAI. It is predicted that glial cell activation via RIG-I will lead to similar levels of neuronal cell death. Although the mechanism by which DAI induces cell death has not been identified, TNF-α and nitric oxide are likely candidates in this pathway.

**Relevance**- Understanding the pathways by which PRRs respond to viral challenge is fundamental to limiting encephalitis. It has been shown that HSV-1 is a major causative agent of encephalitis in adults, in the U.S.. Additionally, a wide range of viruses including rabies and varicella, are responsible for causing this neuroinflammatory disease. Patients with encephalitis have a mortality rate ranging between 40% and 70% (Furr and Marriott, 2012). Prior research has shown that viral replication leads to PRR activation. DAI recognizes double-stranded DNA viruses. This activation leads to a cascade of events, culminating in the production of antiviral and inflammatory cytokines. However, it is unknown if the effects of this DAI-induced cascade have an overall positive or negative effect on the body. Antiviral cytokines act to prevent viral replication, in turn, limiting the widespread effects of the virus and possibly of encephalitis. Inflammatory cytokines play a protective role in the body; however, these immune agents are
also associated with swelling and neuronal death. The activation of PRRs leads to the production of molecules essential to the immune response and to the production of inflammatory cytokines which underlie neuroinflammation. Studying the effects of PRRs will enable us to understand if these viral sensors have more of a protective or detrimental role in the body’s response to viral challenge. This information will allow us to identify areas of the PRR pathway that can be modified to limit the production of damaging inflammatory cytokines. Our results may be useful in limiting neuroinflammation in encephalitis patients.

**Hypothesis**- This research aims to determine if the effects of PRRs in glial cells are more positive or negative. The production of antiviral and inflammatory cytokines will be measured using ELISA analyses. The intracellular viral DNA sensor, DAI, acts synergistically with the viral RNA sensor RIG-I. It is uncertain what effect, if any, the knockdown of RIG-I will have on the action of DAI. It is predicted that RIG-I knockdown will lead to a decrease in overall DAI activity. It is expected that DAI activation will lead to the production of antiviral and inflammatory cytokines, but that the magnitude of this response will be decreased. Viral replication and cell membrane integrity will be quantified. Using this information, further studies will help elucidate how one can decrease the production of inflammatory cytokines via the manipulation of such PRRs.

**Methodology**- Experiments will be performed to test the effects mediated by these PRRs in both resting state and virally challenged astrocytes and microglia. Cells will be infected with the DNA virus HSV or the RNA virus VSV; following this, an inflammatory response is predicted to occur and/or antiviral cytokines will be produced. Viral replication will then be quantified as will
the integrity of the challenged cells or neuronal cells exposed to glial-cell conditioned medium.

To measure the levels of viral replication and cell viability, multiple methods will be employed. Cell viability will be measured by assessing membrane integrity and by determining levels of apoptosis. This will be done using propidium iodide (PI) and annexin V. PI cannot permeate live cells; therefore if membrane integrity is compromised, red fluorochrome PI stain will be visualized in the cells. In addition, annexin V will be used to measure rates of apoptosis. When cells undergo apoptosis phosphatidylserine is expressed on the cell surface. Annexin V conjugated to green fluorochrome bonds to this membrane component and allows levels of apoptosis to be measured. These methods will enable us to determine if the virus replicates under both normal conditions and following PRR knockdown and will additionally allow us to measure the relative level of neurotoxic mediator production by virally challenged glial cells. These techniques will allow us to determine the extent to which PRRs affect the production of antiviral cytokines.

It has been shown that DAI activation is associated with neuronal cell death and the production of antiviral cytokines. It is predicated that prior to apoptosis, cells release TNF-α and Nitric oxide. Following viral infection of the glial cells, levels of nitric oxide will be quantified. This will be calculated using the Greiss Test, a colorimeter nitric oxide assay. ELISAs will be employed to analyze the concentration of TNF-α in cells, following PRR activation. Additionally, ELISAs will be used to better understand which proteins are secreted due to the PRR response. Using these methods will help identify if the action of PRRs causes a net positive or negative effect in the body’s response to viral challenge.
Bibliography

