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Characterisation of TLR4 and TLR7/8-Induced Pro-Inflammatory Signals in Cortical Astrocyte Cultures

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Neuroinflammation is a principal pathological feature of a number of neurological diseases. Activated astrocytes and microglia play a key role in the inflammatory response by augmenting the expression and release of inflammatory mediators. Toll-like receptors (TLR), expressed on glial cells, are pattern recognition receptors that initiate the innate immune response to CNS injury. While neuroinflammatory signals in the CNS are often studied using lipopolysaccharide (LPS) acting through extracellular TLR4 receptors, emerging data suggest that endosomal TLR7/8 receptors may also be important in these processes and in neurodegeneration[1]. In the present study, LPS and the TLR7/8-selective agonist, R848 (resiquimod) were used to investigate the function of these receptors in astrocyte-enriched cultures (consisting of 60% GFAP positive astrocytes, prepared as described previously[2]). All data described are $n \geq 3$ using independent glial preparations and statistical analysis for each data set was carried out using an unpaired student t-test (GraphPad Prism 6). LPS (1 pg/ml - 10 µg/ml) produced a significant ($p<0.05$ compared to control) time- and concentration-dependent release of inflammatory cytokines including tumour-necrosis factor–α (TNF-α, EC_{50} = 2.0 ng/ml, n = 5) and interleukin-6 (IL-6, EC_{50} = 6.3 ng/ml, n = 5) at 4 and 18 h respectively, as determined using ELISA. Immunoblot analysis also showed that LPS (10 µg/ml) induced a robust, time-dependent increase in the expression of inducible nitric oxide (iNOS) which reached significance ($p<0.05$ compared to control samples, n = 3) at 24 h, while GAPDH expression levels were unchanged. R848 (2 ng/ml - 3 µg/ml) also induced cytokine release in a concentration-dependent manner (EC_{50} = 79.4 and 100 ng/ml for TNF-α and IL-6, respectively, n = 4), but with lower maximal efficacy (64 ± 8.7% and 39 ± 6.4% relative to LPS for TNF-α and IL-6). Further characterisation showed that while the synthetic glucocorticoid, prednisolone, potently attenuated TLR4 and TLR7/8-mediated TNF-α release (pIC_{50} = 8.0 ± 0.2 and 8.3 ± 0.1, respectively), this response was insensitive to classical NSAIDs such as aspirin (up to 10 µM). ER-358063, a small molecule compound shown to exhibit inhibitory activity at c-jun NH2-terminal kinases (JNK1-3)[3], produced a robust inhibition of both LPS and R848-induced TNF-α responses with a similar potency (pIC_{50} = 6.2 ± 0.1 and 6.5 ± 0.1, respectively, n = 4). This study suggests that both functional TLR7/8 and TLR4 receptors are expressed in rat cortical astrocyte-enriched glial cultures; although cytokine signals mediated by the TLR7/8-selective agonist, R848, are markedly lower than those activated by LPS. Despite these receptors exhibiting distinct subcellular locations, inhibitor experiments point towards a convergence of glucocorticoid sensitive-nuclear factor κB transcription and JNK activation in mediating both TLR4 and TLR7/8 mediated cytokine responses in these cells.

1. Lehmann SM et al. Nat Neurosci. 15: 827, 2012

2. Cohen, J & Wilkin, G.P Neural Cell Culture—A Practical Approach, 85–96, 1995.

3. Graczyk PP Med Chem. 50: 5773, 2007