A heavy toll on the outcome of ischemic brain stroke

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Ischemic stroke causes cells in the brain to respond excessively at the site of injury, as well as immune cells outside the brain to actively migrate into the injured brain tissue and exacerbate the inflammatory process. The unfortunate result is an increased neuronal cell death and functional behavioral impairments to the subject. Our understanding of the inflammatory process following stroke continuously increases, as many of the factors that mediate these excessive inflammatory responses are constantly being described. Toll-like receptors (TLR), a family of innate immune receptors, are of the most central and potent contributors to inflammation during infection, tissue damage and disease (Kawai and Akira, 2010). A unique attribute of TLRs is the large variety of exogenous microbial associated molecular patterns (MAMPs) that encompass almost all types of microbial flora that the organism encounters. In addition, an ever increasing milieu of endogenous tissue-damage (or danger) associated molecular patterns (DAMPs) are continuously being discovered (Uematsu and Akira, 2008). TLR activation induces intracellular signaling pathways that result in production of inflammatory cytokines as well as type I interferons (IFNs) (Cervantes et al., 2012). TLRs are heavily involved in the pathogenesis of cerebral ischemia. Preconditioning the brain using MAMPs that activate TLRs 2, 4, 7 and 9 decreases the severity of ischemic insults (Hickey et al., 2007; Hua et al., 2008; Leung et al., 2012; Rosenzweig et al., 2004; Stevens et al., 2008; Tasaki et al., 1997). This process is thought to depend on a reprogrammed transcriptional response to stroke injury, which comprises of a consensus set of genes, whose transcriptional regulation is dominated by IFN regulatory elements (Stevens et al., 2011). Deficiency for TLRs 2 or 4 confers protection against cerebral ischemia (Cao et al., 2007; Caso et al., 2007, 2008; Lehnardt et al., 2007; Tang et al., 2007), in a mechanism involving deactivation of ERK1/2, JNK1/2, p38, downregulation of iNOS and inhibition of the executioner caspase-3 (Kilic et al., 2008). Interestingly, both MAMP-induced preconditioning and a deficiency for TLRs result in reduced stroke severity. In a striking contrast, activating TLR4 using MAMPs immediately prior to or following cerebral ischemia exacerbates the injury (Denes et al., 2011; McColl et al., 2007).

TLR8 is expressed on intracellular vesicular membranes and is commonly involved in recognition of bacterial RNA. TLR8 not only is involved in the production of type I IFNs in response to viral pathogens, but also is triggered upon ligation of bacterial RNA. This is probably due to TLR8’s ability to recognize both single-stranded RNA and short double-stranded RNA. In addition to various types of oligoribonucleotides, a variety of synthetic chemical agonists such as imidazoquinolines were shown to activate TLR8 (Cervantes et al., 2012).

The first observations that TLR8 is expressed in neurons and plays a critical role in determining neuronal cell fate were shown in two elegant works by Ma et al. (2006, 2007). MA and colleagues illustrated important roles for TLR8 deficiency and TLR8 activation in determining the fate of neurons and their development. The current study by Tang et al. (2013) expands these studies, and provides a functional relevance for TLR8 in the context of cerebral ischemia. In their manuscript, Tang and colleagues show how ischemia increases TLR8 expression levels in neurons in vitro. An increased TLR8 expression is also indicated in brain tissue subjected to ischemic stroke in vivo. Activating TLR8 using the synthetic ligand R-848 reduces neuronal viability under nutrient deprivation in vitro and exacerbates ischemia/reperfusion injury in vivo (Tang et al., 2013). Perhaps of higher importance, reducing the expression of TLR8 using shRNA in a neuronal-related cell-line exposed to oxygen-glucose deprivation, an in vitro model for ischemia, significantly reduces cell death. While this is in full agreement with previous reports by Ma et al., Ma and colleagues found no activation of JNK by R-848 in healthy neurons, whereas Tang et al. found that under ischemic stress, R-848 dose dependently activated JNK signaling in neuronal cultures. This suggests that in the context of stress, TLR8 possibly plays a detrimental role in the viability of neurons via the JNK signaling cascade. The issue of JNK activation by TLR8 under stress in neurons will probably be addressed in follow-up studies. This point can be addressed by stably decreasing TLR8 expression levels using viral particles expressing TLR8-specific shRNA in cultures of cortical neurons.

Several important aspects, however, are yet to be assessed with respect to TLR8-mediated JNK activity. It is unclear whether inhibiting JNK signaling with specific antagonists prevents TLR8 activation from exacerbating ischemic damage in neurons. While neuronal cells in culture exhibit increased TLR8 expression following ischemia, it is unclear whether the observed increase in TLR8 expression in the brain occurred in neurons, astrocytes, microglia or other infiltrating immune cells such as T cells. Similarly, while JNK signaling was shown by Tang et al. to be activated following ischemia in culture, it is yet to be shown which cells are responsible for this effect in vivo. In the past several years, many critical roles have been described for T cells in regulating cognitive behavior in the healthy brain (Kipnis et al., 2004), as well as...
under pathological conditions such as stroke (Magnus et al., 2012). Tang and colleagues have also shown that T cells are actively infiltrating into the brain tissue following activation of TLR8 using R-848. The possible role of T cell infiltration in TLR8-mediated pathogenesis of ischemic stroke can be conclusively shown by grafting T cells from TLR8−/− mice into gamma-irradiated WT mice treated with R-848. This work also illustrates an important but yet unanswered question: which endogenous ligands are responsible for mediating signaling via TLR8 in the absence of MAMPs or exogenous synthetic ligands such as R-848. The most probable candidates are endogenous RNA species such as mRNA, tRNA or rRNA, however, this is yet to be shown. Elucidating this question will enable the development drugs of potential therapeutic abilities for pathologies such as cerebral stroke or other neurodegenerative disorders.

References


