Myeloid but not endothelial expression of the CB2 receptor promotes atherogenesis in the context of elevated levels of the endocannabinoid 2-arachidonoylglycerol

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Topic(s):
Basic Science - Vascular Biology and Physiology: Leukocytes, Inflammation, Immunity

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Background:
The endocannabinoid 2-arachidonoylglycerol (2-AG) is an inflammatory mediator and ligand to the cannabinoid receptors CB1 and CB2, which are expressed on myeloid and endothelial cells. 2-AG has recently been described to promote atherogenesis in ApoE-deficient mice. While the CB2 receptor has previously been considered to solely exert anti-inflammatory and atheroprotective effects, newer data have raised the notion, that CB2 might exert atherogenic effects in the context of elevated 2-AG plasma levels. In the present study, we investigated the atherogenic mechanisms of 2-AG and the role of the CB2 receptor on myeloid and endothelial cells in atherogenesis using cell-specific knockout mouse models.

Methods:
Two mouse models with atherogenic background and distinct cell-specific knockouts of the CB2 receptor on myeloid (ApoE-/-/LysMcreCB2fl/fl) or endothelial cells (ApoE-/-/Tie2creCB2fl/fl) were created. Mice were treated with JZL184, which inhibits 2-AG-degrading enzyme monoacylglycerol lipase, and thereby elevates 2-AG plasma levels, or with vehicle (DMSO), while being fed a high-fat diet for four weeks. Plaque volume and plaque composition were analyzed. In vitro, macrophages were treated with 2-AG and mRNA levels of adhesion molecules, scavenger receptors and chemokines, the production of reactive oxygen species (ROS) and the release of myeloperoxidase (MPO) were determined using qPCR, fluorometric assays and ELISA respectively.

Results:
Elevated levels of 2-AG promote atherogenesis in ApoE-deficient mice (JZL184 vs. DMSO: 39.6 ± 2.1% vs. 32.6 ± 2.4%; n = 14; p < 0.05). The atherogenic effect of 2-AG is abrogated in mice lacking myeloid CB2 receptor expression (35.0 ± 2.0% vs. 34.0 ± 2.5%; n = 14-16; p > 0.05) but not in mice lacking endothelial CB2 receptor expression (37.1 ± 3.1% vs. 20.9 ± 2.6%; n = 10-12; p < 0.01). In vitro, 2-AG significantly increases transcription of adhesion molecule ICAM-1 (2.09 ± 0.42 -fold; n = 5-6; p < 0.05), chemokine receptor CCR-1 (2.04 ± 0.46 -fold; n = 10-11; p < 0.05) and scavenger receptor CD36 (8.02 ± 1.89 –fold; n = 3; p < 0.05) in 2-AG-treated macrophages. These effects are mitigated by pharmacological inhibition of CB2. Furthermore, 2-AG significantly increases myeloperoxidase (MPO) release in monocytes in a CB receptor-dependent fashion (451 ± 23 pg/ml vs. 151 ± 8.3 pg/ml; n = 3-4; p < 0.01) and promotes ROS production (2698 ± 24 pdu vs. 1981 ± 27 pdu; n = 8; p < 0.01).

Conclusion:
Elevated 2-AG levels show an atherogenic effect in vivo which is dependent on the presence of the CB2 receptor on myeloid cells. Our in vitro data reveal 2-AG to promote pro-inflammatory signaling in macrophages...
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Conclusion:
Elevated 2-AG levels show an atherogenic effect in vivo which is dependent on the presence of the CB2 receptor on myeloid cells. Our in vitro data reveal 2-AG to promote pro-inflammatory signaling in macrophages and elucidate a previously unrecognized link between the endocannabinoid system and MPO in monocytes. In summary, cell-specific effects of the endocannabinoid system will have to be taken into account to facilitate its exploitation as an anti-atherosclerotic drug target.