

Regulation of Astrocyte Inflammatory Responses by the Parkinson's Disease-Associated Gene DJ-1

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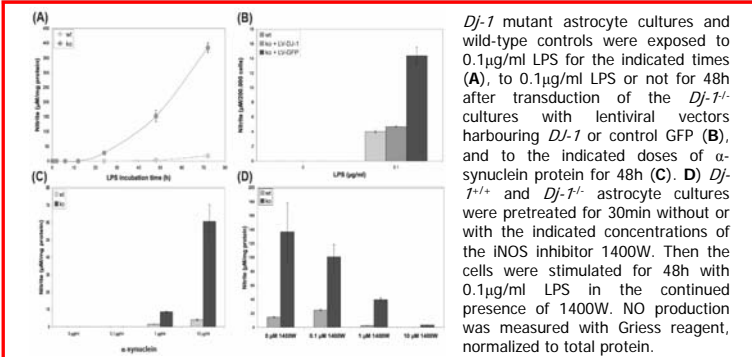
Abstract

Mutations in the DJ-1 gene cause autosomal-recessive Parkinson's disease (PD). In addition to neuron-intrinsic protective functions, its up-regulation in reactive astrocytes also suggests a glial contribution of DJ-1. Here we show in astrocyte-rich primary cultures derived from *Dj-1* knockout mice that DJ-1 regulates pro-inflammatory responses. When treated with the bacterial endotoxin lipopolysaccharide (LPS) as well as the PD-linked protein α -synuclein, *Dj-1* knockout astrocytes generated >10 times more nitric oxide (NO) than littermate controls. The excessive NO production was a direct consequence of *Dj-1* ablation, because lentiviral reintroduction of *Dj-1* specifically restored the NO response to LPS. Enhanced NO production in *Dj-1* knockout astrocytes was mediated by hyper-stimulation of type II NO synthase (iNOS). In addition to iNOS, *Dj-1*^{-/-} astrocytes also induced stronger cyclooxygenase-2 and interleukin-6, all proinflammatory mediators implicated in PD pathogenesis. These effects coincided with phosphorylation of p38 mitogen-activated protein kinase (MAPK), and inhibition of this pathway with SB203580 suppressed NO production and iNOS induction. Moreover, enhanced phosphorylation of p38^{MAPK} was detected in DJ-1 deficient *Caenorhabditis elegans* exposed to pathogenic gram-negative bacteria (*Pseudomonas aeruginosa*), which stimulates LPS-dependent p38^{MAPK} signaling in *C. elegans*. Finally, primary neuron cultures grown on *Dj-1* knockout astrocytes were significantly sensitized to LPS, directly demonstrating the neurotoxic potential of DJ-1 deficiency in astrocytes. Collectively, our findings identify DJ-1 as an evolutionary conserved regulator of pro-inflammatory responses and suggest that loss of DJ-1 contributes to PD pathogenesis by deregulation of astrocytic neuroinflammatory damage.

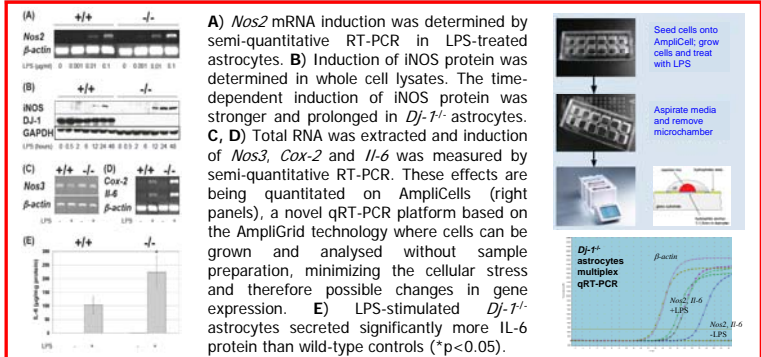
Introduction

A large genomic deletion removing most of the coding region in the *DJ-1* gene was originally linked to PARK7 autosomal-recessive Parkinson's disease (PD). DJ-1 is a multifunctional redox-sensitive protein that may mediate neuroprotection by dampening mitochondrial oxidative stress, regulation of anti-apoptotic and antioxidative gene expression, and regulating kinase pathways. However, *Dj-1* knockout mice do not show overt neurodegeneration, only enhanced striatal denervation when exposed to the PD toxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, perhaps due to excessive dopamine reuptake. Thus, DJ-1 appears to be dispensable for neuronal survival under basal conditions. Interestingly, DJ-1 is strongly upregulated in reactive astrocytes in human PD brain. Moreover, we also found astroglial DJ-1 upregulation in other chronic neurodegenerative diseases and in an α -synuclein transgenic mouse model of PD. Finally, DJ-1 was found in reactive astrocytes of chronic infarct regions after stroke. These neuropathological findings point to a major role for DJ-1 in reactive astrocytes, which we have addressed here in astrocyte-rich primary cultures derived from *Dj-1* knockout mice.

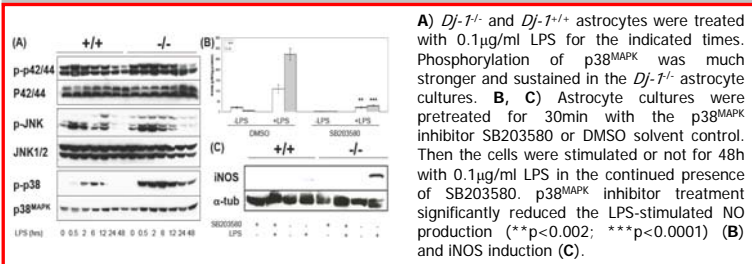
Excessive formation of NO in *Dj-1*^{-/-} astrocytes



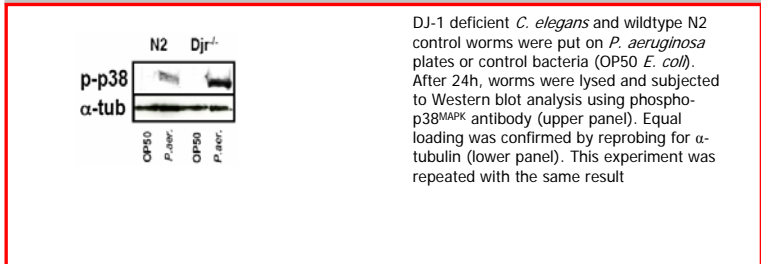
Induction of pro-inflammatory mediators in *Dj-1*^{-/-} astrocytes



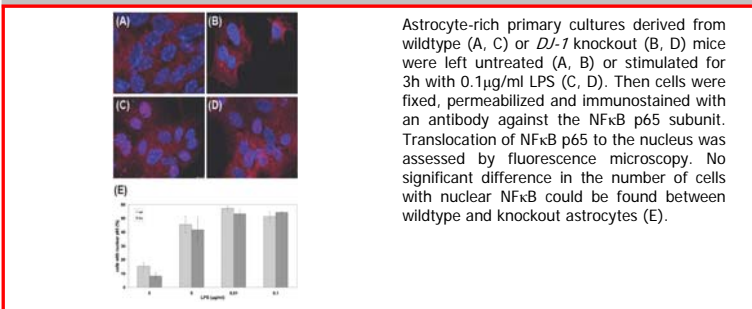
Involvement of the p38^{MAPK} in DJ-1 regulated responses to LPS



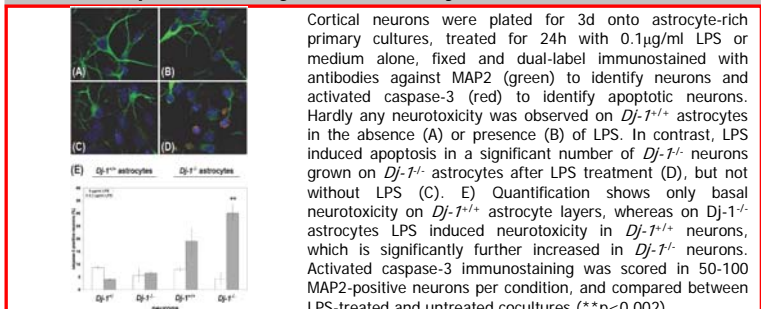
Overstimulation of p38^{MAPK} in *Dj-1*^{-/-} *C. elegans*



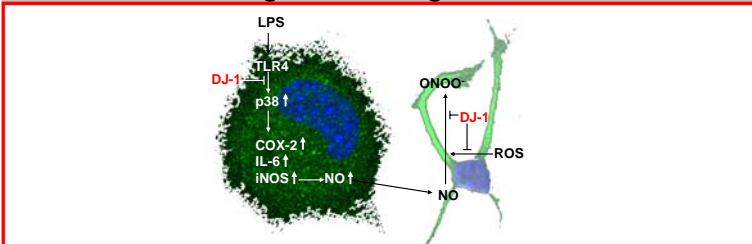
No effect of DJ-1 knockout on NF κ B nuclear translocation



Neurotoxic potential of *Dj-1* ko in astrocyte-neuron co-cultures



Model of the DJ-1 regulated astrogliosis



Conclusions

- DJ-1 plays a key role in the regulation of astrocyte inflammatory response
- Dj-1* knockout leads to excessive NO production by deregulated NOS2 induction via the p38^{MAPK} pathway
- COX-2 and IL-6 are stronger induced by LPS in *Dj-1*^{-/-} astrocytes
- Dj-1*^{-/-} astrocytes have a neurotoxic potential towards surrounding neurons
- This study provides a direct link of a PD gene (DJ-1) to neuroinflammatory processes in (astro)glial cells.