In vitro Approaches to Study Inflammatory Processes

Irene Schilcher, Tina Loeffler, Stefanie Flunkert, Birgit Hutter-Paier

QPS Austria GmbH, Parkring 12, 8074 Grambach, Austria

BACKGROUND

Immune activation in the central nervous system (CNS) and production of neurotoxic mediators are linked to various neurodegenerative diseases including Multiple Sclerosis, Alzheimer's disease and Parkinson's disease. Neuroinflammation, a response within the brain or spinal cord, is mediated by the production of different stimuli for example cytokines and interleukines.
Activated glial cells are key players for the inflammatory process in the CNS, representing the resident immune cells in this area and playing a critical role in the regulation of brain homeostasis during development, adulthood and aging.

MATERIALS and METHODS

Organotypic slices from the hippocampus as well as primary microglial cultures were prepared from early postnatal wildtype mouse pups. Furthermore, a mouse microglial cell line (BV-2) was used to investigate neuroinflammation. To stimulate inflammation, all three systems were treated with Lipopolysaccharide (LPS), followed by measuring the cytokine release into the supernatant over time by Mesoscale Discovery (MSD) analyses. To inhibit cytokine release, Ibudilast (Ibu) or Dexamethasone (Dexa) served as reference items.

RESULTS

Mouse hippocampal slices revealed increased cytokine production after 12 and 24 h of LPS treatment. This effect was reversed after the addition of Ibudilast.
LPS stimulation of primary microglia for 12 h resulted in an increased release of TNF-alpha, IL-6 and IL-10 into the supernatant. Cytokine production was inhibited after co-treatment with Dexamethasone.
Cytokine levels in the supernatant of LPS treated BV-2 cells were found to be increased over time. TNF-alpha and IL-6 release was inhibited after 6 h Dexamethason treatment.

RESULTS

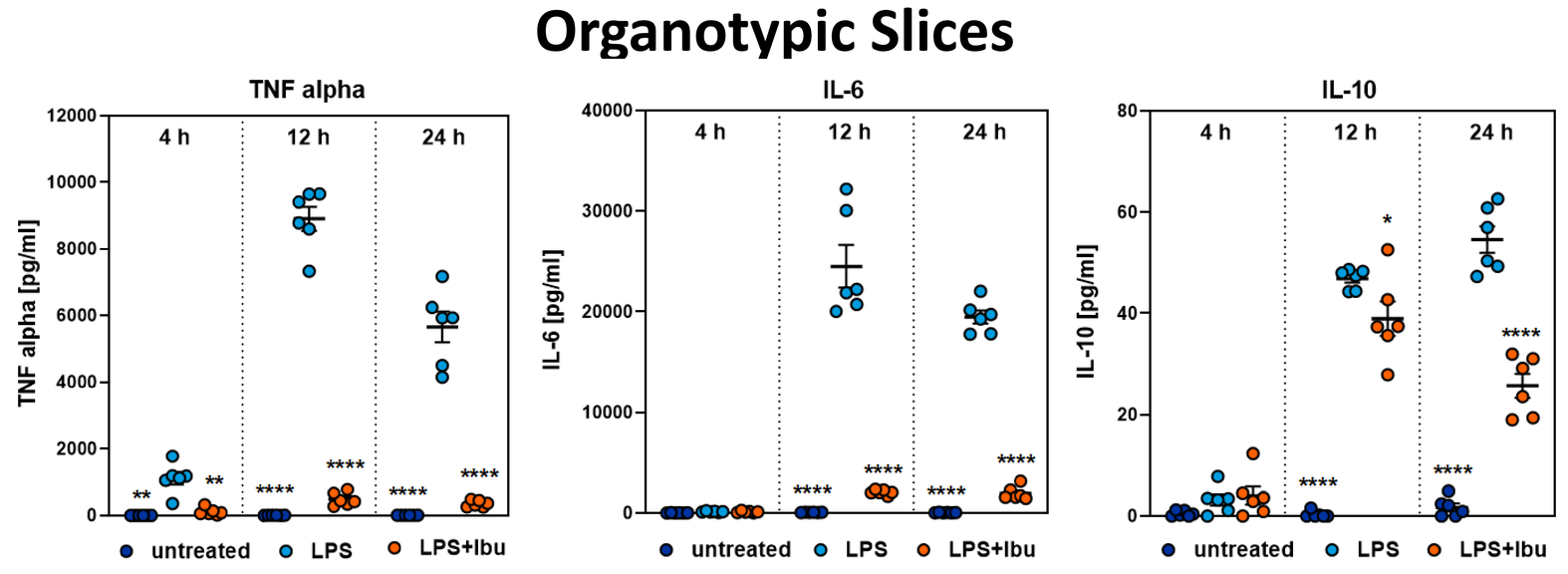

Figure 1: Cytokine release by mouse hippocampal slices after LPS stimulation over time
Data are displayed as aligned dot blots with group means (n=6 per group). Two-way ANOVA followed by Bonferroni Multiple Comparison Test (*posthoc* test) compared to LPS group *p<0.05, **p<0.01, ****p<0.0001.

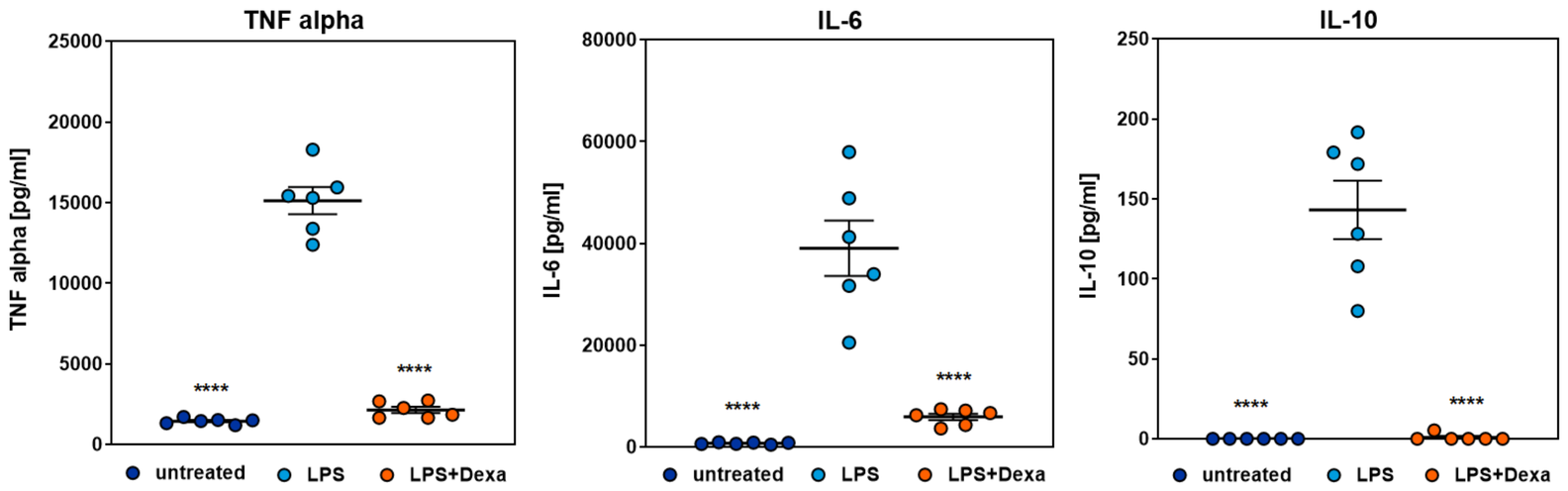

Figure 2: Cytokine release by mouse primary microglia after LPS stimulation for 12 h
Data are displayed as aligned dot blots with group means (n=6 per group). One-way ANOVA followed by Bonferroni Multiple Comparison Test (*posthoc* test) compared to LPS group ****p<0.0001.

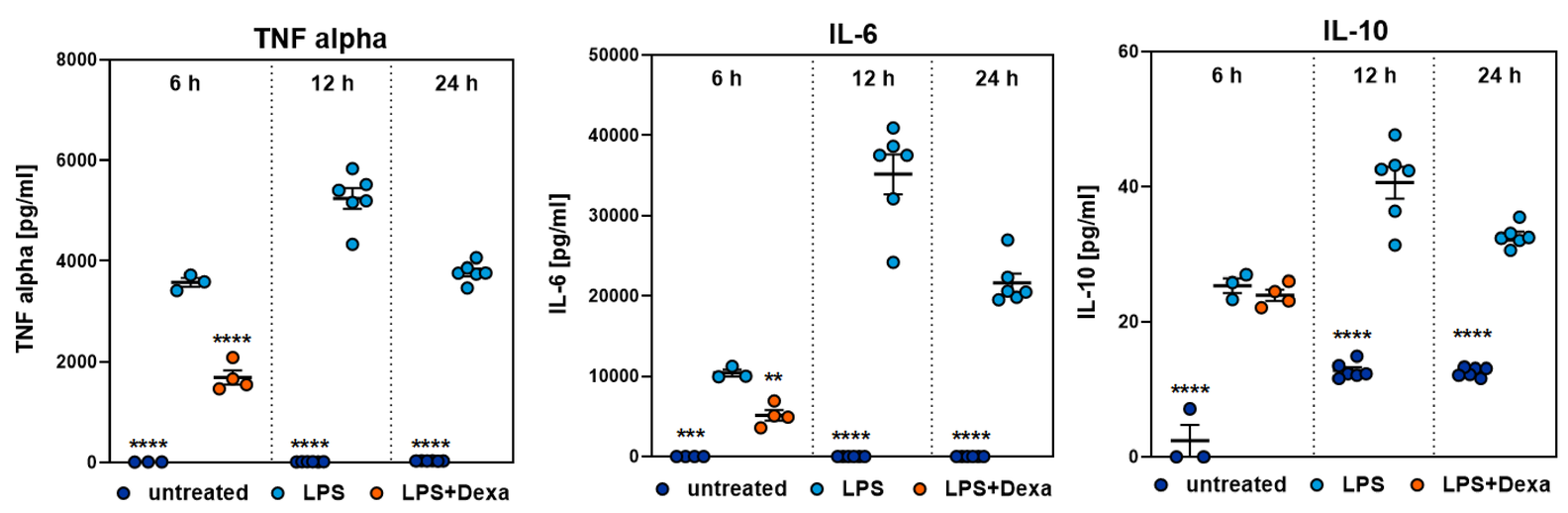

Figure 3: Cytokine release by mouse microglial cell line after LPS stimulation over time
Data are displayed as aligned dot blots with group means (n=3-6 per group). Two-way ANOVA followed by Bonferroni Multiple Comparison Test (*posthoc* test) compared to LPS group **p<0.01, ***p<0.001, ****p<0.0001.

SUMMARY and CONCLUSION

All three tested *in vitro* approaches serve as good models to evaluate the effect of different compounds on neuroinflammation and its related neurodegenerative diseases.

Contact for more information about the model:
Birgit Hutter-Paier, PhD | Director Neuropharmacology
| QPS Austria GmbH | Parkring 12 | 8074 Grambach |
Austria
birgit.hutter-paier@qps.com | www.qpsneuro.com