

Differential Immune Activation in Astrocytes of IL-6 Genotypes

Laurel M Bolin,¹ Erik Nelson,¹ Iwona Strycharska-Orczyk,¹ Hernan Cardenas,¹ Ivan Huang,² Michele Malit,² and Aiguo Zhang², ¹The Parkinson's Institute, 1170 Morse Avenue, Sunnyvale, CA 94089 USA and ²Bio-Rad Laboratories, Inc., Hercules, CA 94547 USA

Introduction

Neuroinflammation and its cellular manifestation, reactive gliosis, may contribute to nigrostriatal degeneration in Parkinson's disease. Neuropathologic examination of substantia nigra in individuals with Parkinson's disease and that of humans and mice exposed to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) indicates reactive gliosis accompanies dopaminergic neuron death. MPTP is a contaminant of fentanyl analog designer drugs identified by its selective neurotoxicity to dopaminergic neurons (Langston et al. 1983), which produces clinical symptoms similar to those of Parkinson's disease. Astrocytes convert MPTP to its neurotoxic metabolite 1-methyl-4-phenylpyridinium (MPP⁺) through the action of monoamine oxidase B (Langston et al. 1984). MPP⁺ is taken up by the dopaminergic neurons via the dopamine transporter and interferes with mitochondrial energy metabolism (Javitch et al. 1985). This selective neurodegeneration in the MPTP-lesioned nigrostriatal system permits the study of reactive astrocytosis and microgliosis in response to dopaminergic neuron death in a mouse model. In IL-6-deficient (–/–) mice, MPTP-induced dopamine depletion and neural cell death are greater than in control IL-6 (+/+) mice, suggesting that IL-6 is neuroprotective in this model (Bolin et al. 2002). Additionally, IL-6 (–/–) astrocyte activation correlates with increased dopaminergic neuron death (Cardenas and Bolin 2003). Therefore, to better understand neuroinflammatory regulation in the absence of IL-6, immune activation was examined in central nervous system immunoeffector cells. Using the Bio-Plex suspension array system, cytokine repertoires of lipopolysaccharide (LPS)-stimulated astrocytes isolated from IL-6 (+/+) and IL-6 (–/–) mice were analyzed.

Methods

Primary astrocytes were isolated from neonatal mouse cortices of both IL-6 genotypes as described by Zietlow et al. (1999). Confluent astrocyte cultures were stimulated in triplicate with a range of LPS concentrations (0, 0.1, 1, 10, and 100 ng/ml) over a time course of 1, 6, 18, and 48 hr.

Culture supernatants were collected and snap frozen. Plate configurations included both IL-6 (+/+) and IL-6 (–/–) astrocyte supernatants from the complete LPS dose-response at the same time point to minimize intra-assay differences.

Supernatant cytokine levels were measured with Bio-Plex mouse cytokine assays. Beads coated with capture antibodies (5,000 beads per cytokine) were incubated with premixed standards or sample supernatants (50 µl) in 96-well filter plates. Plates were shaken for 30 sec at high speed (1,000 rpm), then incubated at room temperature for 30 min at low speed (300 rpm). Following incubation, premixed detection antibodies (1 µg/ml) were added, and plates shaken and incubated as before. After washing, streptavidin-phycoerythrin (2 µg/ml) was added to the wells and the plates incubated for 10 min at room temperature with shaking. After washing, the beads were resuspended in 125 µl of Bio-Plex cytokine assay buffer and read by the Bio-Plex array reader. Data were analyzed with Bio-Plex Manager™ software version 2.0 with 4PL and 5PL curve fits.

Results

The Bio-Plex suspension array system permitted the simultaneous measurement of 18 different cytokines and chemokines across multiple dosages of LPS as seen in Figure 1 for wild-type astrocytes. As expected, IL-6 secretion was observed in wild-type astrocytes with no detection in

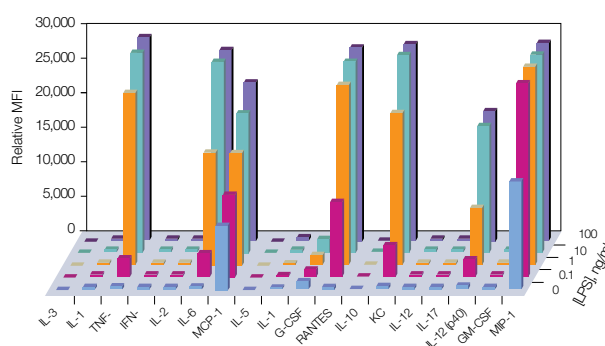


Fig. 1. Cytokine and chemokine expression profile. Culture supernatants of IL-6 (+/+) primary mouse astrocytes were treated with LPS for 1, 6, 18, and 48 hr, and cytokine and chemokines simultaneously analyzed using the Bio-Plex suspension array system. Shown is an LPS dose-response at the 6 hr time point. MFI, median fluorescent intensity.

IL-6 (-/-) cell supernatants (data not shown). IL-3, IL-5, IL-9, IL-12 (p70), IL-17, and IFN- γ were not detected in LPS-stimulated astrocyte supernatants of either IL-6 genotype. IL-6 (-/-) astrocyte secretion of the inflammatory cytokines IL-1 α , IL-1 β , and TNF- α was greater than that measured in supernatants of IL-6 (+/+) astrocytes under the same stimulatory conditions. For IL-12 (p40), G-CSF, and GM-CSF, the opposite trend was observed, with IL-6 (+/+) astrocytes secreting more of these cytokines than IL-6 (-/-) astrocytes. IL-10 secretion was attenuated in both genotypes with small but significantly greater levels in IL-6 (-/-) than wild-type astrocytes stimulated with LPS (data not shown). Vigorous secretion of the chemokines MCP-1, MIP-1 α , KC, and RANTES was observed in astrocyte supernatants of both IL-6 genotypes (Figure 2).

Discussion

These data are consistent with a differential inflammatory activity of IL-6 (-/-) astrocytes compared to IL-6 (+/+) astrocytes stimulated with LPS. The increased inflammatory cytokine secretion suggests a greater potential for astrocytic-driven neuroinflammation in the absence of IL-6 immunomodulation. Whether this in vitro data has in vivo correlates in the MPTP-lesioned nigrostriatal system is intriguing and the focus for continuing investigation. For example, the trend toward greater specific chemokine secretion, i.e., MIP-1 α , by IL-6 (+/+) astrocytes is particularly interesting in light of the compromised microgliosis observed in IL-6 (-/-) mice lesioned with MPTP (Cardenas and Bolin 2003). This data also suggests new targets for exploration of inflammatory processes in nigrostriatal degeneration. Thus, the Bio-Plex suspension array system has been a discovery platform in the study of immunoeffector cell activation in the mouse model of Parkinson's disease.

References

- Bolin LM et al., Increased vulnerability of dopaminergic neurons in MPTP-lesioned interleukin-6 deficient mice, *J Neurochem* 83, 167-175 (2002)
- Cardenas H and Bolin LM, Compromised reactive microgliosis in MPTP-lesioned IL-6 knock out mice, *Brain Res* 985, 89-97 (2003)
- Javitch JA et al., Parkinsonism-inducing neurotoxin, N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine: uptake of the metabolite N-methyl-4-phenylpyridine by dopamine neurons explains selective toxicity, *Proc Natl Acad Sci USA* 82, 2173-2177 (1985)
- Langston JW et al., Chronic Parkinsonism in humans due to a product of meperidine-analog synthesis, *Science* 219, 979-980 (1983)
- Langston JW et al., 1-Methyl-4-phenylpyridinium ion (MPP⁺): identification of a metabolite of MPTP, a toxin selective to the substantia nigra, *Neurosci Lett* 48, 87-92 (1984)
- Zietlow R et al., The effect of microglia on embryonic dopaminergic neuronal survival in vitro: diffusible signals from neurons and glia change microglia from neurotoxic to neuroprotective, *Eur J Neurosci* 11, 1657-1667 (1999)

The Bio-Plex suspension array system includes fluorescently labeled microspheres and instrumentation licensed to Bio-Rad Laboratories, Inc. by the Luminex Corporation.

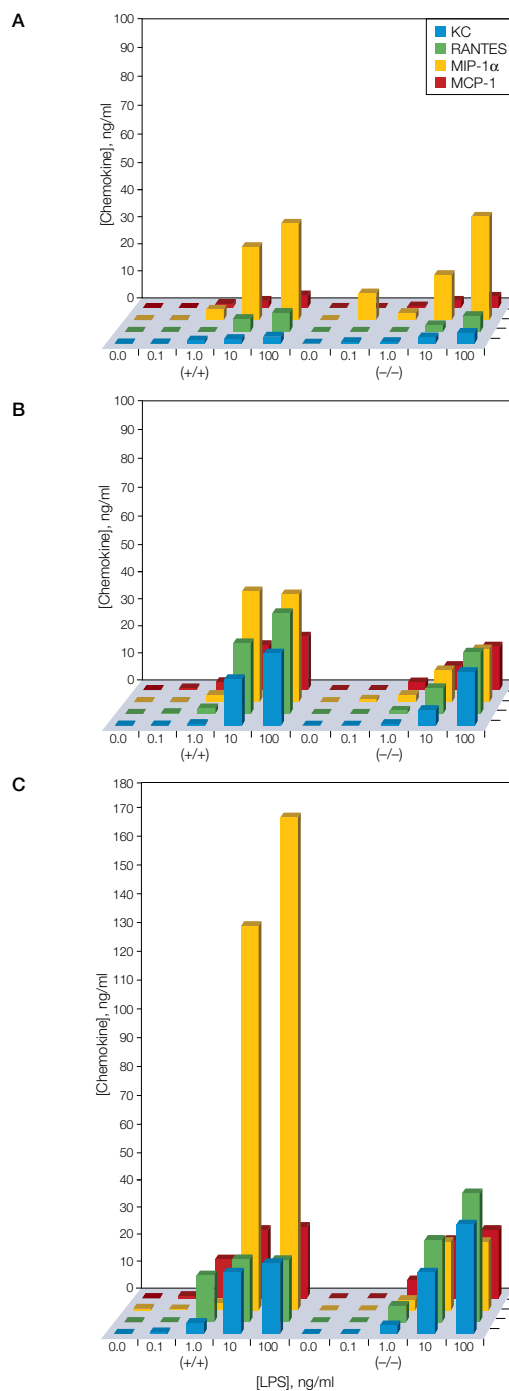


Fig. 2. Simultaneous determination of chemokine secretion. IL-6 (+/+) and (-/-) primary mouse astrocytes were stimulated with LPS for 6 hr (A), 18 hr (B), and 48 hr (C), and chemokine levels measured using the Bio-Plex suspension array system.

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