Cell Research (2011) 21:1391-1392.

www.nature.com/cr

RESEARCH HIGHLIGHT

New blood brothers: the GPR55 and CB_2 partnership

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Cell Research (2011) **21**:1391-1392. doi:10.1038/cr.2011.77; published online 3 May 2011

Endocannabinoids are increasingly being recognized as key lipid-derived regulators of immune function [1]. Although the peripheral cannabinoid type 2 receptor (CB_2) is thought to orchestrate many of these actions, additional non-CB_1/CB_2-mediated effects of cannabinoids have been identified in immune cells [1, 2], where several orphan G protein-coupled receptors (GPCRs), including the effusive GPR55, are implicated [3]. Despite numerous studies addressing the cannabinoid sensitivity of GPR55, the area remains a pharmacological minefield, with much inconsistent and conflicting data. Emerging provocatively from behind the marijuana smokescreen is a rather surprising, endogenous, lipid ligand at GPR55: namely lysophosphadityl-inositol (LPI; [4]). In this issue of *Cell Research*, Balenga and colleagues [5] demonstrates that LPI and the cannabinoid antagonist AM251, have prominent effects on neutrophils that are mediated by GPR55, providing the first compelling evidence for functional GPR55 in blood. In addition, they show that GPR55 forms an intimate but mercurial partnership with the CB_2 receptor and that crosstalk between these lipid-sensing GPCRs is likely to be important in fine-tuning the immune response to abrogate excessive tissue injury.

Cannabinoids exert varied effects in blood and hematopoietic cells, including modulation of proliferation, apoptosis, cytokine production, reactive oxygen species (ROS) generation, chemotaxis and migration. Indeed, both plant-derived cannabinoids and synthetic CB_2 receptor ligands exert well-established anti-inflammatory actions, which are likely be useful therapeutically [1]. However, the cannabinoid immunosuppressant dogma does not always hold true, and recent data suggests that the generation of ROS in neutrophils, a key inflammatory event, is in fact promoted by the endocannabinoid 2 arachidonoyl glycerol (2-AG) acting via the CB_2 receptor [5]. The role of cannabinoids in regulating chemotaxis and directed migration in neutrophils is also controversial, although Balenga and coworkers [5] demonstrate 2-AG-mediated chemotaxis, other studies have not observed this effect [*2*].

There is increasing evidence for non-CB_1/CB_2 pharmacological targets for cannabinoids and associated lipids in the immune system [1, 2, 6]. For example, the endocannabinoid virodhamine and the atypical cannabinoid O-1602 inhibit fMLP-induced migration of human neutrophils via a novel receptor [2] and O-1602 also promotes microglial migration via an interaction with the orphan receptor, GPR18, which was recently heralded as a molecular incarnation of the "abnormal cannabidiol" receptor [6]. Moreover, Schicho and coworkers demonstrated that O-1602 inhibits neutrophil recruitment in a model of experimental colitis, via a mechanism that is independent of GPR55 [7], thereby implicating an additional target, most likely GPR18. Notwithstanding these observations, as now shown by Balenga and colleagues [5], GPR55 is knocking on the door of this exclusive "novel lipid-sensing cannabinoid GPCR club", where they demonstrate that both LPI and the aryl pyrazole cannabinoid antagonist AM251 acts as a GPR55 agonists, promoting RhoA-dependent neutrophil chemotaxis, which is reassuringly consistent with previous data using recombinant GPR55 expressed in HEK293 cells [8].

The function of endogenous GPR55 is only just emerging, however, there is evidence for expression of GPR55 mRNA in immune tissues [9] and microglia, where it may regulate ERK/MAP kinase signaling following activation [10]. Importantly, the study by Balenga and colleagues [5] is now the first to identify a function for GPR55 in leukocytes. GPR55 is unusual in that the majority of its cellular effects are thought to be mediated by $G_{12/13}$ G-proteins and Rho-family GTPases, including RhoA, Rac1 and Cdc42 [9], although a linkage to G_q may occur in certain contexts [3]. The latest report is also consistent with this idea, where LPI/GPR55-provoked effects on cytoskeletal remodeling/chemotaxis are mediated via the $G\alpha_{13}$/RhoA signaling pathway ([5]; see Table 1). Interestingly, no clear effect of GPR55 activation alone on Rac1 or Cdc42 was detected in neutrophils, but

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Table 1 GPR55 and CB_2-mediated signaling in human neutrophils

	LPI	AM251	2AG	LPI/2-AG	AM251/2-AG
Chemotaxis (RhoA)	++	++	++	+++	+++++
Rac1	–	n.d.	++	++	n.d.
Cdc42	–	n.d.	+	+++	n.d.
Rac2	↓	n.d.	+	↓↓	n.d.
ROS generation	–	n.d.	+++	+	n.d.

GPR55 was activated either with AM251 (3 μM) or LPI (1-3 μM). CB_2 receptors were stimulated using 2-AG (1 μM). Data was extracted from reference [5]. Key: positive effect (+), no effect (–), inhibition (↓), not tested (n.d.).

GTP-Rac2 levels were lowered by LPI treatment, which is important for CB_2 receptor crosstalk.

Importantly, Belanga and coworkers identify novel functional interactions between GPR55 (activated with LPI or AM251) and CB_2 (activated with 2-AG) receptors in neutrophils. In these cells signaling pathways involving RhoA and Cdc42 are enhanced when both receptors are activated and this is recapitulated using analysis of NFAT activation in HEK293 cells expressing both receptors. In some cases, there is a clear synergism, for example with AM251- and 2-AG-mediated effects on RhoA- mediated chemotaxis (see Table 1). A negative interaction between GPR55 and CB_2 is however observed at the level of ROS generation during the "respiratory burst"- a mechanism used by neutrophils to resolve infection. This effect involves crosstalk at the level of the small GTPase Rac2, where LPI markedly represses the response to 2-AG [5]. Negative cooperativity between CB_2 receptors and a novel SR141716A-sensitive, non-CB_1/CB_2 pharmacological target inhibiting migration has also been noted previously in neutrophils [2]. Many of the features of this latter site are consistent with the "abnormal cannabidiol" receptor although pharmacological crossover with GPR55 (for example when using O-1602 or abnormal cannabidiol) is likely to complicate the picture.

Thus in summary, CB_2 and GPR55 initially work together with mutually enhanced Rho signaling and directed migration to seek out sites of inflammation. Ultimately they disengage, and in a graceful process of functional repression, GPR55 acts to limit CB_2 receptor-mediated collateral damage. In future studies, it will be interesting to extrapolate this GPCR crosstalk into other systems, for example in microglia that express both GPR55 and CB_2 receptors. *In vivo* studies involving GPR55 knockout animals will also help to reveal the role of this receptor in regulating immune function and in models of inflammatory disease. A more detailed profiling of GPR55 expression in different immune cells is required, together with further evaluation of the repertoire of molecular targets for cannabinoids, LPI and related lipids in the immune system.

References

1 Pacher P, Mechoulam R. Is lipid signaling through cannabinoid 2 receptors part of a protective system? *Prog Lipid Res* 2011; **50**:193-211.
2 McHugh D, Ross RA. Endogenous cannabinoids and neutrophil chemotaxis. *Vitam Horm* 2009; **81**:337-365.
3 Nevalainen T, Irving AJ. GPR55, a lysophosphatidylinositol receptor with cannabinoid sensitivity? *Curr Top Med Chem* 2010; **10**:799-813.
4 Oka S, Nakajima K, Yamashita A, Kishimoto S, Sugiura T. Identification of GPR55 as a lysophosphatidylinositol receptor. *Biochem Biophys Res Commun* 2007; **362**:928-934.
5 Balenga NA, Aflaki E, Kargl J, *et al*. GPR55 regulates cannabinoid 2 receptor-mediated responses in human neutrophils. *Cell Res* 2011; **21**:1452-1469.
6 McHugh D, Hu SS, Rimmerman N, *et al*. N-arachidonoyl glycine, an abundant endogenous lipid, potently drives directed cellular migration through GPR18, the putative abnormal cannabidiol receptor. *BMC Neurosci* 2010; **11**:44.
7 Schicho R, Bashashati M, Bawa M, *et al*. The atypical cannabinoid O-1602 protects against experimental colitis and inhibits neutrophil recruitment. *Inflamm Bowel Dis* 2011; **17**:1651-1664.
8 Henstridge CM, Balenga NA, Ford LA, Ross RA, Waldhoer M, Irving AJ. The GPR55 ligand L-alpha-lysophosphatidylinositol promotes RhoA-dependent Ca^{2+} signaling and NFAT activation. *FASEB J* 2009; **23**:183-193.
9 Ryberg E, Larsson N, Sjögren S, *et al*. The orphan receptor GPR55 is a novel cannabinoid receptor. *Br J Pharmacol* 2007; **152**:1092-1101.
10 Pietr M, Kozela E, Levy R, *et al*. Differential changes in GPR55 during microglial cell activation. *FEBS Lett* 2009; **583**:2071-2076.