Expression and Functions of the CB₂ Receptor in Human Leukocytes

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The cannabinoid CB₂ receptor was cloned from the promyeloid cell line HL-60 and is notably expressed in most, if not all leukocyte types. This relatively restricted localization, combined to the absence of psychotropic effects following its activation, make it an attractive drug target for inflammatory and autoimmune diseases. Therefore, there has been an increasing interest in the past decades to identify precisely which immune cells express the CB₂ receptor and what are the consequences of such activation. Herein, we provide new data on the expression of both CB₁ and CB₂ receptors by human blood leukocytes and discuss the impact of CB₂ receptor activation in human leukocytes. While the expression of the CB₂ mRNA can be detected in eosinophils, neutrophils, monocytes, B and T lymphocytes, this receptor is most abundant in human eosinophils and B lymphocytes. We also review the evidence obtained from primary human leukocytes and immortalized cell lines regarding the regulation of their functions by the CB₂ receptor, which underscore the urgent need to deepen our understanding of the CB₂ receptor as an immunoregulator in humans.

Keywords: CB₂ receptor, eosinophil, neutrophil, monocyte, lymphocyte, inflammation, asthma, allergy

INTRODUCTION

The cannabinoid receptors 1 and 2 (CB₁ and CB₂) are two G protein-coupled receptors that function through binding a vast array of ligands including phytocannabinoids and endocannabinoids (Di Marzo et al., 1998; Turcotte et al., 2015). The CB₁ receptor, highly expressed in the brain, was the first cannabinoid receptor identified through its responsiveness to Δ⁹-tetrahydrocannabinol (Δ⁹-THC) and cloned (Devane et al., 1988; Matsuda et al., 1990). Its activation induces psychotropic effects and its involvement shown in, among others, motor function, cognition and memory (Howlett and Abood 2017). It is also widely recognized as worsening obesity and related diseases (Di Marzo 2018). The CB₂ receptor was later cloned from HL-60 cells and identified on its 44% aminoacid homology with the CB₁, as well as its similar binding profile to the endocannabinoid N-arachidonoyl-ethanolamine (AEA) and Δ⁹-THC (Munro et al., 1993). Soon after, Galiègue et al. documented that it was expressed by human leukocytes (Galiègue et al., 1995). This consolidated the concept that the CB₂ is the peripheral cannabinoid receptor and, for many, the inflammatory cannabinoid receptor. In fact, the CB₂ receptor has been found in all leukocyte populations tested so far [see...
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**CB₂ Receptor vs. Human Leukocytes**

**EXPRESSION OF THE CB₁ AND CB₂ RECEPTORS BY HUMAN BLOOD LEUKOCYTES**

Galiègue et al. paved the way to our understanding of CB₂ expression by human leukocytes by showing its mRNA was expressed in human leukocytes, with the following order of relative abundance: tonsillar B cells > natural killer cells > monocytes > granulocytes > T4 lymphocytes > T8 lymphocytes (Galiègue et al., 1995). While very informative and useful, the data from Galiègue et al. did not include eosinophils while including tissue instead of blood B lymphocytes. This was somewhat pointed out in following studies (Turcotte et al., 2016), as it might have led to some inconsistencies. For example, while some documented the expression of the CB₂ receptor in human granulocytes (neutrophils and contaminating eosinophils) (Galiègue et al., 1995; Kurihara et al., 2006), others did not (Oka et al., 2004; Graham et al., 2010). This raised the possibility that contaminating cells might have been responsible for the previously documented CB₂ signal in neutrophils, and possibly other cell types. Noteworthy, it was later reported that eosinophil-depleted neutrophils weakly expressed the CB₂ receptor mRNA, while eosinophils (the main neutrophil suspension contaminant) expressed it at high levels, raising the strong possibility that discrepancies regarding CB₂ expression in neutrophils could be the result of contaminating eosinophils in granulocyte preparations (Chouinard et al., 2013). CB₂ expression was also reported in human eosinophils in other studies (Frei et al., 2016; Larose et al., 2017; Freundt-Revilla et al., 2018; Dothel et al., 2019).

In an attempt to better define CB₂ expression in human blood leukocytes, we revisited its expression by qPCR using mRNA from leukocytes that were isolated from the blood of healthy volunteers. CB₁ receptor expression was assessed in parallel. Hypothalamus samples were utilized as positive controls for the CB₁ receptor. In our hands, all tested leukocytes expressed the CB₁ receptor mRNA although to a lesser extent than hypothalamus samples (Figure 1A). In contrast, while we detected the expression of the CB₂ receptor mRNA in all leukocyte and hypothalamus samples, human eosinophils and B lymphocytes displayed the strongest signals (Figure 1B). Thus, these cell types are likely the origin of CB₂ expression found in mixed populations such as granulocytes (neutrophils and eosinophils, often abbreviated as PMN) and PBMCs (monocytes, B and T lymphocytes). This underlines the importance of separating granulocytes and PBMCs when studying the CB₂ receptor. The small, but detectable levels of CB₂ receptor mRNA in hypothalamus samples are consistent with other studies reporting its expression in this tissue (Sanchez et al., 2001; Van Sickle et al., 2005; Ellert-Miklaszew ska et al., 2007).

**FACTORS INFLUENCING CB₂ RECEPTOR EXPRESSION IN HUMAN LEUKOCYTES**

Some factors were documented as influencing CB₂ receptor expression in human leukocytes. CB₂ expression can increase

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**FIGURE 1** | Expression of the CB₂ and CB₁ receptors mRNA in human leukocytes isolated from the blood. Human venous blood was collected from healthy volunteers with the informed consent of all participants in blood collection tubes containing K₂EDTA as anticoagulant. Granulocytes (GRAN), eosinophils (EOS) and neutrophils (NEU) were isolated as in Chouinard et al. (2013). PBMCs were obtained from the PBMC layer and taken as is or otherwise processed for monocyte (MONO), B and T lymphocytes (LYMP) isolation using the EasySep™ monocyte isolation kit, CD19 positive Selection Kit II and CD3 positive selection Kit II respectively, as per the manufacturer’s protocol. Purity of the different isolated leukocytes was always >97% with the exception of B Lymphocytes (90%) with MONO being the main contaminant. Hypothalamus (HYPO) samples were obtained from the Douglas-Bell Canada Brain Bank (McGill University, Montréal, Canada). mRNA was next isolated from the different preparations with TRIzol as per the manufacturer’s protocol. 500 ng of total RNA was reverse transcribed using a High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, CA, USA) as recommended. qPCR analyses were finally performed on a CFX Connect Real-Time PCR System, using the following primers (forward - reverse): GAPDH (5′-ACATCGTGACACACATG-3′-5′-TGTAGTTGAGGTCAATGAAGGG-3′) 18S (5′-GGGAAGGGAGCGAGCGACCA-3′-5′-GGGGAGAGCGAGCGACCA-3′) CB₁ (5′-TTCCCTCTTGTGAAGGCACTG-3′-5′-TCTTGACCGTGCTCTTGATGC-3′) CB₂ (5′-CAAGGCGTCTTCTGCTGTA-3′-5′-OGGGTGAGCAGACAGCTTGTGA-3′). Data represent the mean (+SEM) of 4-6 donors and was obtained using the CFX Maestro Software (Bio-Rad).
during inflammation as it is the case in eosinophils from symptomatic allergic donors compared to healthy controls (Frei et al., 2016; Larose et al., 2017), in monocytes of patients after ischemic stroke (Greco et al., 2021), in myeloid and plasmacytoid dendritic cells of patients with multiple sclerosis (Chiurchiu et al., 2013; Sanchez Lopez et al., 2015) and in T lymphocytes of Non-Hodgkin’s lymphomas (Rayman et al., 2007; Robinson et al., 2013). On the other hand, LPS decreased CB2 receptor expression in isolated dendritic cells and B lymphocytes (Lee et al., 2001; Do et al., 2004). Finally, the CB2 receptor was not detected in resting macrophages, was present at high levels in responsive and primed cells and was greatly diminished in fully activated cells (Cabral 2010). The latter observation suggests that the CB2 receptor might have a time-specific function in macrophages during inflammation.

Numerous CB2 receptor antibodies have been developed but most (if not all) are failing to provide reliable signals in different applications (immunohistochemistry, cytofluorometry and immunoblot), while not always having been characterized with the appropriate controls (control peptide blockade, CB2 receptor-devoid cells, cross reactivity). Thus, until a clear consensus is achieved on which antibodies are sufficiently reliable, data on CB2 protein should be interpreted with caution. With that in mind, the CB2 receptor protein localization can vary. Indeed, Castaneda et al. reported that the CB2 receptor protein was found intracellularly in most leukocytes with only B lymphocytes expressing it at the extracellular membrane (Castaneda et al., 2013). CB2-positive B lymphocytes were mainly located in the mantle of secondary lymphoid follicles, which contain immature B lymphocytes while some positive cells also appeared in the germinal centers of secondary follicles, which contain mature B lymphocytes, suggesting an heterogeneous distribution of the receptor during B lymphocytes maturation stages (Gallegue et al., 1995). Immunohistochemical analysis using an N-terminal specific anti-CB2 antibody revealed high protein expression in the germinal centers of secondary follicles while a C-terminal specific anti-CB2 antibody (only recognizing a non-phosphorylated inactive receptor) showed positivity primary follicle, the mantle and marginal zones of the secondary follicles where resting cells reside (Rayman et al., 2004). Therefore, active CB2 seems mainly present on B lymphocytes in the germinal centers.

**IMPACT OF CB2 RECEPTOR ACTIVATION IN HUMAN LEUKOCYTES**

The early studies investigating the roles of the CB2 receptor, notably those involving cmr2-deficient mice, led to the idea that it is mainly anti-inflammatory (Turcotte et al., 2016). However, recent studies are emerging and indicate that the outcome of CB2 receptor signaling may differ depending on the experimental model/disease. A good example is experimental asthma. Indeed, early work indicated that the CB2 receptor agonist WIN 55,212-2 inhibited ovalbumin-induced plasma extravasation in guinea pig airways (Fukuda et al., 2010). In contrast, the CB2 receptor agonist JWH-133 aggravated ovalbumin-induced asthma in mice while having no effect in dinitrofluorobenzene-induced asthma (Bozkurt et al., 2016; Frei et al., 2016). When house dust mites were utilized as allergen, cmr2-deficient mice were resistant to allergic responses (Ferrini et al., 2017) while an innate lymphoid cell-2 dependent model involving IL-25, IL-33 and/or Alternaria alternate had lower symptoms, decreased eosinophil number, and airway resistance (Hurrell et al., 2021). In humans, CB2 receptor expression was increased in nasal polyps of aspirin-exacerbated disease patients (Corrado et al., 2018) while being decreased in epithelial cells of asthmatic patients (Fantauzzi et al., 2020).

While we address some leukocytes individually below, the overall impact of CB2 receptor activation on human leukocytes is summarized in **Table 1**. However, we underscore that the selectivity of the pharmacological tools targeting CB2 receptors (agonists, antagonists, inverse agonists) has been often questioned, as exemplified by the work of Soethoudt et al. (2017).

### Human Eosinophils

Eosinophils participate in innate immunity against parasites and in the development/persistence of diverse inflammatory responses, notably allergies and asthma. Studies involving human eosinophils and CB receptors are scarce. Their treatment with either the endocannabinoid 2-AG and/or CB2 receptor agonists stimulated their migration or potentiated their migration toward other chemoattractants (Oka et al., 2004; Kishimoto et al., 2006; Larose et al., 2014; Frei et al., 2016). Importantly, these effects were prevented by the CB2 receptor antagonists AM630 and/or SR144528. Consistent with a CB2-mediated increased in eosinophil migration, cannabis use has been linked to some cases of acute eosinophilic pneumonia, although no demonstration has proven that this involved the CB2 receptor (Sauvaget et al., 2010; Liebling and Siu 2013; Natarajan et al., 2013; Ocal et al., 2016; Mull et al., 2020). Interestingly, while JWH-133 led to a moderate chemotactic response in human eosinophils, it had no effect on mouse eosinophils (Frei et al., 2016). Altogether, the current data support that the CB2 receptor stimulates eosinophil migration. This could eventually lead to increased parasitic defenses but also to a worsening of eosinophil-related inflammatory diseases.

### Human B Lymphocytes

B lymphocytes maturation and differentiation are complex processes. Following their activation, naive cells (spleen marginal zone) proliferate and differentiate into short-lived plasma cells, while cells from the follicles undergo massive proliferation and form germinal centers, where long-lived plasma and memory cells are formed (Basu et al., 2013). Very little is known about the role of the CB2 receptor in human B lymphocytes but their treatment with CP 55,940 increased their proliferation, a phenomenon blocked by SR144528 (Carayon et al., 1998). In mice, activation of the CB2 receptor has been associated with B lymphocyte differentiation, migration, proliferation and antibody class switching (Jorda et al., 2002; Tanikawa et al., 2007; Agudelo et al., 2008), suggesting the receptor is part of the B lymphocytes immune programing.
| Leukocytes or cell lines | Agonist | Antagonist or inverse agonist | Effects | Impact on signaling | References |
|-------------------------|---------|-----------------------------|---------|---------------------|-----------|
| Eosinophils Blood       | 2-AG    | SR144528 (1 μM)             | Induce migration in presence of 1 μM NDGA (lipooxygenase inhibitor) | Inhibited by the Lyn inhibitor PP2 | Oka et al. (2004) |
|                         | 1 μM (1 h) | SR144528 (1 μM) | 2-AG-induced migration in presence of 1 μM NDGA is attributed to chemotaxis rather than chemokinesis | Not inhibited by pertussis toxin (PTX; Gαi-dependent), p38 or PI3K inhibitors | Kishimoto et al. (2006) |
|                         | 3 μM (2 h) | SR144528 (10 μM) | Induce migration in presence of IL-5 | ↑ CCL24-induced shape change and migration | Larose et al. (2014) |
|                         | 250 nM (5 h) | SR144528 (1 μM) | ↑ CCL24-induced shape change and migration | ↑ CCL24-induced CD11b upregulation | Frei et al. (2016) |
| CP 55,940               | 1 μM (2 h) | -                          | No effect on migration | ↑ Adhesion to ICAM-1 | Frei et al. (2016) |
|                         | 100–250 nM (5 h) | SR144528 (1 μM) | Induce migration | ↑ CCL24-induced shape change and migration | Haruna et al. (2017) |
|                         | S-777469 | SR144528 (1 μM) | Induce migration in presence of 1 μM NDGA | ↑ 2-AG-induced migration | Oka et al. (2004) |
|                         | 100–500 nM (4 h) | SR144528 (1 μM) | ↑ 2-AG-induced migration | 2-AG-induced migration | Haruna et al. (2017) |
| Leukemia EoL-1 cells   | 2-AG    | SR144528 (1 μM)             | Induce migration in presence of 1 μM NDGA | Migration inhibited by MEK1 inhibitors (U-0126, PD98,059) and the ROCK inhibitor Y-27632 | Oka et al. (2004) |
|                         | S-777469 | SR144528 (1 μM)             | ↑ 2-AG-induced migration | Not inhibited by pertussis toxin (PTX; Gαi-independent), p38 or PI3K inhibitors | Carayon et al. (1998) |
|                         | 100–500 nM (4 h) | SR144528 (1 μM) | ↑ 2-AG-induced migration | ↑ CCL24-induced shape change and migration | Carayon et al. (1998) |
|                         | CP 55,940 | 100–300 nM (72 h) | ↑ Proliferation | ↑ CCL24-induced upregulation | Gustafsson et al. (2006) |
|                         | CP 55,940 | 100–300 nM (72 h) | ↑ Proliferation of both naïve and germinal centrosome B lymphocytes | ↑ Adhesion to ICAM-1 | Rayman et al. (2004) |
| Tonsillar               | CP 55,940 | 100–300 nM (72 h) | ↑ Proliferation of both naïve and germinal centrosome B lymphocytes | ↑ CCL24-induced shape change and migration | Gustafsson et al. (2006) |
| Raj cell line           | 2-AG    | SR144528 (10 nM)            | Induce moderate migration | ↑ CCL24-induced shape change and migration | Gustafsson et al. (2006) |
|                         | 300 nM (4 h) | SR144528 (10 nM) | ↑ Migration following stimulation with an anti-αCD40 antibody | ↑ Adhesion to ICAM-1 | Gustafsson et al. (2006) |
| Rec-1 cell line         | WIN 55,212–2 | 10 μM (4 h) | ↑ Apoptosis (caspase-3 activity) | ↑ CCL24-induced upregulation | Gustafsson et al. (2006) |
|                         | WIN 55,212–2 | 10 μM (4 h) | ↑ Apoptosis (caspase-3 activity) | ↑ IL-6 induced secretion of soluble IgM | Gustafsson et al. (2006) |
|                         | WIN 55,212–2 | 10 μM (4 h) | ↑ Apoptosis (caspase-3 activity) | ↑ IL-6 induced p-STAT3 | Gustafsson et al. (2006) |
| SKW 6.4 cell line       | -       | SR144528 (5–10 μM)          | ↑ IL-6 induced secretion of soluble IgM | ↑ IL-6 induced p-STAT3 | Feng et al. (2014) |
|                         | -       | AM630 (5 μM)                | ↑ IL-6 induced secretion of soluble IgM | ↑ IL-6 induced p-STAT3 | Feng et al. (2014) |
| Neutrophils Blood       | 2-AG    | SR144528 (1 μM)             | No effect on migration in presence of NDGA | ↑ IL-6 induced secretion of soluble IgM | Oka et al. (2004) |
|                         | 300 nM (20 min) | SR144528 (1 μM) | No motility or morphologic alterations | ↑ IL-6 induced p-STAT3 | Kurihara et al. (2006) |
|                         | JWH-015 | 100 nM-10 μM (20 min) | No motility or morphologic alterations | ↑ IL-6 induced p-STAT3 | Kurihara et al. (2006) |
|                         | JWH-133 | 1 μM (2 h) | No effect on neutrophil function | ↑ IL-6 induced p-STAT3 | Zhou et al. (2020) |
|                         | 100 nM (5 h) | SR144528 (1 μM) | No effect on IL-8-induced migration | ↑ IL-6 induced p-STAT3 | Frei et al. (2016) |

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### TABLE 1 (Continued) CB₂-mediated effects on human leukocytes and related human cell lines.

| Leukocytes or cell lines | Agonist | Antagonist or inverse agonist | Effects | Impact on signaling | References |
|--------------------------|---------|------------------------------|---------|-------------------|------------|
| T lymphocytes            | 100 nM-1 μM (30 min) | AM630 (500 nM) | ↓ LPS-induced VEGF-A | Braile et al. (2021) |
|                          |         |                             | ↓ LPS-induced endothelial permeability | |
| Blood                    | AEA     | 0.5–5 μM (6 h) | SR144528 (1 μM) | Proliferation | Cencioni et al. (2010) |
|                          | JWH-015 | 20 μM (1 h) | AM630 (500 nM) | ↓ IL-2, TNF-α and IFN-γ | Ghosh et al. (2006) |
|                          |         | 250 nM (2 h) | AM630 (500 nM) | ↓ IL-2 | Borner et al. (2009) |
|                          |         | 1 μM (6 h) | SR144528 (1 μM) | ↓ IL-2, TNF-α and IFN-γ | Cencioni et al. (2010) |
|                          | JWH-133 | 0.001–10 μM (30 min) | - | ↓ CXCL12-induced chemotaxis | Costantino et al. (2012) |
|                          |         | 100 nM-1 μM (1–30 min) | AM630 (1 μM) | ↓ HIV-1 infection in primary CD4 T cells | Coopman et al. (2007) |
|                          | Δ²-THC  | 5 μg/ml (18 h) | SR144528 (1 μM) | ↓ CXCL12-induced chemotaxis | Costantino et al. (2012) |
| Jurkat cells             | 10–40 μM (3–24 h) | AM630 (1 μg/ml) | ↓ Cell viability | Huang et al. (2019) |
|                          | JWH-015 | 20 μM (1 h) | AM630 (500 nM) | ↓ CXCL12-induced chemotaxis | Ghosh et al. (2006) |
|                          |         | 250 nM (2 h) | AM630 (500 nM) | ↓ Transendothelial migration | Borner et al. (2009) |
|                          | LV50    | 10 μM (4–72 h) | SR144528 (1 μM) | ↓ Migration (chemotaxis toward 2-AG) | Capozzi et al. (2018) |
|                          | Δ²-THC  | 1–5 μM (1–2 h) | SR144528 (2 μM) | ↓ Apoptosis (annexin V) | Herrera et al. (2006) |
| Monocytes                | 2-AG    | 10 nM-10 μM (4 h) | SR144528 (1 μM) | ↑ Migration (chemotaxis toward 2-AG) | Kishimoto et al. (2003) |
| Blood                    |         | 500 nM (18 h) | AM630 (5 μM) | ↓ LPS-induced IL-1β and TNFα | Gertsch et al. (2006) |
|                          | (E)-β- | 5–20 μM (80 min) | SR144528 (1 μM) | ↓ CCL2- and CCL3-induced migration | Montecucco et al. (2008) |
| caryophyllene            | JWH-015 |         | | ↓ CCR2 and CCR1 mRNA expression | |
|                          |         | 1–10 μM (20 min) | - | ↓ IFNγ-induced ICAM-1 induction | |
|                          | JWH-133 | 1 μM (18 h) | SR144528 (1 μM) | ↑ p-ERK1/2 | Rizzo et al. (2019) |
|                          |         | 0.1–10 μM (days 4, 7 and 10) | - | | Gertsch et al. (2006) |
|                          |         |                 | | | Williams et al. (2014) |

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### TABLE 1 (Continued) CB2-mediated effects on human leukocytes and related human cell lines.

| Leukocytes or cell lines | Agonist | Antagonist or inverse agonist | Effects | Impact on signaling | References |
|--------------------------|---------|-------------------------------|---------|---------------------|------------|
| U937 cells               | 2-AG    | 1 μM (5 min) SR144528 (3 μM) | ↓ HIV-1 viral infection during  | ↑ Adhesion to fibronectin | Gokoh et al. (2005a) |
|                          |         |                               | differentiation in monocyte  |                     | Raborn et al. (2014) |
|                          |         |                               | derived macrophages         |                     |            |
| CP 55,940                | 1 nM-1 μM (2 h) SR144528 (1 μM) | ↓ HIV-1 transactivating  | ↓ oxLDL-induced CD36 |                     | Raborn et al. (2014) |
|                          |         | protein-enhanced adhesion of  | ↓ oxLDL-induced TNF-α, IL-12 |                     |            |
|                          |         | cells to extracellular matrix | and IL-10                  |                     |            |
|                          |         | protein, such as collagen IV  |                     |                     |            |
|                          |         | and laminin                  |                     |                     |            |
| WIN 55,212–2            | 1–10 μM (2 h) AM630 (1 μM) | ↓ Adhesion to HUVECs        |                     |                     | Zhao et al. (2010) |
| Mast cells               | JWH-015 | 10^−8–10^−6 M (2 h) - | ↓ Calcium ionophore A23187- |                     | Iuvone et al. (2006) |
| Endometrial              |         |                               | induced degranulation       |                     |            |
| Macrophages              | JWH-015 | 50 nM (30 min) SR144528 (50 nM–0.1 μM) | ↓ oxLDL-induced  |                     | Chiurchiu et al. (2014) |
| Monocyte-derived         | Lenabasum | 0.1–30 μM (Day 0, 3, and 6) - | No effect                  |                     | Tarique et al. (2020) |
| macrophages (healthy  |         |                               |                     |                     |            |
| subjects)                | Lenabasum | 0.1–30 μM (Day 0, 3, and 6) - | Macrophage polarization into |                     | Tarique et al. (2020) |
| Monocyte-derived         |         |                               | pro-inflammatory M1 phenotype |                     |            |
| macrophages (patients  |         |                               | ↓ IL-6 and TNF-α secretion  |                     |            |
| with cystic fibrosis)    |         |                               |                     |                     |            |
| Lung                     | JWH-133 | 1 μM (10 min) AM630 (0.5 μM) | ↓ LPS-induced VEGF-A and | ↑ p-ERK1/2          | Staiano et al. (2016) |
|                          |         |                               | VEGF-C                     |                     |            |
|                          |         |                               | ↓ LPS-induced IL-6          |                     |            |
|                          |         |                               | Induce morphological changes |                     |            |
|                          |         | such as the extension of  |                     | - Inhibited by PTX (Gαs- | Gokoh et al. (2005b) |
|                          |         | pseudopods                   |                     | dependant)          |            |
|                          |         |                               | ↑ Actin polymerization      |                     |            |
|                          |         |                               |                     |                     |            |
| HL-60-derived            | 2-AG    | 1 μM (1 min) SR144528 (1 μM) | ↓ Migration of A549 cells  |                     | Chiurchiu et al. (2013) |
| macrophage               |         |                               |                     |                     |            |
| THP-1-derived            | JWH-015 | 1–5 μM (12 h) - | ↓ Migration of A549 cells  |                     | Chiurchiu et al. (2013) |
| Macrophage M2            |         |                               |                     |                     | Chiurchiu et al. (2013) |
| Myeloid                  | AEA     | 2.5 μM (4 h) SR144528 (1 μM) | ↓ R848-induced TNF-α, IL- |                     | Chiurchiu et al. (2013) |
| Plasmacytoid             | JWH-015 | 1 μM (4 h) SR144528 (1 μM) | ↓ R848-induced TNF-α, IL- |                     | Chiurchiu et al. (2013) |
| (healthy subjects)       | AEA     | 2.5 μM (4 h) SR144528 (1 μM) | ↓ R848-induced TNF-α, IFN-α |                     | Chiurchiu et al. (2013) |
| 2-AG                     | 10 μM (18 h) SR144528 (1 μM) | ↓ CpG-induced IFNa          |                     |                     | Rahaman et al. (2019) |
| JWH-015                  | 1 μM (4 h) SR144528 (1 μM) | ↓ TLR9 activation           |                     |                     | Henriquez et al. (2019) |
|                          |         | ↓ R848-induced TNF-α and  |                     |                     |            |
|                          |         | IFN-α                        |                     |                     |            |
|                          |         | 0.01–1 μM (5 h) - | ↓ CpG-induced IFNa and TNFα |                     | Henriquez et al. (2019) |
|                          |         |                               |                     |                     |            |
|                          |         | 0.001–0.1 μM (5 h) - | ↓ CpG-induced IFNa and TNFα |                     | Henriquez et al. (2019) |
|                          |         |                               |                     |                     |            |
|                          |         | 2.5 μM (4 h) SR144528 (1 μM) | No effect               |                     | Chiurchiu et al. (2013) |
| Plasmacytoid             | AEA     | 2.5 μM (4 h) SR144528 (1 μM) | No effect               |                     | Chiurchiu et al. (2013) |
| (patient with multiple   | JWH-015 | 1 μM (4 h) SR144528 (1 μM) | No effect               |                     | Chiurchiu et al. (2013) |
| sclerosis)               |         |                               |                     |                     |            |
playing an important role in B lymphocyte repertoire formation (Pereira et al., 2009).

**Human Neutrophils**
Neutrophils are first responders of the innate immune system, playing crucial roles in acute inflammatory responses and host defense. They employ several strategies to fight microbes, including the phagocytosis and killing of pathogens with the help of their granule content. Studies showing a CB2-receptor-mediated effect of human neutrophils were not conclusive and contaminating eosinophils in neutrophil preparations might have caused a red herring situation, eosinophils being responsible for most of the CB2 receptor signal/effects (Figure 1 and Expression of the CB2 and CB2 Receptors by Human Blood Leukocytes). In fact, numerous studies indicated that endocannabinoids as well as selective and non-selective CB2 receptor agonists do not diminish human neutrophil functions (migration, superoxide generation and degranulation) via the CB2 receptor and when they display an inhibitory effect on their functional responses it is mostly related to a mechanism distinct from the CB1 and CB2 receptors (Deusch et al., 2003; Kraft et al., 2004; Oka et al., 2004; McHugh et al., 2008; Chouinard et al., 2011; Montecucco et al., 2012; Zhou et al., 2020), which is consistent with their lack/very low expression of the CB2 receptor. In contrast, JWH-133 inhibited the release of VEGF-A but not CXCL8 from LPS-stimulated human neutrophils, a phenomenon prevented by the CB2 receptor antagonist AM630 (Braile et al., 2021).

- *In vivo* studies indicated that mouse neutrophils are more responsive to CB2 receptor activation than human neutrophils. As such, *Cnr2*−/− mice models reported increased neutrophil numbers at inflammatory sites (Alferink et al., 2016; Kapellos et al., 2017; Kapellos et al., 2019). Accordingly, CB2 activation by selective agonists suppressed neutrophil recruitment to the inflammation site (Horvath et al., 2012; Andrade-Silva et al., 2016; Wang et al., 2016; Parlar et al., 2018; Kapellos et al., 2019). However, it is not clear whether the reported evidence is a matter of mouse neutrophil responsiveness or of indirect CB2-dependent effects mediated by other cells (Kraft and Kress 2005). At this point, we cannot exclude that a CB2-dependent mechanism prevents neutrophil recruitment into by impairing their transmigration into the tissues and by affecting other cells (e.g., endothelial cells) as proposed earlier (Nilsson et al., 2006).

**Human T Lymphocytes**
Cytotoxic CD8 T lymphocytes are responsible for the elimination of invading/dysfunctional cells while CD4 T lymphocytes produce a myriad of inflammatory mediators and are referred to as helper lymphocytes (Th). Although CB2 receptor expression was barely detected in circulating T lymphocytes (Figure 1), several studies reported that CB2 receptor expression is increased in activated T lymphocytes and that its activation decreases their proliferation (Borner et al., 2009; Cencioni et al., 2010; Capozzi et al., 2018). This is accompanied with decreased IL-2 production and increased apoptosis (Herrera et al., 2006; Borner et al., 2009; Cencioni et al., 2010; Capozzi et al., 2018; Huang et al., 2019). Interestingly, CB2 receptor activation seems to exert divergent effects depending on the T lymphocyte subtype with the tendency to decrease human Th1 and Th17 functions, while promoting those of Th2. For instance, Δ9-THC decreased in a CB2-dependent manner the percentage of human T lymphocytes expressing IFN-γ, and intracellular levels of IFN-γ per cells (Th1), while increasing levels of IL-4 and IL-5 (Th2) (Yuan et al., 2002). Accordingly, a decrease in IL-17 levels was found in JWH-015-treated T lymphocytes (Cencioni et al., 2010). Finally, the CB2 agonist Lenabasum reduced TNF-α in both CD8 and CD4 T lymphocytes (Th1). The treatment also decreased IL-17 levels (Th17) as well as Th1 and Th17 respective signature transcription factors T-bet and RORγT (Tiberi et al., 2021).

**Human Monocytes**
Blood monocytes migrate into tissues where they differentiate into macrophages or convert into non-classical monocytes (Guilliams et al., 2018). 2-AG is a CB2-dependent human monocyte chemoattractant (Kishimoto et al., 2003) and induces the adhesion of human mononuclear U937 cells to fibronectin (Gokoh et al., 2005a). However, JWH-015 decreased the CCL2- and CCL3-induced migration of human monocytes by decreasing their receptors’ expression (Montecucco et al., 2008). JWH-015 also reduces human monocyte differentiation and U937 cells adhesion to extracellular matrix proteins, both induced by HIV-1 (Raborn et al., 2014; Williams et al., 2014). Finally, CB2 receptor engagement in human monocytes was shown to decrease the LPS-induced IL-1β and IL-6 production (Gu et al., 2019; Rizzo et al., 2019).

**Human Macrophages**
Macrophages are resident cells that are remarkably versatile, exerting important roles in development, homeostasis, tissue repair and immunity. The endocannabinoid 2-AG was found to induce shape changes of HL-60-derived macrophages in a CB2-dependent manner (Gokoh et al., 2005b). Additionally, CB2 receptor activation with JWH-015 or JWH-133 decreased the LPS-induced VEGF-A, VEGF-C IL-6 release, as well as the oxLDL-induced release of TNF-α, IL-12 and IL-10 (Chiurchiu et al., 2014; Staiano et al., 2016). In mice, the CB2 receptor was shown to switch the polarization of M1 macrophage into M2 macrophage (Duerr et al., 2014; Denaes et al., 2016; Du et al., 2018). Such a phenomenon has been partially observed in humans by Tarique et al. who showed that Lenabasum decreased the polarization (M1) of monocyte-derived macrophage obtained from cystic fibrosis patients (Tarique et al., 2020).

**Human Mast Cells**
Mast cells are strategically located at the interface with the external environment, acting as key initiators of local inflammatory responses (Elieh Ali Komi et al., 2020). The first evidence that they could be regulated by the CB2 receptor came from the rat basophilic leukemia cell line (RBL-2H3) expressing the CB2 receptor (Facci et al., 1995). However, while the authors showed that N-palmitoylethanolamine (PEA) inhibited serotonin release AEA did not. However, PEA interacts with
PPARα (Lo Verme et al., 2005) and its initial effects are likely linked to PPARs. In humans, the treatment of isolated mast cells with JWH-015 decreased their degranulation in vitro (Iuvone et al., 2008).

**Human Dendritic Cells**

Dendritic cells are sentinels of the immune system bridging the innate and adaptive immunity by ingesting pathogens and transporting antigens to lymphoid tissues. Stimulation of CB2 receptor with CB2 receptor agonists reduced their cytokine production. Indeed, AEA and JWH-015 decreased R848-induced levels of TNF-α, IL-12p40 and IL-6 by myeloid dendritic cells while AEA, 2-AG, JWH-015 and JWH-133 decreased levels of R848-and/or CpG-induced IFN-α by plasmacytoid dendritic cells by a mechanisms involving NF-κB and IKK signalization (Chiurchiu et al., 2013; Henriquez et al., 2019; Rahaman et al., 2019).

**CONCLUSION**

It is becoming clear that the CB2 receptor plays important roles in the regulation of several inflammatory processes. However, while the first studies investigating the role of this receptor in mice led to the concept that its function was mainly anti-inflammatory, new evidence is challenging this concept, notably in allergic diseases, which usually involve cells such as eosinophils and B lymphocytes, whose functional responses to CB2 receptor activation simulates them, in human-based studies. Moreover, the scarcity of human studies investigating the CB2 receptor makes our understanding of the latter difficult at this point and underscores the urgency of performing additional work involving human samples/cells to deepen our understanding of CB2-receptor-driven inflammatory responses and establish to what extent we can translate findings from experimental models to the clinic. It is thus urgent to further characterize the functions of the CB2 receptor in human leukocytes and inflammatory diseases.

**REFERENCES**

Agudelo, M., Newton, C., Widen, R., Sherwood, T., Nong, L., Friedman, H., et al. (2008). Cannabinoid Receptor 2 (CB2) Mediates Immunoglobulin Class Switching from IgM to IgG in Cultures of Murine-Purified B Lymphocytes. *J. Neuroimmune Pharmacol.* 3 (1), 35–42. doi:10.1007/s11481-007-9088-9

Allerink, J., Specht, S., Arends, H., Schumak, B., Schmidt, K., Ruland, C., et al. (2016). Cannabinoid Receptor 2 Modulates Susceptibility to Experimental Cerebral Malaria through a CCL17-dependent Mechanism. *J. Biol. Chem.* 291 (37), 19517–19531. doi:10.1074/jbc.M116.746594

Andrade-Silva, M., Correa, L. B., Candéa, A. L., Cavalher-Machado, S. C., Barbosa, H. S., Rosas, E. C., et al. (2016). The Cannabinoid 2 Receptor Agonist β-caryophyllene Modulates the Inflammatory Reaction Induced by Mycobacterium Bovis BCG by Inhibiting Neutrophil Migration. *Inflamm. Res.* 65 (11), 869–878. doi:10.1007/s00011-016-0969-3

Basu, S., Ray, A., and Dittel, B. N. (2013). Cannabinoid Receptor 2 (CB2) Plays a Role in the Generation of Germinal Center and Memory B Cells, but Not in the Production of Antigen-Specific IgG and IgM, in Response to T-dependent Antigens. *PLoS One* 8 (6), e67587. doi:10.1371/journal.pone.0067587

**DATA AVAILABILITY STATEMENT**

The original contributions presented in the study are included in the article, further inquiries can be directed to the corresponding author.

**ETHICS STATEMENT**

The studies involving human participants were reviewed and approved by the Comité d’éthique de la recherche de l’Institut universitaire de cardiologie et de pneumologie de Québec. The participants provided their written informed consent to participate in this study.

**AUTHOR CONTRIBUTIONS**

Conceptualization: MS, VR, VD, and NF; Investigation: MS and VR; Data curation—formal analysis: MS, VR, and NF; Writing—original draft: MS and NF; Writing—review, editing, and revision: MS, VR, VD, and NF.

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Börner, C., Smida, M., Höllt, V., Schraven, B., and Kraus, J. (2009). Cannabinoid Receptor Type 1- and 2-mediated Increase in Cyclic AMP Inhibits T Cell Receptor-Triggered Signaling. *J. Biol. Chem.* 284 (51), 35450–35460. doi:10.1074/jbc.M109.006338

Bozkurt, T. E., Kaya, Y., Durlu-Kandili, N. T., Onder, S., and Sahin-Erdemli, I. (2016). The Effect of Cannabinoids on Dinitrofluorobenzene-Induced Experimental Asthma in Mice. *Respir. Physiol. Neurobiol.* 231, 7–13. doi:10.1016/j.resp.2016.05.012

Braile, M., Cristinizzano, L., Marcella, S., Varricchi, G., Marone, G., Modesto, L., et al. (2021). LPS-mediated Neutrophil VEGF-A Release Is Modulated by Cannabinoid Receptor Activation. *J. Leukoc. Biol.* 109 (3), 621–631. doi:10.1002/jlb.3A0520-187R

Brown, S. M., Wager-Miller, J., and Mackie, K. (2002). Cloning and Molecular Characterization of the Rat CB2 Cannabinoid Receptor. *Biochem. Biophys. Acta* 1576 (3), 255–264. doi:10.1016/S0005-2760(02)00341-x

Cakir, M., Tekin, S., Doganyigit, Z., Cakan, P., and Kaymak, E. (2019). The Protective Effect of Cannabinoid Type 2 Receptor Activation on Renal Ischemia-Reperfusion Injury. *Mol. Cel Biochem* 462 (1-2), 123–132.

Capozzi, A., Mattei, V., Martellucci, S., Manganelli, V., Saccomanni, G., Garofalo, T., et al. (2018). Anti-proliferative Properties and Proapoptotic Function of
New CB2 Selective Cannabinoid Receptor Agonist in Jurkat Leukemia Cells. *Int. J. Mol. Sci.* 19 (7), 1938. doi:10.3390/ijms19071938

Carayon, P., Marchand, J., Dussossoy, D., Deroqc, J. M., Jbilo, O., Bord, A., et al. (1998). Modulation and Functional Involvement of CB2 Peripheral Cannabinoid Receptors during B-Cell Differentiation. *Blood* 92 (10), 3605–3615. doi:10.1182/blood.v92.10.3605.3615

Castaneda, J. T., Harui, A., Kiertscher, S. M., Roth, J. D., and Roth, M. D. (2013). Differential Expression of Intracellular and Extracellular CR2(Cb2) Receptor Protein by Human Peripheral Blood Leukocytes. *J. Neuroimmunol. Pharmacol.* 8 (1), 323–332. doi:10.1007/s11481-012-9430-8

Cenciøni, M. T., Chiurchiù, V., Catanzaro, G., Borsellino, G., Bernardi, G., Battistini, L., et al. (2010). Anamndamide Suppresses Proliferation and Cytokine Release from Primary Human T-Lymphocytes Mainly via CB2 Receptors. *PLoS One* 5 (1), e6888. doi:10.1371/journal.pone.0006886

Chiurchiù, V., Cenciøni, M. T., Biscchia, E., De Bardi, M., Gasperini, C., Borsellino, G., et al. (2013). Distinct Modulation of Human Myeloid and Plasmacytoid Dendritic Cells by Anamndamide in Multiple Sclerosis. *Ann. Neurol.* 73 (5), 626–636. doi:10.1002/ana.23875

Chiurchiù, V., Lanuti, M., Catanzaro, G., Fezza, F., Rapino, C., and Maccarrone, M. (2014). Detailed Characterization of the Endocannabinoid System in Human Macrophages and Foam Cells, and Anti-inflammatory Role of Type-2 Cannabinoid Receptor. *Atherosclerosis* 233 (1), 55–63. doi:10.1016/j.atherosclerosis.2013.12.042

Chouinard, F., Lefebvre, J. S., Navarro, P., Ferland, C., Lalancette-Flamand, J., and Maccarrone, M. (2013). Expression of Endocannabinoid System Components in Human Airways Epithelial Cells: Impact of Sex and Chronic Respiratory Disease Status. *Eur. J. Respir. Open* 6 (4). doi:10.1183/23120541.00128-2020

Feng, R., Milcarek, C. A., and Xie, X. Q. (2014). Antagonism of Cannabinoid Receptor 2 Pathway Suppresses IL-6–induced Immunoglobulin IgM Secretion. *BMC Pharmacol.* 15, 30. doi:10.1186/2050-6510-15-30

Ferrini, M. E., Hong, S., Sterle, A., Sterle, D., Stelia, N., Roberts, K., et al. (2017). CB2 Receptors Regulate Natural Killer Cells that Limit Allergic Airway Inflammation in a Murine Model of Asthma. *Allergy* 72 (6), 937–947. doi:10.1111/1398-9995.13658

Frei, R. B., Luschinig, P., Parzmair, G. P., Peinhard, M., Schanz, M., Fauland, A., et al. (2016). Cannabinoid Receptor 2 Augments Eosinophil Responsiveness and Aggravates Allergen-Induced Pulmonary Inflammation in Mice. *Allergy* 71 (7), 944–956. doi:10.1111/1398-9995.12858

Freundt-Revilla, J., Heinrich, F., Zoerner, A., Gesell, F., Beyerbach, M., and Crespi, M., et al. (2018). The Endocannabinoid System in Canine Steroid-Responsive Meningitis-Arteritis and Intraspinal Spirocercosis. *PLoS One* 13 (2), e0197197. doi:10.1371/journal.pone.0197197

Fukuda, H., Abe, T., and Yoshihara, S. (2010). The Cannabinoid Receptor Agonist JWH-133 Inhibits the Activation and Differentiation of Human Polymorphonuclear Leukocytes. *Eur. J. Biochem.* 267 (10), 2955–2962. doi:10.1111/j.1742-5251.2010.06336.x

Gokoh, M., Kishimoto, S., Oka, S., Metani, Y., and Sugiura, T. (2005a). 2-Arachidonoylglycerol, an Endogenous Cannabinoid Receptor Ligand, Enhances the Adhesion of HL-60 Cells Differentiated into Macrophage-Like Cells. *J. Immunol.* 168 (1), 319–327. doi:10.4049/jimmunol.1680033

Gokoh, M., Kishimoto, S., Oka, S., Mori, M., Waku, K., Ishima, Y., et al. (2005b). Cannabinoid Receptor CB2 Modulates the CXCR2/CXCR4-Mediated Chemotaxis of T Lymphocytes. *J. Neuroimmunol.* 179 (1–3), 124–131. doi:10.1016/j.jneuroim.2005.01.003

Gokoh, M., Kishimoto, S., Oka, S., Metani, Y., and Sugiuara, T. (2005c). 2-Arachidonoylglycerol, an Endogenous Cannabinoid Receptor Ligand, Enhances the Adhesion of HL-60 Cells Differentiated into Macrophage-Like Cells and Human Peripheral Blood Monocytes. *FEBS Lett.* 579 (28), 6473–6478. doi:10.1016/j.febslet.2005.10.030

Gokoh, M., Kishimoto, S., Oka, S., Morii, M., Waku, K., Ishima, Y., et al. (2006a). Cannabinoid Receptor CB2 Modulates the CXCL12/CXCR4-Mediated Chemotaxis of T Lymphocytes. *Mol. Immunol.* 43 (14), 2169–2179. doi:10.1016/j.molimm.2006.01.005

Gokoh, M., Kishimoto, S., Oka, S., Metani, Y., and Sugiuara, T. (2005a). 2-Arachidonoylglycerol, an Endogenous Cannabinoid Receptor Ligand, Enhances the Adhesion of HL-60 Cells Differentiated into Macrophage-Like Cells and Human Peripheral Blood Monocytes. *FEBS Lett.* 579 (28), 6473–6478. doi:10.1016/j.febslet.2005.10.030

Gokoh, M., Kishimoto, S., Oka, S., Morii, M., Waku, K., Ishima, Y., et al. (2006b). Cannabinoid Receptor 2 Selectively Inhibits Antigen-Induced Plasma Extravasation in guinea Pig Airways. *Int. Arch. Allergy Immunol.* 152 (3), 295–300. doi:10.1159/000283042

Galié, S., Mary, S., Marchand, J., Dussossoy, D., Carrière, D., Carayon, P., et al. (1995). Expression of central and Peripheral Cannabinoid Receptors in Human Immune Tissues and Leukocyte Subpopulations. *Eur. J. Biochem.* 232 (1), 54–61. doi:10.1111/j.1432-1327.1995.tb20780.x

Gertsch, J., Leonti, M., Raduner, S., Racz, I., Chen, J. Z., Xie, X. Q., et al. (2008). Beta-caryophyllene Is a Dietary Cannabinoid. *Proc. Natl. Acad. Sci. U S A.* 105 (26), 9099–9104. doi:10.1073/pnas.0803601105

Ghosh, S., Preet, A., Groopman, E. J., and Ganju, R. K. (2006). Cannabinoid Receptor CB2 Modulates the CXCL12/CXCR4-Mediated Chemotaxis of T Lymphocytes. *Mol. Immunol.* 43 (14), 2169–2179. doi:10.1016/j.molimm.2006.01.005

Gokoh, M., Kishimoto, S., Oka, S., Metani, Y., and Sugiuara, T. (2005b). 2-Arachidonoylglycerol, an Endogenous Cannabinoid Receptor Ligand, Enhances the Adhesion of HL-60 Cells Differentiated into Macrophage-Like Cells and Human Peripheral Blood Monocytes. *FEBS Lett.* 579 (28), 6473–6478. doi:10.1016/j.febslet.2005.10.030
