Evaluation of Human *ex vivo* and Rodent *in vivo* Models of Inflammation for CNS Anti-Inflammatory Drug Development

Transpharmation
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L.R. Davison[1], D. Kozareva[1], F. Dunphy-Doherty[1], J.A. Prenderville[1]

[1]*Transpharmation Ireland Ltd., Trinity College Institute of Neuroscience, Dublin, Ireland*

Introduction

Lipopolysaccharide (LPS) is a cell-wall component of gram-negative bacteria and a ligand of Toll-like receptor 4. TLR-4 activation by LPS leads to downstream production and release of pro-inflammatory cytokines through the NFκB pathway. The administration of LPS, therefore, is commonly used to study inflammation-associated disease both in human peripheral blood mononuclear cell (PBMC) and whole blood cultures, as well as *in vivo* in rodents.
Peripheral blood mononuclear cells constitute both innate and adaptive immune cell types that historically have been utilised as a means to identify disease biomarkers and test novel therapeutics for cancers, metabolic and peripheral immune disorders *in vitro*. More recently, PBMCs have garnered much interest as a source of peripheral biomarkers for CNS disorders. Microglial-mediated inflammation is a common theme in CNS disorders and recent research has indicated that central inflammation in these disorders may be mirrored in the periphery through PBMC activation and expression of gene and protein biomarkers. Both PBMCs and whole blood may be obtained from healthy and disease patients to assess the efficacy of novel anti-inflammatory therapeutics on peripheral immune cells, with whole blood having the added benefit of identifying therapeutics that are highly plasma-protein bound in blood, which is a significant obstacle to peripheral administration of CNS-targeted drugs.
The use of rodents for anti-inflammatory drug screening is well established and is the driving force in pre-clinical drug efficacy and toxicity testing for progression to clinical trials. LPS administered peripherally can be used to induce both peripheral and central inflammation, from which novel therapeutics may be tested for anti-inflammatory effects in the CNS. Here, we show that LPS induces inflammatory cytokine production *ex vivo* in humans and *in vivo* in rodents, and that various anti-inflammatory therapeutics effectively attenuate LPS-induced inflammation.

Methods

Methods 1: PBMCs

- PBMCs from healthy donors, male (n=3) and female (n=2)
- 45 mins SB239063 or TAK-242 pre-treatment, 24 hours 100ng/ml LPS
- Supernatant cytokines analysed via Meso Scale Diagnostics assay

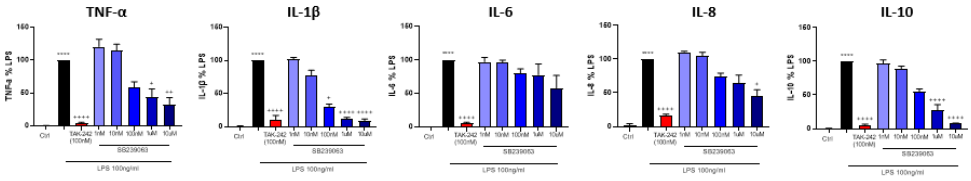
Methods 2: Whole Blood

- n=6 healthy donors
- ± 1 hour pre-treatment with TAK-242/MCC950
- 4 hour LPS treatment (8 concentrations) ± 5mM ATP for final 30 mins
- Supernatant cytokines analysed via Meso Scale Diagnostics assay

Methods 3: Rat i.p. injections

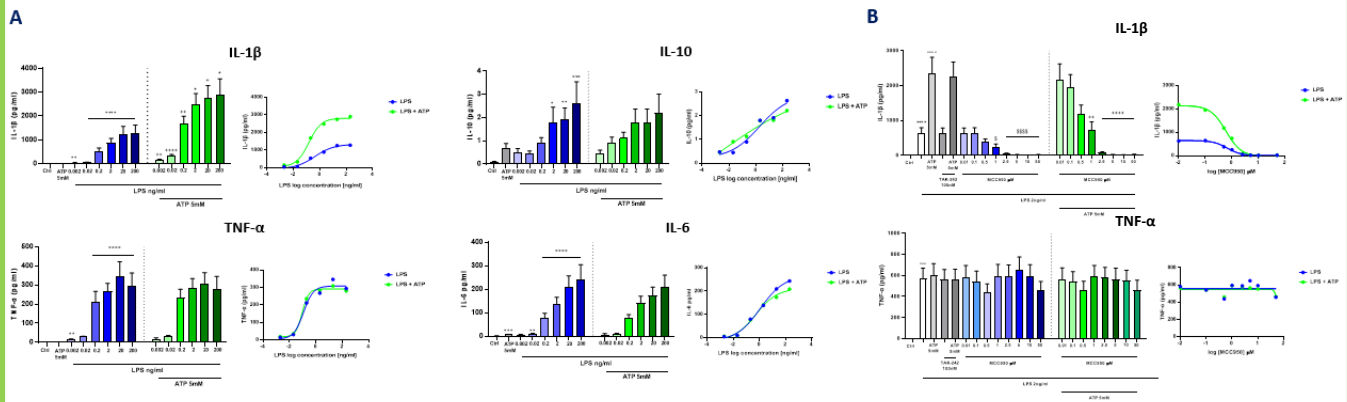
- n=10 rats per group
- 2 hour pre-treatment with dexamethasone (1mg/kg p.o.), then 1 hour 0.1mg/kg LPS i.v.
- Plasma cytokines analysed via Meso Scale Discovery

Results 1



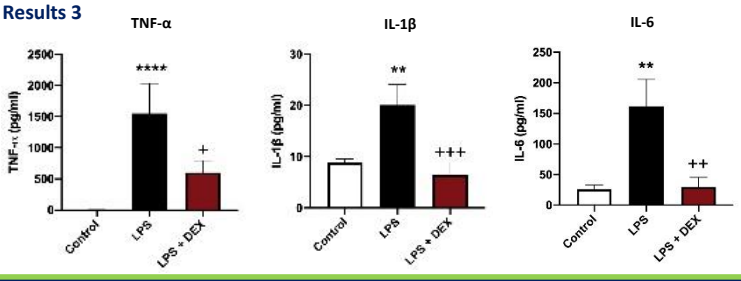
Pre-treatment of human PBMCs with the P38 MAPK inhibitor SB239063 produced a dose-dependent reduction in LPS-induced cytokine expression.
10μM SB239063 treatment significantly reduced LPS-induced expression of all cytokines measured bar IL-6. Data presented as mean ± SEM, One-way ANOVA with Fisher's LSD post-hoc test. ****P<0.0001 vs. control, ⁺P<0.05, ⁺⁺P<0.01, ⁺⁺⁺⁺P<0.0001 vs. LPS. n=5

Results 2



Pre-treatment of human whole blood with the specific NLRP3-inhibitor MCC950 significantly attenuated LPS/ATP-induced NLRP3 inflammasome activation.
A) LPS-treatment of human whole blood produced a dose-dependent increase in cytokine expression which was further increased by the addition of 5mM ATP for IL-1β alone. **B)** Pre-treatment of human whole blood with MCC950 produced a significant reduction in both LPS and LPS/ATP-induced IL-1β but not TNF-α. Data presented as mean ± SEM, One-way ANOVA with Fisher's LSD post-hoc test. *P<0.05 **P<0.01, ***P<0.001 ****P<0.0001 vs. control, ⁺P<0.05, ⁺⁺P<0.01, ⁺⁺⁺⁺P<0.0001 vs LPS (LPS/ATP for figure B), $P<0.05 $$$$P<0.0001 vs. LPS. n=6

Results 3



Pre-treatment with dexamethasone significantly reduced LPS-induced cytokine production peripherally in rats.
A) Pre-treatment with dexamethasone produces a significant reduction in LPS-induced cytokine production in rat plasma. Data presented as mean ± SEM, One-way ANOVA with Fisher's LSD post-hoc test. **P<0.01, ****P<0.0001 vs. control, ⁺P<0.05, ⁺⁺ P<0.01 ⁺⁺⁺P<0.001 vs. LPS. n=10

Results

- Results 1: Pre-treatment of human PBMCs with the P38 MAPK inhibitor SB239063 produced a dose-dependent reduction in LPS-induced cytokine expression.
- Results 2: Pre-treatment of human whole blood with the specific NLRP3-inhibitor MCC950 significantly attenuated LPS/ATP-induced NLRP3 inflammasome activation
- Results 3: Pre-treatment with dexamethasone significantly reduced LPS-induced cytokine production peripherally in rats

Conclusion

- LPS is a potent pro-inflammatory stimulant that can induce robust inflammation both in human peripheral immune cell cultures and peripherally in rodents
- Both *ex vivo* human immune cultures and rodents are useful models of inflammation that allow for novel inhibitors of a number of pro-inflammatory signalling pathways to be evaluated for anti-inflammatory efficacy in a semi high-throughput manner
- Future studies will investigate efficacy of anti-inflammatory therapeutics in disease-patient immune cultures e.g. multiple sclerosis, Alzheimer's disease.