Modulation of LPS-induced alterations in peripheral cytokine levels by the endocannabinoid system

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Several studies have reported the immunosuppressive effects of exogenously administered cannabinoids on peripheral, circulating cytokines (Smith et al., 2000; Croci et al., 2003, Roche et al. 2006). The aim of the present study was to investigate *in vivo* regulation of circulating pro-inflammatory cytokine levels by the endocannabinoid system.

Male Sprague-Dawley rats (220-260g, groups of 6-9) were housed singly and habituated to handling and i.p. injection for 4 days. Rats then received an acute i.p. injection of the fatty acid amide hydrolase inhibitor URB597 (0.6 mg kg^{-1}), putative monoacylglycerol lipase (MGL) inhibitor URB754 (13 mg kg^{-1}), AM251 (3 mg kg^{-1}), AM630 (3 mg kg^{-1}), URB597 + AM251, URB597 + AM630, URB754 + AM251, URB754 + AM630 or vehicle (ethanol: tween 80: saline) 30 minutes prior to i.p. administration of lipopolysaccaride (LPS) (100 μg/kg) or saline. Under CO_2 anaesthesia, blood was taken via cardiac puncture 2 hours post-LPS administration. Plasma levels of interleukin (IL)-1β, tumour necrosis factor α (TNFα) and IL-6 were determined using ELISA. Data were analysed by ANOVA and Student-Newman-Keuls *post-hoc* test and expressed as mean (pg ml^{-1}) ± SEM.

LPS administration increased TNF-α (610 ± 110; $P<0.01$), IL-1β (382 ± 84; $P<0.01$), and IL-6 (10692 ± 1461; $P<0.01$) in the plasma compared to saline-treated controls (TNF-α: 4 ± 1, IL-1β: 28 ± 8, IL-6: 129 ± 32). URB597 potentiated the LPS-induced increase in TNF-α levels (831 ± 164; $P<0.05$). The immunostimulatory effect of URB597 was attenuated by both the CB_1 and CB_2 receptor antagonists, AM251 (143 ± 32; $P<0.01$) and AM630 (221 ± 69; $P<0.01$) respectively. URB754 attenuated the LPS-induced increase in IL-1β (162 ± 39; $P<0.01$), TNF-α (85 ± 28; $P<0.01$) and IL-6 (3968 ± 877; $P<0.01$), an effect not altered by administration of the CB_1 and CB_2 receptor antagonists. The LPS-induced increase in TNF-α was attenuated by both AM251 (121 ± 37; $P<0.01$) and AM630 (261 ± 92; $P<0.01$) when administered alone and AM251 also partially attenuated the increase in plasma IL-6 (5542 ± 946; $P<0.05$).

The effects of URB597 suggest that anandamide plays a role in facilitating pro-inflammatory cytokine release via CB_1 and CB_2 receptors. Immunosuppressive effects of the selective cannabinoid receptor antagonists may be due to blockade of the facilitatory effects of anandamide or unmasking of the effects of endocannabinoids at alternative receptor targets. The inability of the CB_1 or CB_2 antagonists to block the effects of URB754 indirectly supports recent evidence that this compound is not a selective inhibitor of MGL, the enzyme responsible for degradation of 2-arachidonoyl glycerol. Elucidating the role of the endocannabinoid system in immune regulation may facilitate development of novel pharmacological agents for the treatment of chronic inflammatory disorders.

Croci et al. (2003) *Br. J. Pharmacol.* **140**:115-122
Roche et al. (2006) *Neuroimmunology* **181**:57-67
Smith et al. (2000) *J. Pharmacol. Exp. Ther*. **293**:136-150

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