

# MyD88 knockout mice exhibit neuroprotection in LPS-HI induced brain injury in neonatal mice.

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Inflammation can alter the vulnerability to a later insult (so-called sensitization or preconditioning). For instance, we have previously shown that lipopolysaccharide (LPS) exposure can increase the sensitivity to hypoxia-ischemia (HI) during development. In the adult, the effects of LPS are mediated by its binding to toll-like receptor 4, which requires recruitment of the myeloid differentiation factor 88 (MyD88) adaptor protein for downstream signal transduction. However, the role of MyD88 in neonatal brain injury is unknown. Thus, the aim of this study was to examine the effects of LPS in combination with HI on brain injury in neonatal MyD88 knockout (KO) mice.

Method: Littermates of mixed genotype (MyD88 KO and wild type (WT)) were subjected to left carotid artery ligation and 10% O<sub>2</sub> for 40min (HI) on PND9. LPS (0.1mg/kg, i.p.) or saline were given at 14h before HI. At 3 days post HI, brains were perfused and examined macro- and microscopically. Immunostaining for myelin basic protein (MBP), microtubule associated protein-2 (MAP-2), and induction of ionized calcium binding adapter molecule 1 (Iba-1) were examined.

Results: In WT mice, LPS in combination with HI showed a significant increase in infarct volume (LPS/HI: 18.07±2.42 mm<sup>3</sup>, N=11 vs. saline/HI: 5.57±1.22 mm<sup>3</sup>, N=12, P < 0.001), and tissue loss (LPS/HI: 57.46 ± 3.93 mm<sup>3</sup>, N=11 vs. saline/HI: 35.53 ± 3.13 mm<sup>3</sup>, N=12, P < 0.001), but not in atrophy (LPS/HI: 39.39 ± 2.76 mm<sup>3</sup>, N=11 vs. saline/HI: 29.96 ± 2.59 mm<sup>3</sup>, N=12, P > 0.05). There was also a significant decrease in immunostaining for MBP in the subcortical white matter (LPS/HI: 74.50 ± 2.60% loss, N=10 vs. saline/HI: 31.00 ± 8.22% loss, N=11, P < 0.001). In the MyD88 KO animals there were no differences between the treatment groups in infarct volume (LPS/HI: 5.91 ± 1.15 mm<sup>3</sup>, N=11 vs. saline/HI: 3.36 ± 0.40 mm<sup>3</sup>, N=13, P > 0.05), atrophy (LPS/HI: 27.51 ± 2.46 mm<sup>3</sup>, N=11 vs. saline/HI: 26.65 ± 2.00 mm<sup>3</sup>, N=13 P > 0.05), tissue loss (LPS/HI: 33.41 ± 3.49 mm<sup>3</sup>, N=11 vs. saline/HI: 30.00 ± 2.19 mm<sup>3</sup>, N=13, P > 0.05), and MBP immunostaining (LPS/HI: 27.27 ± 6.58 % loss, N=11 vs. saline/HI: 22.50 ± 6.06 % loss, N=12, P > 0.05). Data from the Iba-1 staining is still under evaluation.

Conclusions: These data demonstrate that the MyD88 pathway is important in neonatal LPS-HI induced brain injury. However, further studies are needed to investigate the precise mechanism and the role of microglia in this process.