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The impact of cannabinoid receptor 2 (CB2) deficiency on neutrophil recruitment and inflammation

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Abstract

Neutrophil trafficking into damaged or infected tissues is essential for the initiation of inflammation, clearance of pathogens and damaged cells, and ultimately tissue repair. Neutrophil recruitment is highly dependent on the step-wise induction of adhesion molecules and pro-migratory chemokines and cytokines. A number of studies in animal models have shown the efficacy of CB_2 agonists in limiting inflammation in a range of pre-clinical models of inflammation, including colitis, atherosclerosis, multiple sclerosis and ischemia-reperfusion injury. Recent work in pre-clinical models of inflammation raises two questions; by what mechanisms do CB_2 agonists provide anti-inflammatory effects during acute inflammation and what challenges exist in the translation of CB_2 modulating therapeutics into the clinic.

Neutrophil recruitment in acute inflammation

Neutrophil migration into inflamed tissues is driven by soluble factors released by activated endothelial and tissue resident immune cells. As inflammation reduces vascular flow rates, neutrophils in the blood undergo margination, selectin-mediated weak adhesion and rolling (Zarbock and Ley, 2009). The process of neutrophil firm adhesion and migration is mediated by G-protein coupled receptors (GPCRs) and integrin ligation (Lefort and Ley, 2012). Interactions between lymphocyte function-associated antigen 1 (LFA-1), an αβ-integrin, and its ligand intracellular adhesion molecule 1 (ICAM-1) facilitates neutrophil recruitment by inducing pro-migratory changes including; further integrin clustering, cytoskeletal changes, increases in intracellular calcium levels and weakening of endothelial cell: endothelial cell junctions (Ley et al., 2007).

Following migration through the endothelium, neutrophils will cross the pericyte sheath and vascular basement membrane into inflamed tissues (Nourshargh et al., 2010). Remodelling of the tissue is essential for neutrophil recruitment under physiological and pathophysiological conditions; enzymatic degradation of the basement membrane by matrix metalloproteinase-9 (MMP-9) being an example (Yabluchanskiy et al., 2013). Clinically, stroke patients have been found to have increased circulating levels of MMP-9, this is accompanied by remodelling of the blood brain barrier and neutrophil infiltration (Turner and Sharp, 2016). In murine models, MMP-9 deficient mice had been observed to have reduced counts of airway infiltrating neutrophils following influenza infection; decreased neutrophil recruitment was accompanied by increased viral titres in these mice (Bradley et al., 2012).

Recently, there has been increasing interest in the effect of neurotransmitters/neurochemicals on leukocyte migration. The cannabinoid system has received continuous attention, now more so than ever, against the backdrop of increased recreational and medicinal use of cannabis, cannabinoids and cannabinoid-like compounds (Kerage et al., 2019).

Cannabinoid receptor regulation of inflammation

Cannabinoid receptors 1 and 2 (CB_1 and CB_2) are class A rhodopsin-like GPCRs with endogenous lipid ligands. CB_1 is highly expressed throughout the central nervous system (CNS) accompanied by restricted expression of CB_2 in the brain (Laprairie et al., 2012;Chen et al., 2017). CB_1 expression has also been reported in a number of peripheral tissues, such as the liver, pancreas and skeletal muscle. CNS expression of CB_1 mediates the psychoactive effects of cannabinoids such as delta-9-tetrahydrocannabinol (Δ^9-THC) (Mackie, 2007). CB_1 expression outside the CNS may play an important role in appetite regulation and tissue repair (Dodd et al., 2010;Cooper and Regnell, 2014;Krott et al., 2016). CB_2 is most widely expressed in cells of the immune system, including but not restricted to, B-cells, monocytes, neutrophils and in particular, cells derived from macrophages (Galiègue et al., 1995;Van Sickle et al., 2005;Ofek et al., 2006). CB_1 and CB_2 activation cause the downstream activation of a number of signalling pathways (Figure.1) (Mackie, 2008).

Pharmacological activation of cannabinoid receptors has been reported to improve the clinical outcomes of a number of chronic inflammatory conditions in mice. For example, by increasing the half-life of 2-arachidonoylglycerol (2-AG), an endogenous CB_1 and CB_2 ligand, Alhouayek *et al.* reported a marked reduction in colonic and

peripheral inflammation in mice subjected to chemically induced colitis. The authors demonstrated that increased circulating levels of 2-AG reduced weight loss, decreased pro-inflammatory cytokine levels, improved colonic histological endpoints and intestinal barrier integrity (Alhouayek et al., 2011). The synthetic CB_2 ligand JWH-133 displayed similar efficacy in attenuating chemically induced colitis in IL-10^{-/-} mice. Singh *et al.* have shown that JWH-133 treated colitis mice presented with reduced activated leukocytes infiltrating the intestinal lamina propria and mesenteric lymph nodes (Singh et al., 2011). Synthetic activation of CB_2 by using low oral doses of Δ^9-THC in atherosclerotic mice has also been shown to be effective at ameliorating disease progression; immune cells recovered from Δ^9-THC treated mice had diminished proliferative capacity and reduced interferon-gamma secretion. Increases in interferon-gamma secretion was of particular significance as it correlates to vascular plaque progression from stable to unstable state, thereby enhancing risk of mortality (Van der Wal and Becker, 1999;Steffens et al., 2005). More recently, Zhao *et al*. have shown that CB_2 activation with the synthetic ligand, WIN55212-2, also reduced plaque burden and immune cell infiltration in atherosclerotic mice. Interestingly, the authors demonstrated that this reduction in vascular disease was due to decreased adhesion molecule expression (Zhao et al., 2010). Alterations in the recruitment of immune cells, including neutrophils, T-cells and macrophages by CB_2 agonists has also been reported in a number of pre-clinical models of human inflammatory conditions, including, sepsis (Csóka et al., 2009;Sardinha et al., 2014), multiple sclerosis (Maresz et al., 2007;Wen et al., 2015) and ischemia-reperfusion injury (Di Filippo et al., 2004;Bátkai et al., 2012).

For the most part, the vast majority of pre-clinical studies focus entirely on the use of CB_2 deficient mice or CB_2 specific pharmaceuticals. Importantly, Wen *et al.*

demonstrated that the anti-inflammatory effects of the CB$_2$ agonist WWL70 in wild-type mice undergoing experimental autoimmune encephalomyelitis were lost in CB$_2$ deficient mice verifying the efficacy of CB$_2$ agonists in targeting immune cell recruitment (Wen et al., 2015).

Effects of CB$_2$ deficiency on neutrophil recruitment

Kapellos *et al.* have built further on previous pre-clinical studies by studying the phenotype of immune cell trafficking in CB$_2$ deficient animals. The majority of the pre-clinical studies on the effects of cannabinoid receptors on myeloid cell biology *in vitro* and *in vivo* have used pharmacological tools rather than global or cell type specific knockout mice. A clear example of the dangers of relying solely on cannabinoid receptor agonists and antagonists is provided by the studies of Taylor *et al.* who demonstrated that macrophage chemotaxis in response to well characterised CB$_2$ agonists occurred with equal potency in CB$_2$ deficient mice (Taylor et al., 2015).

Under steady state, mice with global deficiencies in CB$_2$ were shown to have elevated neutrophil counts in the bone marrow (Kapellos et al., 2017). The observed changes in neutrophil counts may result from endogenous CB$_2$ agonists contributing to either myelopoiesis and/or the egress of myeloid cells from the bone marrow under normal physiological conditions (McHugh et al., 2008;Pasquevich et al., 2015). Despite not observing any significant changes in neutrophil recruitment to the lung following the induction of endotoxemia, Kapellos and colleagues reported a greater retention of neutrophils in the spleen of CB$_2$ deficient mice 2 hours following LPS administration. Levels of pro-inflammatory cytokines and soluble factors were observed to be significantly elevated in the spleens of CB$_2$ deficient mice, namely, CXCL10, CCL3,

IL-6 and MMP-9. Additionally, MMP-9 was found to be significantly elevated in the serum of CB_2 deficient animals. The authors concluded that CB_2 deficiency delayed the host response to LPS by decreasing neutrophil migration into and within lymphoid organs, reducing immune cell cross-talk with neutrophils, and pro-longing host exposure to LPS (Kapellos et al., 2017).

In a subsequent study using the dorsal air pouch model of inflammation, Kapellos and colleagues observed enhanced neutrophil recruitment in $CB_2^{-/-}$ air pouches following zymosan injection. Enhanced neutrophil recruitment was independent of any changes in circulating blood cell counts. Importantly, the authors confirmed the effect of CB_2 deficiency was directly affecting neutrophils as bone marrow chimera experiments demonstrated that CB_2 deficiency on haematopoietic cells, but not stromal cells, led to increased immune cell recruitment in response to inflammation. Following zymosan administration, CB_2 deficient neutrophils overexpressed pro-migratory genes and their air pouch exudates were observed to have increased levels of pro-inflammatory cytokines and factors, like MMP-9, CCL22 and CXCL10. Interestingly, purified CB_2 deficient neutrophils were reported to have greater adherence to immobilised ICAM. In the same study the authors explored the effects of pharmacological modulation of CB_2 signalling on human neutrophils. Human neutrophils treated with the CB_2 agonist, JWH133, were shown to have reduced adhesion and transmigration across cytokine stimulated endothelial cell monolayers (Kapellos et al., 2019).

While CB_2 is largely seen as a GPCR that mediates anti-inflammatory effects, there are some studies which propose a pro-inflammatory role for CB_2 agonists both *in vivo* and *in vitro* (Turcotte et al., 2015). A potential explanation for these differences in various pre-clinical models of inflammation are variations in endocannabinoid levels

between different tissue microenvironments in combination with which cell types are responsible for driving immune cell recruitment and the progression of inflammation.

Outlook and future perspectives

Currently, the recreational and medicinal use of cannabinoids, whether in the form of cannabis, synthetic cannabinoids or cannabis extracts such as cannabidiol (CBD), THC or cannabis oils, is at an all-time high with ever increasing societal acceptance and changes in local and national legislation. Cannabis oils and cannabis itself, require special attention as the both contain a mixture of CBD, THC or Δ^9-tetrahydrocannabivarin (Δ^9-THCV), all three are able to interact with both CB_1 and CB_2. Irrespective of the ability of cannabinoids to modulate neutrophil recruitment via interactions with CB_2, there are some important considerations before translating basic science findings to the clinic.

A challenge in producing compounds to modulate CB_1 or CB_2 signalling in vivo is the specificity of any one compound for either receptor. For example, Gp-1a was reported as a selective agonist for CB_2 but Soethoudt *et al.* demonstrated its promiscuity in being able to bind both cannabinoid receptors. With regards to Δ^9-THC and 2-AG, they have been reported to have a >100-fold binding bias for CB_2, however, interactions with CB_1 were still observed (Mussinu et al., 2003;Soethoudt et al., 2017). In addition to issues with specificity, CBD, a CB_1 and CB_2 ligand, behaves very differently dependent on its concentration. It has been reported that CBD behaves as an antagonist of CB_1 at µM concentrations but an inverse agonist at nM concentrations (Thomas et al., 2007). Current data also indicates that cannabinoids may interact at sites other than the orthosteric sites on cannabinoid receptors (Pertwee, 2008). What

impact concentration and off target effects can have on CB_2 interactions across a range of different CB_2 ligands still requires further investigation. However, the use of pre-clinical models lacking global expression of CB_2 is one way to better understand the potential immune modulating effects of CB_2 activation in humans without the biochemical limitations currently associated with the use of pharmacological tool compounds alone for research.

Aside from biochemical considerations, genetic studies in mice have highlighted a potential pitfall of CB_2 agonists as therapeutics. CB_2 deficiency seemingly prolonged host exposure to LPS, decreased viral clearance and broke down immune cell cross talk, such as neutrophil migration to the marginal zone of the spleen for involvement in antigen presentation to T-cells (Kesteman et al., 2008;Bradley et al., 2012;Kapellos et al., 2017). CB_2 deficiency also reduced levels of the factor MMP-9, similar to the effects seen in animals treated with the CB_2 antagonist, SR144528 (Netherland et al., 2010). In line with this, CB_2 agonists have been reported to inhibit MMP-9 release in pre-clinical models of inflammation (Netherland et al., 2010;Kapellos et al., 2019). This raises questions, as with all anti-inflammatory therapeutics; will CB_2 agonists leave individuals more susceptible to infections?

With regards to the clinical efficacy of a CB_2 agonist, lessons can be drawn from the CB_1 antagonist, rimonabant. Rimonabant was developed as a first in class drug intended for treating obesity by aiding weight-loss. After gaining FDA approval in 2006, the drug was withdrawn worldwide after two years. Detrimentally, rimonabant induced psychiatric alterations inducing depression, anxiety and suicidal ideation (Leite et al., 2009). With the restricted expression of CB_2 in the brain, it remains unclear how a CB_2 specific agonist would impact the psychiatric health of recipients (Rogers, 2015).

Despite this potential issue a number of CB_2 agonists, such as GW842166X and LY2828360, have been taken to clinical trials in the context of pain management (Dhopeshwarkar and Mackie, 2014). Interestingly, these compounds were found not to provide meaningful analgesia despite the efficacy observed in pre-clinical models of pain management (Ostenfeld et al., 2011;Pereira et al., 2013). However, The CB_2 agonist, lenabasum, successfully passed phase 2 trials in the context of dermatomyositis where the compound was observed to provide greater improvement in a dermatological damage index after four weeks of administration (Werth et al., 2018). Lenabasum is currently being used in a phase 3 clinical trial for patients with dermatomyositis with a completion date of September 2021 (Werth et al., 2019). For now, the administration of candidate CB_2 agonists during clinical trials has been short term in nature, whether long-term use of CB_2 agonists recreates the psychiatric side-effects remains to be seen. Overall, whether a CB_2 specific agonist will fare any better than rimonabant, or if the efficacy observed in animals is comparable to that in humans, is something that requires greater investigation in acute inflammation in humans.

Various challenges still remain when considering cannabinoids as efficacious and specific therapeutics but the use of pre-clinical disease models in CB_2 deficient animals highlight that CB_2 agonists merit serious consideration as new class of anti-inflammatory drug candidates.

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Conflict of interest disclosures

The authors declare no conflicts of interest regarding the publication of this paper.

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