Toll-Like Receptors and Astrocyte – Microglia Interaction

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Background: Central nervous system (CNS) inflammation plays a key role in the progression of chronic neurodegenerative disease, although the mechanisms through which this occurs are still unclear. The inflammatory response during most chronic neurodegenerative disorders is dominated by microglia, although astrocytes (which predominate over microglia in absolute numbers) can also assume a reactive state following CNS injury. Pathogen-associated molecular patterns (PAMPs) are molecules associated with groups of pathogens that are recognized by cells of the innate immune system like microglia. One important class of PAMP receptors comprise transmembrane Toll-like receptors (TLRs), which respond to invading pathogens and utilize receptor dimerization in order to achieve specific agonist identification. TLR engagement leads to activation of the transcription factor NF-kB with increased expression of pro-inflammatory cytokines. We have shown that the TLR4 agonist lipopolysaccharide (LPS) induces production of pro-inflammatory molecule genes and products in both enriched (~95%) astrocytes and purified (>99%) microglia from rat cortex or spinal cord. However, astrocyte responses are dependent on the presence of small numbers of contaminating microglia. Because TLRs are involved in injury responses of nervous system tissue and in neuropathic pain, it is important to understand how glia respond to a TLR2 and TLR3 agonists.
Methods: Established methods in our laboratory for the culture and maintenance of newborn rat cortical and spinal cord enriched astrocytes and purified microglia have been described the preceding year. The lysosomotropic agent L-leucyl-L-leucine methyl ester (L-LME) was used to remove residual microglia from the enriched astrocyte population, as confirmed by the loss of microglia-specific marker genes (Real-Time PCR). Target gene expression was analyzed by Real-Time PCR, and mediator output by ELISA. In some cases, protein expression was confirmed by Fluorescence Activated Cell Sorting (FACS).
Results: FACS analysis confirmed the cell subtype composition of the glia cultures (as determined previously by immunocytochemistry), including the relative distribution of microglia/astrocytes at the various stages of preparation. In addition to LPS, the TLR2 agonist zymosan and the TLR3 agonist poly(I:C) (which mimics viral double-stranded RNA) up-regulated expression of pro-inflammatory markers at the gene and product levels in cortical, as well as spinal cord microglia and astrocytes. Responsiveness of the latter cells was determined to reside with contaminating microglia. Interestingly, activation of one TLR was able to influence expression of the others, either by up- or down-regulating transcription. Complementary experiments at the translational level are being examined by Western blotting and FACS. Another aspect of neuroinflammation are the nuclear receptors (for example, peroxisome proliferator-activated receptors and liver X receptors) whose activation is anti-inflammatory and neuroprotective. The possibility that the pro-inflammatory actions of TLRs involves, at least in part, a down-regulation of one or more nuclear receptors is also under investigation.
Conclusions: These results suggest that TLR2 and TLR3, and not only TLR4 are involved in glial cell inflammatory responses. Future experiments will investigate how microglia behave when cultured alone, and in the presence of astrocytes, with the goal of identifying astrocyte-derived factors which may affect microglial cell sensibility to an inflammatory stimulus.