TNF-DEPENDENT CERAMIDE SIGNALING INDUCES DEATH OF DOPAMINERGIC (DA) NEURONS AND DELAYED DN-TNF GENE TRANSFER IN VIVO HALTS PROGRESSIVE LOSS OF NIGRAL DA NEURONS

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Introduction: Neuroinflammatory mechanisms have been implicated in the pathophysiology of Parkinson's disease (PD). We previously demonstrated that neutralization of soluble Tumor Necrosis Factor (solTNF) by dominant-negative TNF (DN-TNF) inhibitor protein (McCoy et al., 2006) or DN-TNF gene transfer (McCoy et al., 2008) significantly attenuated the loss of rat DA neurons both \textit{in vitro} and \textit{in vivo}.

Aims: We investigated the role of the sphingolipid ceramide in mediating TNF neurotoxicity \textit{in vitro} and the extent to which delayed solTNF inhibition \textit{in vivo} halts nigral degeneration.

Methods: Sphingomyelinase (SMase) inhibitors were used to block ceramide generation in TNF-treated DA cells and primary DA neurons and lenti-DN-TNF was injected intranigrally two weeks post 6-OHDA lesion.

Results: We found that TNF-dependent ceramide signaling contributes to DA neuron cytotoxicity \textit{in vitro} by compromising mitochondrial membrane potential, inducing ER stress and activating caspase-dependent apoptosis. \textit{In vivo} 6-OHDA-lesioned rats injected with lenti-GFP displayed progressive loss of TH+ neurons between week 2 and 5 whereas 6-OHDA/lenti-DN-TNF rats displayed attenuated microglial activation and no further loss of TH+ cells after week 2.

Conclusions: Although the etiology of sporadic PD is unknown, identification of cell death pathways activated by neuroinflammation may aid in the development of novel therapeutic strategies to prevent or ameliorate disease progression. We speculate that timely delivery of DN-TNF and perhaps SMase inhibitors in the SNpc may be neuroprotective in at-risk individuals by successfully halting the progressive degeneration of DA neurons so the threshold of 70% DA loss needed to develop clinical symptoms is never reached.