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## Is lipid signaling through cannabinoid 2 receptors part of a protective system?

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### Abstract

The mammalian body has a highly developed immune system which guards against continuous invading protein attacks and aims at preventing, attenuating or repairing the inflicted damage. It is conceivable that through evolution analogous biological protective systems have been evolved against non-protein attacks. There is emerging evidence that lipid endocannabinoid signaling through cannabinoid 2 (CB<sub>2</sub>) receptors may represent an example/part of such a protective system/armamentarium. Inflammation/tissue injury triggers rapid elevations in local endocannabinoid levels, which in turn regulate signaling responses in immune and other cells modulating their critical functions. Changes in endocannabinoid levels and/or CB<sub>2</sub> receptor expressions have been reported in almost all diseases affecting humans, ranging from cardiovascular, gastrointestinal, liver, kidney, neurodegenerative, psychiatric, bone, skin, auto-immune, lung disorders to pain and cancer, and modulating CB<sub>2</sub> receptor activity holds tremendous therapeutic potential in these pathologies. While CB<sub>2</sub> receptor activation in general mediates immunosuppressive effects, which limit inflammation and associated tissue injury in large number of pathological conditions, in some disease states activation of the CB<sub>2</sub> receptor may enhance or even trigger tissue damage, which will also be discussed alongside the protective actions of the CB<sub>2</sub> receptor stimulation with endocannabinoids or synthetic agonists, and the possible biological mechanisms involved in these effects.

### Keywords

Endocannabinoids; Cannabinoid 1 and 2 receptors; Disease; Inflammation; Immune function; Disease

### 1. Introduction

The mammalian body has a highly developed immune system, whose main role is to guard against protein attack and prevent, reduce or repair a possible injury. It is inconceivable that through evolution analogous biological protective systems have not been developed against non-protein attacks. Are there mechanisms through which our body lowers the damage caused by various types of neuronal as well as non-neuronal insults? The answer is of course positive. Through evolution numerous protective mechanisms have been evolved to prevent and limit tissue injury.

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We believe that lipid signaling through cannabinoid 2 (CB<sub>2</sub>) receptors is a part of such a protective machinery and CB<sub>2</sub> receptor stimulation leads mostly to sequences of activities of a protective nature. Inflammation/tissue injury triggers rapid elevations in local endocannabinoid levels, which in turn regulate fast signaling responses in immune and other cells modulating their critical functions. Endocannabinoids and endocannabinoid-like molecules acting through the CB<sub>2</sub> cannabinoid receptor have been reported to affect a large number of pathological conditions, ranging from cardiovascular [1,2], gastrointestinal [3,4], liver [5–7], kidney [8,9], lung [10], neurodegenerative [11–14] and psychiatric [15–20] disorders to pain [21,22], cancer [23–26], bone [27,28], reproductive system [29–31] and skin pathologies [32] (Fig. 1). This receptor works in conjunction with the immune system and with various other physiological systems. As numerous excellent reviews have been published on the actions of the endocannabinoid system on specific topics, in this Review we aim to summarize some of the protective actions of the CB<sub>2</sub> receptor stimulation with endocannabinoids or synthetic agonists, and the possibly biological mechanisms involved in these effects. While CB<sub>2</sub> receptor activation in general mediates immunosuppressive effects, which limit inflammation and associated tissue injury in large number of pathological conditions, in some disease states activation of the CB<sub>2</sub> receptor may enhance or even trigger tissue damage, which will also be discussed alongside the protective actions.

## 2. The endocannabinoid system and endocannabinoids

The endocannabinoid system is a latecomer to the family of neuromodulators. Looking back, its late discovery was an anomaly. Despite the advances made in the chemistry of cannabinoids, the molecular basis of cannabinoid activity remained enigmatic for several decades. The chemistry of the constituents of the cannabis plant was investigated from the mid 19th century intermittently for almost a century [33], but the main psychoactive constituent,  $\Delta^9$ -tetrahydrocannabinol (THC), was isolated in pure form and its structure was elucidated only in 1964 [34]. Mainly due to its lipophilicity, over the next 2 decades it was assumed that its activity was due to some unspecific effects, comparable to the actions of anesthetics and solvents [33]. Some experimental work presumably gave support to this belief [35]. A further, conceptual, assumption also pointed in the same direction. Both enantiomers of THC were found to be active in some simple physiological assays, although the level of activity differed considerably, the natural (–) enantiomer being much more active than the synthetic (+) enantiomer. As natural active sites of enzymes or receptors are chiral, it is generally accepted that only one enantiomer of an active material will react with them. As both THC enantiomers were found to be active, it was assumed that its action did not involve such an active site [33]. However, in the mid-1980's it was shown that, when well purified, the enantiomers of a very potent analog of THC, namely the (–) enantiomer, HU-210, was nearly 5000 times more potent than the (+) enantiomer, HU-211 [36]. The assumption that both enantiomers were active was in fact due to a technical error, presumably of purification!

In 1988 Howlett's group in St. Louis reported the presence of a specific cannabinoid receptor in the brain [37]. Today it is generally known as the CB<sub>1</sub> receptor, which was cloned shortly after this initial discovery [38]. A second receptor, now known as CB<sub>2</sub>, was identified in rat spleen and was also cloned in 1993 [39]. Although numerous other receptors such as the transient receptor potential cation channel subfamily V member 1 (TRPV1), G protein-coupled receptors 55 and 119 (GPR55 and GPR119) may bind some cannabinoids under certain conditions, they are not yet considered cannabinoid receptors as their specificity for cannabinoids is tenuous [10,40–43]. While there is considerable evidence suggesting that targeting TRPV1 receptors has strong therapeutic rational in pain and multiple other disorders [44,45], and recent studies provide support for anandamide being a potential physiological agonist in the brain at these receptors [46–48], compelling *in vitro* or *in vivo*

functional evidence on the role of GPR55 and GPR119 in health and disease, as well as on the effects of endocannabinoids/cannabinoids on these targets have been limited by a lack of selective pharmacological tools [40,43]. Surprisingly, by using multiple novel tools, a recent study from GlaxoSmithKline challenged the possibility of GPR55 being the “third” endocannabinoid receptor [43].

The CB<sub>1</sub> receptor was originally considered to be mainly a CNS receptor, but it is now known to be present in many tissues and organs and its activation leads to both central and peripheral effects [10,49]. Its best known effect is psychoactivity; its activation and the resulting ‘high’ are the most popular upshot. Its specific effects – in the nervous, cardiovascular, gastrointestinal, reproductive etc. systems – are of paramount importance in vertebrate and invertebrate physiology.

The CB<sub>2</sub> receptor has recently been bestowed the title of a ‘cannabinoid receptor with an identity crisis’ [50]. Initially it was presumed to be absent in the central nervous system. High levels of CB<sub>2</sub> mRNA were found in the spleen. However, more recently it was found in microglia, particularly during neuroinflammation, which causes activation of the microglia and enhancement of CB<sub>2</sub> levels [13,50]. Its presence in neurons [51] is still controversial – hence the ‘identity crisis’. CB<sub>2</sub> is coupled to a Gi/o protein receptor and inhibits adenylyl cyclase, which is similarly affected by the CB<sub>1</sub> receptor, but the latter can also be coupled with other G proteins such as Gs and Gq/11 [49]. It modulates Ca<sup>2+</sup> channels, though less than the CB<sub>1</sub> receptor, promotes MAPK activation and ceramide production among many other effects [13,50]. Interestingly, stimulation of CB<sub>2</sub> receptors in immune cells after initial decrease in cAMP production may lead to a sustained, more pronounced increase in cAMP levels, which results in suppression of T cell receptor signaling through a cAMP/PKA/Csk/Lck pathway [52].

The first endogenous cannabinoid, arachidonoyl ethanolamide (anandamide, AEA) was isolated in 1992 from porcine brain [53]. A highly potent, centrally active tritium labeled cannabinoid [54], was bound to the CB<sub>1</sub> receptor and liposoluble extracts were tested for their ability to replace it. An active extract was further purified by silica gel chromatography to yield minute amounts of an active constituent, whose structure was determined by physical methods and by comparison with synthetic material. A second endocannabinoid, 2-arachidonoyl glycerol (2-AG), was identified a few years later by the same method using a peripheral organ [55]. Independently, it was also identified in brain [56]. These two endocannabinoids have been investigated in great detail [10,49,57]. Numerous other endocannabinoids and related endogenous substances have also been identified, but their activities have not been investigated to the same extent [45,58].

Contrary to most neurotransmitters, anandamide and 2-AG are formed postsynaptically mostly when and where needed [10,41,57]. Their formation and metabolism have been investigated in considerable detail as these processes determine the levels of the endocannabinoids and their physiological activities. The best known enzymes involved are fatty acid amine hydrolase (FAAH), diacyl glycerol lipase (DAGL), monoacyl glycerol lipase (MAGL). Some of these enzymes exist in several forms and their levels of activity vary in different tissues [59–63]. Examples of prototypical endogeneous/exogenous cannabinoid receptor ligands (mentioned in this review) with various degrees of activities at CB<sub>2</sub> receptors are presented in the Table 1. For the structures of these ligands and numerous recently developed another ones we would like to refer readers to several recent overviews [64–69].

### 3. CB<sub>2</sub> receptors and endocannabinoids in health and disease

The CB<sub>2</sub> receptor was previously considered to be expressed predominantly in immune and hematopoietic cells. Indeed, CB<sub>2</sub> receptors are expressed in almost all human peripheral blood immune cells with the following rank order of mRNA levels: B cells > NK cells > monocytes > PMNs > T cells, respectively (overviewed in [76]). The CB receptor expression in immune cells can be influenced by various inflammatory, e.g. bacterial lipopolysaccharide (LPS), and other triggers activating these cells, and may therefore largely be dependent on the experimental conditions [76]. Interestingly, inflammatory stimuli may also trigger increased production of endocannabinoids in immune cells (e.g. macrophages, peripheral-blood mononuclear cells and dendritic cells) via activation of various biosynthetic pathways, and/or by reducing expression of the metabolic enzyme(s) responsible for the degradation (overviewed in [77,78]). Earlier studies investigated the immunomodulatory effects of THC (a ligand for both CB<sub>1</sub> and CB<sub>2</sub> receptors) and other natural or synthetic cannabinoids, in mice and/or rats *in vivo* or in immune cells cultured from humans. Overall, these studies have shown suppressive effects of cannabinoid ligands on B, T, and NK cells, and macrophages, which most likely involve both CB<sub>1</sub> and CB<sub>2</sub> receptor-dependent and -independent mechanisms [76,77,79]. Recent studies have also revealed that endocannabinoids may exert important effects on immune functions by modulating T and B lymphocytes proliferation and apoptosis, inflammatory cytokine production and immune cell activation by inflammatory stimuli (e.g. LPS), macrophage-mediated killing of sensitized cells, chemotaxis and inflammatory cell migration, among many others [76,77,80] (Fig. 2). However, these effects (mostly, but not always inhibitory) were largely influenced by the endocannabinoid or synthetic agonist/antagonist, trigger/condition, and cell type used [76,77,81,82]. Similar context and experimental condition-dependent effects of endocannabinoids or synthetic analogs have been reported on microglia activation and migration [81]. Furthermore, cannabinoids may also modulate inducible nitric oxide synthase expression and nitric oxide and reactive oxygen species production in immune cells, which play important role against invading pathogens, as well as in modulating inflammatory response [83]. Endocannabinoids and CB<sub>2</sub> receptors have also been implicated in hematopoietic stem and progenitor cell mobilization [84], with potential implication for bone marrow transplantation. Recent studies have also identified low level of CB<sub>2</sub> receptor expression in specific regions of the brain [51,85], spinal cord and dorsal root ganglia [21,22,86], neurons in the myenteric and submucosal plexus of the enteric nervous system [87–89], colonic epithelial cells [90], bone [27,28,91], myocardium or cardiomyocytes [92–94], human vascular smooth muscle [95], activated hepatic stellate cells [5,96], mouse and human exocrine and endocrine pancreas [97–101], and endothelial cells of various origins [2,102,103], and in reproductive organs/cells [30,104] and in various human tumors [23] (Fig. 2).

Despite the presence of low level of CB<sub>2</sub> receptors, endocannabinoids and their metabolizing enzymes in cardiovascular, gastrointestinal, bone, various neuronal, liver tissues/cells, CB<sub>2</sub> receptors appear to play limited, if any role in normal physiological regulation of these organ systems, which is also supported by the absence of obvious pathological alterations in normal CB<sub>2</sub> receptors knockout mice [82]. This situation can be different (at least in mice) in male reproductive organs where CB<sub>2</sub> receptors are abundantly expressed and may possibly modulate sperm cell differentiation (spermatogenesis) [31], likewise during the development of age-related trabecular bone loss and trabecular expansion [105].

However, under various pathological conditions/disease states the expression of CB<sub>2</sub> receptors can be markedly upregulated in affected tissues/cells, which is often accompanied by elevated endocannabinoid levels as a part of the inflammatory response/tissue injury;

some of these selected pathologies and related CB<sub>2</sub> mediated effect are summarized in Table 2 and Figs. 1–3.

## 4. Effects of CB<sub>2</sub> receptor modulation in disease states

The significance of CB<sub>2</sub> receptor activation in the above mentioned immunomodulatory effects of endocannabinoids and of various cannabinergic ligands is now becoming increasingly recognized, and most likely these effects are largely responsible for the anti-inflammatory properties of endogenous or synthetic ligands observed in a multitude of disparate diseases and pathological conditions, ranging from atherosclerosis, myocardial infarction, stroke, inflammatory pain, gastrointestinal inflammatory, autoimmune and neurodegenerative disorders, to hepatic ischemia/reperfusion injury, inflammation and fibrosis, kidney and bone disorders and cancer, which will be reviewed below briefly (Fig. 1 and Table 2).

### 4.1. Cardiovascular disease

Cannabinoids and their endogenous and synthetic analogs, through activation of cardiovascular CB<sub>1</sub> and other receptors, exert a variety of complex hemodynamic effects (mostly resulting in decreased blood pressure and myocardial contractility) both in vivo and in vitro involving modulation of autonomic outflow, as well as direct effects on the myocardium and the vasculature, the discussion of which is beyond the scope of this synopsis [10,213]. In contrast, activation of cardiovascular CB<sub>2</sub> receptors is devoid of adverse hemodynamic consequences. Despite the presence of functional cannabinoid receptors, endocannabinoids and their metabolizing enzyme in cardiovascular tissues/cells, the endocannabinoid system appears to play limited role in normal cardiovascular regulation under physiological conditions, which is also supported by the normal blood pressure and myocardial contractility and/or baroreflex sensitivity of CB<sub>1</sub>, CB<sub>2</sub> and FAAH knockout mice [10].

In many pathological conditions, such as heart failure, shock, advanced liver cirrhosis, the endocannabinoid system may become overactivated and may contribute to hypotension/ cardiodepression through cardiovascular CB<sub>1</sub> receptors (overviewed in [1,10]). Tonic activation of CB<sub>1</sub> receptors by endocannabinoids may serve as a compensatory mechanism in hypertension [1,214] and may contribute to cardiovascular risk factors in obesity/ metabolic syndrome and diabetes, such as plasma lipid alterations, abdominal obesity, hepatic steatosis, insulin and leptin resistance [215–217]. CB<sub>1</sub> receptor signaling may also promote disease progression in heart failure [92,93,218] and atherosclerosis [117,219,220].

In contrast, CB<sub>2</sub> activation in human coronary endothelial and different inflammatory cells (e.g. neutrophils, monocytes, etc.) attenuates the TNF- $\alpha$  or other triggers-induced endothelial inflammatory response, chemotaxis and adhesion of inflammatory cells to the activated endothelium, and the subsequent release of a variety of proinflammatory mediators [102,115], crucial events implicated in the initiation and progression of atherosclerosis and restenosis, as well as in mediating reperfusion-induced tissue injury [221]. CB<sub>2</sub> receptor activation may also attenuate the TNF- $\alpha$ -induced human coronary artery smooth muscle cell proliferation [95]. Despite the low levels of CB<sub>2</sub> receptors expressed in the myocardium and cardiomyocytes [92,94,108], which can be upregulated in heart failure [125], recent studies have implicated this receptor in cardioprotection [94,108,109]. However, its precise role in cardiomyocyte signaling is still largely unexplored.

#### 4.1.1. Myocardial ischemia/reperfusion (I/R), preconditioning, and stroke—

Ischemia followed by reperfusion is a pivotal mechanism of tissue injury in myocardial infarction, stroke, organ transplantation, and during vascular surgeries. Endocannabinoids

overproduced during various forms of ischemia/reperfusion (I/R) injury have been proposed to protect against myocardial ischemia/reperfusion injury and to contribute to the ischemic preconditioning effect of endotoxin, heat stress, or brief periods of ischemia [107,222], while these lipid mediators (as mentioned above) may also mediate the cardiovascular dysfunction in these pathologies [1,10]. In initial reports, the weight of the evidence for endocannabinoid involvement was limited by the lack of use of selective ligands and/or cannabinoid receptor deficient animal models, and by the absence of direct quantification of tissue endocannabinoid levels and the use of ex vivo models, in which the key immunomodulatory effects of cannabinoids could not be determined. Recent studies using pre-clinical rodent models of myocardial [106,108,109], cerebral [73,121,124] and hepatic [103,131] I/R injury support an important role of endocannabinoids acting via CB<sub>2</sub> receptors in protection against tissue damage triggered by overproduced reactive oxygen and nitrogen species generation during reperfusion [107].

In these models of injury the beneficial effect of CB<sub>2</sub> receptor activation by selective synthetic ligands, such as JWH-133 and HU-308, was largely attributed to decreased endothelial cell activation and suppression of the acute inflammatory response as reflected in attenuated expression of adhesion molecules, secretion of chemokines, leukocyte chemotaxis, rolling, adhesion to endothelium, activation and transendothelial migration and interrelated oxidative/nitrosative stress associated with reperfusion damage (overviewed in [107]). Importantly, it was also demonstrated that CB<sub>2</sub> receptor knockout mice had increased reperfusion damage, implying a protective role of the endocannabinoid signaling mediated through these receptors. Some of these studies also proposed that CB<sub>2</sub> receptor activation may afford direct protection in parenchyma cells such as in cardiomyocytes [108,109], which needs to be confirmed in the future. Collectively, the endocannabinoid system appears to protect against various forms of I/R injury via activation of CB<sub>2</sub> receptors located both on endothelial and inflammatory cells, and perhaps also on some parenchyma cells (e.g. cardiomyocytes), and CB<sub>2</sub> receptor agonists may represent a protective strategy to attenuate reperfusion injury. For more detailed overviews see [2,223].

**4.1.2. Heart failure, cardiomyopathy, septic shock**—While there is considerable evidence that endocannabinoids are overproduced in various forms of shock, heart failure and cardiomyopathy and may mediate detrimental effects via CB<sub>1</sub> receptor signaling in endothelial cells and cardiomyocytes, relatively little is known on the role of CB<sub>2</sub> signaling in these pathological conditions [2,223].

A recent interesting study demonstrated that patients suffering from chronic heart failure exhibited a shift of the myocardial CB<sub>1</sub>-CB<sub>2</sub> receptor ratio towards marked overexpression of CB<sub>2</sub> receptors combined with significantly elevated peripheral blood levels of endocannabinoids indicating an activation of the endocannabinoid system [125]. As already mentioned above, the CB<sub>2</sub> agonist JWH-133 protected against I/R-induced cardiomyopathy [109] and administration of JWH-133 to cirrhotic rats with ascites significantly improved mean arterial pressure, decreased the inflammatory infiltrate, reduced the number of activated stellate cells, increased apoptosis in nonparenchymal cells located in the margin of the septa, and decreased fibrosis compared with cirrhotic rats treated with vehicle [141]. Since the hepatic endocannabinoid levels directly correlate with degree of liver injury and inflammation [131], it is most likely that the CB<sub>2</sub> agonist, by attenuating liver inflammation, also decreased the endocannabinoid levels, thereby resulting in less endocannabinoid-mediated hypotension through the activation of cardiovascular CB<sub>1</sub> receptors in the above mentioned study. This is also supported by the fact that CB<sub>2</sub> agonist administration did not produce significant hemodynamic effects in normal rodents [131].

Two studies investigating the role of CB<sub>2</sub> receptors in a mouse model of bacterial sepsis induced by cecal ligation and puncture yielded opposite results. While in one study CB<sub>2</sub> receptor knockout mice showed decreased survival as compared to wild-type mice [129], the other study demonstrated increased survival of knockouts exposed to bacterial sepsis and decreased bacterial load compared to wild types [128]. Notably, these studies used CB<sub>2</sub> knockout mice on different background, which may, at least in part, explain the discrepancy in the results. It is also important to note that CB<sub>2</sub> receptor activation is generally recognized as an immunosuppressive effect, which protects against overwhelming inflammation in sterile models of tissue injury. However, suppression of the immune system in the presence of live bacteria or other pathogens can promote growth of these pathogens and consequent tissue injury, which is often the reason why many drugs, which are effective in preclinical shock models of sterile inflammation, fail in human sepsis trials. Nevertheless, further studies investigating the role of CB<sub>2</sub> receptors in various forms of sepsis and infections with live pathogens are warranted.

**4.1.3. Atherosclerosis and restenosis**—Proinflammatory cytokines such as TNF- $\alpha$  and bacterial endotoxin(s) are key mediators in the development of atherosclerosis, which induce nuclear factor kappa B (NF- $\kappa$ B)-dependent up-regulation of adhesion molecules and chemokines (e.g. monocyte chemoattractant protein, MCP-1) in endothelial cells promoting recruitment and increased adhesion of monocytes to the activated endothelium followed by their transendothelial migration. These cells also release factors that promote smooth muscle cell proliferation and synthesize extracellular matrix, which also play important role in the development of vascular remodeling that occurs during restenosis (reocclusion of the vessel) in patients who undergo vascular surgery. Synthetic and endogenous cannabinoids have been reported in a context-dependent manner to either inhibit chemokine-induced chemotaxis of various cell types or induce immune cell migration in vitro, at least in part through the activation of CB<sub>1/2</sub> receptors, which raises the possibility that they may either attenuate or promote inflammation by influencing recruitment of immune cells to inflammatory sites [2,81]. In vivo, CB<sub>2</sub> receptor activation may reduce inflammation by interfering with the action of chemoattractants as demonstrated in studies of ischemia–reperfusion injury and in vitro in human monocytes treated with the synthetic CB<sub>2</sub> agonist [2].

CB<sub>1/2</sub> receptor-expressing immune cells, endocannabinoids, and their metabolizing enzymes are present both in human and mouse atherosclerotic plaques [2,111,113,117], however the activation of these receptors may exert opposing effects on the disease progression [220]. In a mouse model of atherosclerosis (ApoE<sup>-/-</sup> mice fed on high fat diet) oral administration of THC resulted in significant inhibition of plaque development in a CB<sub>2</sub>-dependent fashion, which involved reduced lesional macrophage infiltration, as well as attenuated proliferation and interferon- $\gamma$  release by splenocytes isolated from THC-treated mice [113]. Using a different cannabinoid ligand in the same in vivo model, another study concluded that the beneficial effects of CB<sub>2</sub> activation was due to the attenuation of the expression of adhesion molecules VCAM-1, ICAM-1, and P-selectin, which led to reduced macrophage adhesion and infiltration [114], a concept initially proposed by Rajesh et al. demonstrating that CB<sub>2</sub> stimulation attenuated the TNF- $\alpha$ -induced NF- $\kappa$ B and RhoA activation, ICAM-1 and VCAM-1 upregulation, MCP-1/CCL2 release in human coronary artery endothelial cells, as well as transendothelial migration and adhesion of monocytes [102]. An important role of CB<sub>2</sub> receptors was also implicated in inflammation-induced proliferation and migration of human coronary artery smooth muscle cells [95], processes crucially involved in the pathogenesis of both atherosclerosis and restenosis. An interesting recent study found that CB<sub>1</sub> and CB<sub>2</sub> receptor activation may differentially regulate reactive oxygen species production and inflammatory signaling in macrophages [224], which is also supported by recent in vivo studies in model of nephropathy [8,9].

Oxidized LDL is a recognized trigger for atherosclerosis, which accumulates in macrophages within atherosclerotic lesions, resulting in foam cell formation. The ability of oxidized LDL to trigger macrophage apoptosis plays significant role in atherosclerotic plaque stability and the progression of disease. This apoptosis rate was significantly reduced in peritoneal macrophages from CB<sub>2</sub> knockout mice as compared to wild type animals, involving Akt survival pathway in CB<sub>2</sub>-mediated signaling [225]. Further supporting a complex role of CB<sub>2</sub> receptors in atherosclerosis a recent study demonstrated that CB<sub>2</sub> receptor deficiency affected atherogenesis in Ldlr-null mice by increasing lesional macrophage and SMC content, reducing lesional apoptosis and altering extracellular matrix components, in part, by upregulating matrix metalloproteinase 9 [226].

Despite the above mentioned exciting pre-clinical reports, a recent large case-control study enrolling 1968 individuals addressing the involvement of the gene encoding CB<sub>2</sub>, CNR2, in the development of myocardial infarction and several cardiovascular risk factors (e.g. obesity, hypertension, hypercholesterolemia and diabetes mellitus) was not able to find any association of the investigated risk factors with the 13 investigated single nucleotide polymorphisms in the CNR2 gene [227]. Nevertheless, further studies are warranted to explore the role of CB<sub>2</sub> receptors in cardiovascular disease.

#### **4.2. Liver disease (hepatitis, fibrosis, cirrhosis) and its complications (cirrhotic cardiomyopathy and encephalopathy)**

The levels of endocannabinoids and their metabolizing enzymes in the normal liver are comparable to those observed in brain; in both, however, the expression of cannabinoid receptors is very low under physiological conditions. In various liver diseases and their complications, such as hepatitis [133], nonalcoholic fatty liver disease [135], drug-induced toxicity [228], hepatic I/R injury [7,103,131], liver fibrosis and cirrhosis [5,140,141,229], cirrhotic cardiomyopathy and hepatic encephalopathy [139,142,144,146,148,230] dysregulation of the endocannabinoid system (mostly increased levels of endocannabinoid and/or CB<sub>1/2</sub> receptors) have been reported [231] (Table 2 and Fig. 1). The most likely sources of CB<sub>2</sub> receptors in the normal liver are the resident macrophages (Kupffer cells) [232], endothelial and hepatic stellate cells, while under pathological conditions overexpression of CB<sub>2</sub> receptors may also be prominent in hepatocytes and originate from infiltrating inflammatory cells [5–7]. While CB<sub>2</sub> receptor activation in hepatic stellate cells mediates apoptosis and antifibrotic effects [6,96,233], its activation in Kupffer, endothelial and inflammatory cells most likely attenuates inflammation and reactive oxygen and nitrogen species generation associated with hepatic I/R as well as other liver pathologies [7,107]. Selective CB<sub>2</sub> receptor agonists administered prior to experimental hepatic ischemia or right after it, attenuated the I/R-induced rise in serum transaminases by decreasing inflammatory cell infiltration, tissue and serum levels of proinflammatory cytokines/chemokines, hepatic lipid peroxidation, and expression of the adhesion molecule ICAM-1 [103,131]. CB<sub>2</sub> receptor activation also attenuated the extent of the histological damage and PMN cell infiltration 1 day following the ischemic insult, and CB<sub>2</sub><sup>-/-</sup> mice developed aggravated I/R-induced tissue damage and proinflammatory phenotype in agreement with the protective role of CB<sub>2</sub> receptor activation [103,131]. I/R, but not ischemia alone, triggered marked increases in the hepatic levels of endocannabinoids AEA and 2-AG, which originated from hepatocytes, Kupffer and endothelial cells, and positively correlated with the degree of tissue injury and serum inflammatory cytokine and chemokine levels [131]. CB<sub>2</sub> receptor activation may also be protective against autoimmune hepatitis [234] and a beneficial effect of such activation in liver injury and regeneration has also been reported [131,145].

Although substantial evidence (briefly reviewed above) supports an important role for the endocannabinoid system and CB<sub>2</sub> receptors in modulating inflammatory response and tissue



injury in a variety of liver disorders, the exact mechanisms and cellular targets are still largely enigmatic. Additional studies should also address how the endocannabinoid system interacts with immune cells representing the innate immune system (e.g. natural killer cells, gamma delta T cells, and Kupffer cells), which play a pivotal role not only in the host defenses against invading microorganisms and tumor formation, but also in the pathogenesis of various inflammatory liver diseases. Various known polymorphisms of the CB<sub>1/2</sub> receptors should also be closely examined and correlated with liver disease severity. Furthermore, fundamental questions regarding the contrasting roles of CB<sub>1</sub> and CB<sub>2</sub> receptors and endocannabinoids in various liver pathologies (extensively reviewed recently by [5,217,235–237]) should also be answered, to devise clinically meaningful cannabinoid-based medicines for liver disease.

#### 4.3. Gastrointestinal (inflammatory bowel disease) and kidney disorders, pancreatitis

CB<sub>2</sub> receptor expression or immunoreactivity were found on colonic epithelial cells in mice, but not in rats, under baseline conditions, and CB<sub>2</sub> receptor expression was enhanced by the presence of a protective probiotic bacterium (*Lactobacillus acidophilus*) in mouse, rat and human colonic epithelium cells [90]. Immune cells of the gut also express CB<sub>2</sub> receptors. These receptors, as well as tissue endocannabinoid levels can be enhanced in active inflammatory diseases [4]. The expression of the CB<sub>2</sub> in the colonic mucosa is still controversial [88,150], and intriguingly CB<sub>2</sub> receptor expression was also reported on neurons of the submucosal and myenteric plexuses of the enteric nervous system both in rats and humans [87,89]. There is also some evidence [4,238] substantiating a possibly neuromodulatory role of CB<sub>2</sub> receptors in enteric neurotransmission, but overall studies support a role of CB<sub>2</sub> receptors in gut motility only in the stomach, particularly in certain pathophysiological states [4,238].

In experimental models of intestinal inflammation induced by various chemical irritants [151–153] an increase in the CB<sub>2</sub> receptor expression was reported in inflamed gut, and CB<sub>2</sub> agonists (JWH-133, AM1241) attenuated the colitis in wild type, but not in CB<sub>2</sub> knockout mice, which was exacerbated by a CB<sub>2</sub> antagonist AM630. Blockade of the endocannabinoid degrading enzyme FAAH with URB597 or the putative anandamide transporter with VDM11 had anti-inflammatory effect, which could be abolished by CB<sub>2</sub> receptor deficiency, further supporting a protective role for the CB<sub>2</sub> receptor activation in experimental colitis [152]. CB<sub>2</sub> receptors may also play some role in the modulation of the unpleasant visceral sensations (e.g. nausea, pain) in states of gastrointestinal irritation as reviewed recently [4,238], and by controlling gastrointestinal inflammation (a major risk for bowel cancer) may also modulate cancer risk and/or growth.

Michalski et al. [98] have investigated the functional involvement of the endocannabinoid system in modulation of pancreatic inflammation, such as acute pancreatitis. They found an upregulation of cannabinoid receptors and elevated levels of endocannabinoids in the pancreas of patients with acute pancreatitis. They also demonstrated that low doses of HU-210, a potent synthetic agonist at CB<sub>1</sub> and CB<sub>2</sub> receptors, abolished abdominal pain associated with pancreatitis and reduced inflammation and decreased tissue pathology in mice without producing central, adverse effects [98]. Antagonists at CB<sub>1/2</sub> receptors were effective in reversing HU-210-induced antinociception, whereas a combination of CB<sub>1</sub>- and CB<sub>2</sub>-antagonists was required to block the anti-inflammatory effects of HU-210 in pancreatitis [98]. In a follow-up study an important role of the endocannabinoid system in chronic pancreatitis was also revealed [99], and a recent study proposed an opposing time-dependent effects of endocannabinoids and CB<sub>1/2</sub> receptors on the course of the development of acute pancreatitis [101].

A functional endocannabinoid system also exists in the kidney [8,9,239], which can be activated during kidney injury [8,9]. CB<sub>2</sub> receptor activation with a selective receptor agonist attenuates the kidney dysfunction and nephropathy induced by the widely used chemotherapeutic drug cisplatin [8]. The mechanism of the CB<sub>2</sub> protection involves not only attenuation of the cisplatin triggered marked inflammatory response in the kidney (chemokine secretion and signaling by MCP-1, MIPs), endothelial cell activation (adhesion molecule expression), inflammatory cell infiltration, TNF- $\alpha$  and IL-1 $\beta$  levels, but also decrease in the expression of the reactive oxygen species (ROS)-generating NADPH oxidase enzyme isoforms NOX4, NOX2, and NOX1, and the consequent renal oxidative stress, alongside with the attenuation of the cisplatin-induced increased NF- $\kappa$ B-dependent iNOS expression and nitrate stress in the kidneys culminating in apoptotic and necrotic cell death [8]. Furthermore, the cisplatin-induced kidney inflammation, oxidative/nitrosative stress, cell death and dysfunction were enhanced in CB<sub>2</sub><sup>-/-</sup> mice compared to their wild-type CB<sub>2</sub><sup>+/+</sup> littermates, suggesting that the endocannabinoid system may exert protective effects via tonic activation of CB<sub>2</sub> receptors, similar to the effects reported in models of ischemic-reperfusion injury and neuroinflammatory disorders discussed in other parts. In contrast, CB<sub>1</sub> receptor activation appears to exert opposing effects in nephropathy models [9,240].

#### 4.4. Bone disorders

Bones, like all other body tissues, undergo substantial changes throughout life. Initially there is a rapid phase, when bone formation and bone resorption compensate each other. It is followed by a steady-state bone growth phase. Finally an imbalanced age-related bone loss may occur [27,28,241]. Remodeling cycles are observed throughout life. They are initiated by a rapid resorption by osteoclast cells, derived from monocytes, followed by slower bone formation by osteoblast cells. Imbalance in bone remodeling may lead to osteoporosis, a common degenerative disease in developed societies. Weakening of the skeleton and increased fracture risk, primarily in females, may be due to a net increase in bone resorption.

Osteoblasts are mostly regulated by bone morphogenetic proteins. Control of osteoclast formation and activity is governed by numerous factors such as macrophage colony-stimulating factor, receptor activator of NF- $\kappa$ B ligand (RANKL), osteoprotegerin, and interleukin 6. Low levels of gonadal hormones in females and males also cause bone loss. Additional factors such as parathyroid hormone, leptin, calcitonin, insulin-like growth factor I, and neuropeptide Y are also involved in the control of bone formation [27,28,241]. In osteoclasts, CB<sub>1</sub> is barely expressed, however the levels of CB<sub>2</sub> mRNA transcripts are high [105,155]. In vivo, CB<sub>2</sub> protein is present in trabecular osteoblasts [242], as well as in osteoclasts [105].

2-AG affects osteoblasts directly by binding to CB<sub>2</sub> [105,243]. DAGL $\alpha$ , one of the enzymes involved in 2-AG biosynthesis, is expressed in bone-lining cells and in osteoblasts, while DAGL $\beta$ , the second major enzyme involved in this process, was found in both osteoblasts and in osteoclasts [243]. Interestingly, oleoyl serine (an endocannabinoid-like compound which does not bind to either the CB<sub>1</sub> or the CB<sub>2</sub> receptors) has recently been described in bone. It increased bone formation and lowered bone resorption in a mouse osteoporosis model induced by estrogen depletion in ovariectomized animals [156].

Rossi et al. [244] have provided evidence for the role of CB<sub>2</sub> receptors as negative regulators of human osteoclast activity, as well as on the role of TRPV1 receptor in upregulation of CB<sub>2</sub> receptor expression in human osteoclasts obtained from women with osteoporosis; in these cells the role of the endocannabinoid system was also addressed [245].

During their first 2–3 months of life, CB<sub>2</sub><sup>-/-</sup> mice reach a normal peak trabecular bone mass, but later an age-related bone loss is noted [105]. CB<sub>2</sub><sup>-/-</sup> mice have a high bone turnover with

increases in both bone resorption and formation. However, ultimately a net negative balance is reached with age [105]. These effects are somewhat similar to human postmenopausal osteoporosis.

Karsak et al. [154] have examined the contribution of cannabinoid receptors to the regulation of bone mass in humans by investigating in osteoporotic patients the polymorphisms in loci, encoding the CB<sub>1</sub> and the CB<sub>2</sub> receptors. While they found no significant association of the CB<sub>1</sub>-coding exon with the osteoporosis phenotype, they noted that a common variant of the CB<sub>2</sub> receptor contributes to osteoporosis concluding that CNR2 polymorphisms are important genetic risk factors for osteoporosis. Related observations were also published by a Japanese group [246]. The studies by both groups suggest that diagnostic measures to identify osteoporosis-susceptible individuals can be developed based on polymorphisms in CNR2.

As CB<sub>2</sub> receptor activation does not lead to psychoactive effects, and in view of the results summarized above, Bab's group assumed that CB<sub>2</sub>-specific ligands could prevent and/or rescue bone loss, without the occurrence of side effects. Indeed, in the above mentioned mouse osteoporosis model in ovariectomized animals, they found that the specific non-psychoactive CB<sub>2</sub> agonist HU-308 [178], significantly attenuated bone loss in both 'preventive' [105] or 'rescue' protocols [91]. In the preventive approach, the administration of HU-308 was started immediately after ovariectomy. In the 'rescue' protocol the drug was given (daily for 4–6 weeks) starting 6 weeks after ovariectomy, when very significant bone loss had occurred. This treatment led to potent inhibition of bone resorption and stimulation of bone formation. These results strongly indicate that CB<sub>2</sub> agonists may become both antiresorptive and anabolic drugs for osteoporosis [27,28,91].

#### **4.5. Neurodegenerative disorders (multiple sclerosis, Parkinson's, Huntington's, and Alzheimer's diseases, amyotrophic lateral sclerosis) and pain**

A common feature of acute or chronic neurodegenerative diseases, such as multiple sclerosis (MS), Parkinson's disease, Alzheimer's disease, and amyotrophic lateral sclerosis (ALS) is the presence of oxidative and nitrosative/nitrative stress coupled with inflammation of various degrees [83]. Oxidative stress, inflammation, excitotoxicity, mitochondrial dysfunction, and hereditary and environmental factors have all been implicated in the neuronal dysfunction and death contributing to the pathogenesis of these disorders. Oxidative stress appears to provide a key link between environmental factors such as exposure to herbicides, pesticides, and heavy metals with endogenous and genetic risk factors in the pathogenic mechanisms of neurodegeneration (e.g. in Parkinson's disease) [83]. Oxidative/nitrative stress may also contribute to the disruption of the integrity of the blood–brain barrier and trigger reactive changes in glial elements (e.g. astrocytes and microglia represented by resident macrophage like cells in the brain and spinal cord) [83]. The disruption of the blood–brain barrier facilitates the penetration of various toxins and inflammatory cells to the site of brain injury further propagating damage which eventually leads to irreversible degeneration.

Dysregulation of the endocannabinoid system has been reported in virtually all of the above mentioned forms of acute (e.g. traumatic brain injury, stroke, and epilepsy) or chronic neurodegenerative disorders, such as MS, Parkinson's, Huntington's and Alzheimer's diseases, HIV-associated dementia, and ALS (reviewed by [10,11,173–175]). This was mostly reflected by time- and region-dependent increased or decreased endocannabinoid content in the lesions and/or in the cerebrospinal fluids of diseased animals or humans and/or altered cannabinoid receptor expressions in the diseased tissues (reviewed in: [10,11,63,173–175]). Earlier studies proposed that endocannabinoids or plant-derived cannabinoids are neuroprotective agents in the CNS [247–249], and this neuro-protection

involves: (a) attenuation of excitatory glutamatergic transmissions and modulation of synaptic plasticity via presynaptic CB<sub>1</sub> receptors; (b) modulation of excitability and calcium homeostasis via effects on Na<sup>+</sup>, Ca<sup>2+</sup>, K<sup>+</sup> channels, N-methyl d-aspartate (NMDA) receptors, intracellular Ca<sup>2+</sup> stores and gap junctions; (c) CB<sub>1</sub> receptor-mediated hypothermia; (d) antioxidant properties of cannabinoids; (e) modulation of immune responses and the release of inflammatory cytokines/chemokines by CB<sub>1</sub>, CB<sub>2</sub>, and non-CB<sub>1</sub>/CB<sub>2</sub> receptors on neurons, astrocytes, microglia, macrophages, neutrophils and lymphocytes [10]. However, numerous recent studies have also suggested that endocannabinoids through the activation of CB<sub>1</sub> receptors may also promote tissue injury and neurodegeneration (for example in stroke and other forms of I/R injury) [107,174,175], and many of the previously described protective effects of various synthetic CB<sub>1</sub> ligands were in fact attributable to centrally-mediated hypothermia and/or receptor-independent antioxidant/anti-inflammatory effects of the compounds [107].

Since functional CB<sub>2</sub> receptors are predominantly expressed on activated microglia and cells of immune origin in the brain and spinal cord (these cells are progressively present in pathological lesions associated with traumatic brain injury, stroke, MS, Parkinson's, Huntington's and Alzheimer's diseases, and ALS), their modulation by selective CB<sub>2</sub> receptor ligands may represent an interesting therapeutically exploitable possibility [165,174,175,250]. However, the achievement of this goal is largely complicated by the fact that depending on the particular disease type and its stage/progression, the inflammation by itself can be both beneficial and detrimental. Nonetheless, several lines of recent evidence have suggested an important role for CB<sub>2</sub> receptors in regulating central inflammatory response, neurodegeneration and neurogenesis. Firstly, increased immunoreactivity associated with microglial activation and/or immune cell infiltration has been reported in brain lesions of patients with MS [158], Alzheimer's disease [157,159,251], Down's syndrome [252], and in experimental animals exposed to neurotoxin or ischemia [164,253], as well as in mice with experimental autoimmune encephalomyelitis (EAE) [160], a model of MS. Secondly, CB<sub>2</sub> receptor agonists exert beneficial effects in pre-clinical models of stroke [73,121,254], spinal cord injury [123], Parkinson's disease (MPTP toxicity) [164], MS [161], Huntington's disease (malonate toxicity) [168], amyotrophic lateral sclerosis (hSOD1G93A transgenic mice) [162,163], and Alzheimer's disease [159]. Thirdly, CB<sub>2</sub><sup>-/-</sup> mice have larger cerebral injury and dysfunction following cerebral I/R [121], and they are more sensitive to the toxic effects of neurotoxin MPTP [164], and have more inflammation in an experimental model of MS [161]. Finally, CB<sub>2</sub> receptor agonists promote neural progenitor proliferation, suggesting that this receptor may also play an important role in the neurogenesis [12,255]. On the contrary, gliosis induced by β-amyloid(1–42) injection into the frontal cortex (a model of Alzheimer's disease) is potentiated by a CB<sub>2</sub> receptor-selective agonist [256].

Collectively, most of the evidence discussed above supports the view that selective CB<sub>2</sub> agonists may be useful in treating various neurodegenerative disorders by attenuating glial activation and inflammatory response (cytokine and chemokine secretion, etc.), alongside with decreasing reactive oxygen and nitrogen species generation, and possibly promoting neurogenesis.

Acute or chronic inflammatory response in the brain, spinal cord or at peripheral sites may profoundly affect synaptic transmission leading to pain. In addition to a well-known role of CB<sub>1</sub> receptors in pain, recent evidence also implicates CB<sub>2</sub> receptors in the antihyperalgesic activity of cannabinoids in models of acute and chronic, neuropathic pain, especially of inflammatory origin [177–192], however the detailed discussion of these studies is beyond the scope of this synopsis and is a subject of recent reviews [22,193].

#### 4.6. Psychiatric disorders (schizophrenia, anxiety and depression)

Numerous reports strongly indicate that cannabis use – presumably heavy use – may cause psychotic reactions in healthy individuals [257,258]. Cannabis use in adolescence or early adulthood carries a close to two fold increase in the risk of subsequent development of schizophrenia [194], which most likely may involve chronic dysregulation of the CB<sub>1</sub> receptors and/or receptor signaling. It has also been hypothesized that irregularities of the endocannabinoid system may lead to schizophrenia [195], however this assumption needs additional confirmation. The levels of anandamide in the blood are higher in patients with acute schizophrenia than in healthy volunteers [196], and the clinical remission is associated with decrease of these levels, as well as with decrease of the mRNA encoding the CB<sub>2</sub> receptor. These results are also consistent with higher levels of anandamide in the cerebrospinal fluid (CSF) of patients in the initial (prodromal) states of psychosis compared to healthy volunteers [259]. Interestingly, patients with lower levels of anandamide had a higher risk of later development of schizophrenia, indicating that the endocannabinoid system may have a protective role (opposite to the effects of chronic cannabis use). A recent study has investigated the genetic associations between CNR2 gene polymorphisms and schizophrenia and functions of potentially associated single nucleotide polymorphisms (SNPs) in cultured cells and human postmortem brain [15]. The analysis of two populations revealed significant associations between schizophrenia and SNPs. Two alleles were significantly increased among 1920 patients with schizophrenia compared with 1920 control subjects. A lower response to CB<sub>2</sub> ligands in cultured CHO cells transfected with one of the alleles, and significantly lower CB<sub>2</sub> receptor mRNA and protein levels were found in human cadaver brain with the genotypes of the second allele. In the same study, the effects of the CB<sub>2</sub> receptor antagonist AM630 on mouse behavior were also investigated [15]. AM630 exacerbated MK-801- or methamphetamine-induced disturbance of prepulse inhibition (PPI, an animal model of schizophrenia) and hyperactivity in mice. On the basis of these studies the authors concluded that people with low CB<sub>2</sub> receptor function are at increased risk for the development of schizophrenia. In contrast, in a rat model of social isolation (another model of schizophrenia) AM630 failed to affect any of the cognitive deficits (object recognition and contextual fear conditioning) studied [16], suggesting that CB<sub>2</sub> receptors do not play significant role in these paradigms.

Experimental treatment of depression and anxiety with cannabis, and later with THC, has been reported for over 150 years since Jacques-Joseph Moreau de Tours proposed its use in melancholia [260]. The results published over decades stretch from highly positive to adverse [197,198]. In view of the well known biphasic effects of cannabinoids [261], these observations are not unexpected. Since the discovery of the endocannabinoid system numerous publications tried to address its role in anxiety and depression. Because the CB<sub>2</sub> receptor was considered to be absent in the brain, the original assumption was that the CB<sub>1</sub> receptors are responsible for mediating all CNS effects of THC and endocannabinoids, which has been covered by outstanding recent overviews [262,263]. A few typical examples of the lessons learnt from pre-clinical and clinical studies in this regard: (a) knockout mice displayed increased anxiety-like behavior compared to wild-type controls under conditions that are stressful to the animals and have increased sensitivity to develop anhedonia in a model of depression [264]; (b) subjects with a life-time diagnosis of major depression had increased CB<sub>1</sub> receptor mRNA levels in the dorsolateral prefrontal cortex of [265]; (c) clinical trials of rimonabant, a CB<sub>1</sub> antagonist for the treatment of obesity, reported increased dose-dependent anxiety and depression in some patients as adverse events [266].

With the discovery of the presence of CB<sub>2</sub> receptor in the brain [51,267] several groups have addressed the possible effects on depression by stimulation of this receptor. Onaivi et al. found that there is a high incidence of Q63R polymorphism in the CB<sub>2</sub> gene in Japanese depressed subjects [17]. Garcia-Gutierrez et al. [18] using transgenic mice overexpressing

the CB<sub>2</sub> receptor (CB<sub>2</sub>xP mice) found decreased depressive-like behaviors in the tail suspension and a novelty-suppressed feeding tests, compared to wild type mice. The expression of brain-derived neurotrophic factor (BDNF) is downregulated in the hippocampus of wild type mice exposed to unpredictable chronic mild stress. In contrast, no changes in BDNF gene and protein expressions were observed in stressed CB<sub>2</sub>xP mice. It is well known that BDNF plays an important role in adult neurogenesis and that reduction of hippocampal neurogenesis occurs in patients with mood disorders. Hence, the observations described by Garcia-Gutierrez et al. indicate a possible mechanism of the anti-depressant action of the CB<sub>2</sub> receptor system [18]. However, acute administration of the CB<sub>2</sub> receptor antagonist AM630 exerted antidepressant-like effects in a forced swimming test in wild type, but not in CB<sub>2</sub>xP mice. This effect, apparently contradictory to the results described above, was explained on the basis of a possible increase of CB<sub>2</sub> receptor levels by an antagonist, a phenomenon previously seen with other receptors [268]. Hu et al. [19] have compared the antidepressant action of the CB<sub>2</sub> agonist GW405833 with the action of desipramine, a widely used antidepressant, in a chronic model of neuropathic pain, which causes depression-like behavior in animals. In this study mechanical hypersensitivity (a pain assay), time of immobility and climbing behavior in a swimming assay (depression assays) were used. Rats with chronic neuropathic pain displayed a significant mechanical hypersensitivity and a significant increase in time of immobility. Both desipramine and GW405833 significantly reduced immobility; however only GW-405,833 showed anti-nociceptive properties. Though, contrary to desipramine, GW-405,833 did not change the climbing behavior. Increased expression of the CB<sub>2</sub> receptor has also been shown to lead to reduction of anxiogenic-like behavior in mice [20]. The response to stress was also lower. Surprisingly, the action of anxiolytic drugs was impaired in the same study.

Even though the above mentioned studies envision the possibility of modulating schizophrenia and affective disorders by CB<sub>2</sub> agonists, it would be extremely important to confirm the exact localization and function of CB<sub>2</sub> receptors in the brain, which is still a very controversial issue. It should also be considered that multiple lines of evidence support a view that the depression itself can be a consequence of a chronic inflammatory process and immune system dysfunction, conversely many conventional antidepressants exert anti-inflammatory effects [269]. From this perspective modulation of the chronic inflammation by CB<sub>2</sub> receptor ligands may have a reasonable therapeutic rationale. The multifaceted nature of human mood disorders should also be kept in mind and the lack of reliable animal models of these diseases. For example, multiple pre-clinical studies based on these animal models also predicted a possible usefulness of CB<sub>1</sub> inverse agonists/antagonists as antidepressants [270]. Nevertheless, modulating mood disorders with cannabinergic ligands remains a provocative possibility, which warrants future studies.

#### **4.7. Skin, allergic and autoimmune disorders (allergic dermatitis, asthma bronchiale, rheumatoid arthritis, scleroderma, etc.)**

Multiple lines of recent evidence supports the existence of a functional endocannabinoid system in skin which is involved in maintenance of the balanced control of the growth, proliferation, differentiation, apoptosis, and cytokine/mediator/hormone production of various cell types (e.g. keratinocytes, sebocytes, immune cells, etc.) of the skin and appendages (e.g. sebaceous gland, hair follicle) [32]. AEA and/or 2-AG are detectable in rodent skin [177,199] and in human organ-cultured hair follicles [271] and sebocytes [272] alongside with AEA transporter, synthetic and metabolizing enzymes (NAPE-PLD and FAAH) in cultured keratinocytes [273], and in murine epidermal cells/skin [199]. Both cannabinoid receptors are identified on cultured human primary keratinocytes [32,273,274], and CB<sub>2</sub> expression is detectable in human sebaceous gland-derived sebocytes [272].

Disturbance of the above mentioned tight control may promote the development of multiple pathological conditions/diseases of the skin, such as acne, seborrhea, allergic dermatitis, itch and pain, psoriasis, hair growth disorders, systemic sclerosis, and cancer, among others (reviewed in [32]). CB<sub>1</sub> and CB<sub>2</sub> receptors were described in several murine and human skin cell populations (e.g. mast cells, cutaneous nerve fibers, epidermal keratinocytes, and cells of the adnexal tissues [182,199,207,271,272,275,276]). While hair growth appears to be under CB<sub>1</sub>-mediated endocannabinoid signaling control (CB<sub>1</sub> antagonists promote hair growth in mice [277]), CB<sub>2</sub> receptors may be involved in control of lipid production and cell death in sebocytes [272].

Although it is generally recognized that the endocannabinoid system has immunosuppressive, thus anti-inflammatory effects in many models of sterile inflammation [10,77], some controversies do exist regarding the skin contact dermatitis. Using well-established preclinical models for acute and chronic contact dermatitis, Oka et al. [201] demonstrated elevated 2-AG levels in the inflamed skin. They also showed that the symptoms of skin inflammation were dramatically attenuated by CB<sub>2</sub> (but not CB<sub>1</sub>) antagonists/inverse agonists [201]. Ueda et al. using a different approach to induce allergic contact dermatitis also reported suppression of the cutaneous inflammatory response by orally administered CB<sub>2</sub> antagonists/inverse agonists [200,278], as well as in the CB<sub>2</sub> deficient mice [200]. In contrast, Karsak et al. [199] suggested that the endocannabinoid system has a protective role in allergic inflammation of the skin. They demonstrated increased levels of endocannabinoids in skin of mice with contact dermatitis and showed that mice lacking both CB<sub>1</sub> and CB<sub>2</sub> (or treated with antagonists of these receptors) displayed a markedly exacerbated allergic inflammatory response. Notably, the CB<sub>2</sub> agonist HU-308 alone was not sufficient to afford any protection against allergic skin inflammation. The existence of the endocannabinoid-mediated protection was also supported by attenuated allergic response in the skin of FAAH-deficient mice, which have increased levels of the endocannabinoid AEA. Furthermore, the skin inflammation was decreased by locally administered THC [199]. The reasons for the above mentioned conflicting findings are not clear. They may be due to the different approaches utilized to induce the dermatitis. Recently, Zheng et al. [208], using CB<sub>1/2</sub> double knockout mice, provided evidence that endocannabinoids may promote the UVB-induced cutaneous inflammatory processes, since these mice showed remarkable resistance to inflicted damage. Collectively, the above mentioned studies support a therapeutic potential in allergic skin inflammation of CB<sub>2</sub> inverse agonists/antagonists or perhaps ligands which raise endocannabinoid levels or simultaneously target both CB<sub>1</sub> and CB<sub>2</sub> (like THC). Notably, THC has been reported to exert various receptor-independent anti-inflammatory and antioxidant effects, which could contribute to its local anti-inflammatory property [279].

Akhmetshina et al. [202] investigated the role of CB<sub>2</sub> receptors in bleomycin-induced dermal fibrosis (an animal model of systemic sclerosis/scleroderma (a systemic autoimmune disorder)). They found that CB<sub>2</sub> knockout mice or control mice treated with CB<sub>2</sub> antagonist AM630 developed increased dermal thickness and leukocyte infiltration in skin. Using bone marrow transplantation they also elegantly demonstrated that the leukocytes expressing CB<sub>2</sub> were critically involved in the development of experimental fibrosis [202]. Conversely, the CB<sub>2</sub> agonist JWH-133 attenuated dermal fibrosis and inflammation upon treatment implying a therapeutic potential of selective CB<sub>2</sub> agonists for the treatment of early inflammatory stages of systemic sclerosis. Utilizing a different mouse model of systemic sclerosis Servettaz et al. reported that treatment with WIN-55,212 or with the selective CB<sub>2</sub> agonist JWH-133 prevented the development of skin and lung fibrosis as well as reduced fibroblast proliferation and the development of autoanti-bodies. Consistently, the importance of CB<sub>2</sub> in the development of systemic fibrosis and autoimmunity was also confirmed using CB<sub>2</sub> knockout mice [203].

CB<sub>2</sub> agonists could theoretically exert multiple beneficial effects on rheumatoid arthritis (an autoimmune disorder), and perhaps some other degenerative joint disorders including: (a) immuno-suppression (inhibition of proliferation, apoptosis, suppression of cytokine and chemokine production in immune cells, and induction of T regulatory cells) and consequent attenuation of autoimmune inflammatory response; (b) attenuation of fibrosis in connective tissues (attenuation of fibroblast proliferation); (c) decrease in pain and neurogenic inflammation; and (d) anabolic effects on bone metabolism. As already discussed in previous parts, large number of studies support a possibly therapeutic utility of selective CB<sub>2</sub> agonists in chronic inflammatory pain (in fact, many pre-clinical models of inflammatory pain are based on injections of irritants triggering joint or skin inflammation in rodents), as well as in various bone and inflammatory disorders. CB<sub>1</sub> and CB<sub>2</sub> receptors and FAAH activity are detectable in the synovia of patients with osteoarthritis and rheumatoid arthritis, and the synovial fluid of these patients (but not normal volunteers) contains measurable endocannabinoid (AEA and 2-AG) levels, suggesting that a functional endocannabinoid system exists in the synovium, which may be therapeutically exploited for the treatment of pain and inflammation associated with osteoarthritis and rheumatoid arthritis in humans [204].

A recent study using a murine model of allergen-induced airway inflammation (asthma bronchiale) demonstrated that THC treatment of C57BL/6 wild type mice dramatically reduced airway inflammation as determined by reduced total cell counts in bronchoalveolar lavage fluid [280]. These effects were greatest when mice were treated during both the sensitization and the challenge phases. Besides, systemic immune responses were significantly suppressed in mice which received THC during the sensitization phase. However, no changes in lung inflammation were observed using pharmacological blockade of CB<sub>1</sub> and/or CB<sub>2</sub> receptors in the same model or using CB<sub>1/2</sub> receptor double-knockout mice. Furthermore, neither significant change in the cell patterns in BAL nor in immunoglobulin levels were found as compared to wild type mice. These result indicated that THC exerted CB<sub>1/2</sub> receptor-independent anti-inflammatory effects. These results in agreement with a previous study demonstrating that THC administered before sensitization to allergen ovalbumin and then before challenge, significantly attenuated the elevation of IL-2, IL-4, IL-5, and IL-13 steady-state mRNA expression elicited by ovalbumine challenge in the lungs, as well as the elevation of serum and mucus IgE overproduction [281]. Other recent studies demonstrated that the mixed cannabinoid CB<sub>1/2</sub> receptor agonist WIN-55,212-2 inhibited antigen-induced plasma extravasation and neurogenic inflammation in guinea pig airways, which could be reversed by CB<sub>2</sub>, but not CB<sub>1</sub> antagonist, implicating a CB<sub>2</sub>-mediated anti-inflammatory effect [205,206]. Endocannabinoids and synthetic ligands through the activation of CB<sub>2</sub> receptors may also play an important role in controlling the mast cell mediator release, and these cells are critical in the initiation of the inflammatory process associated with asthma, as well as with allergic skin and other diseases [282].

Numerous earlier studies have implicated a possible role of the endocannabinoid system in the control of airway smooth muscle relaxation, but this is still a controversial issue, which was previously reviewed in detail [10]. Thus, the effects of cannabinoids on respiratory function are rather complex, but evidence for their possibly usefulness as adjuncts treatment of allergic asthma is emerging.

#### 4.8. Cancer

Cannabinoids have well documented palliative effects in cancer patients such as appetite stimulation, inhibition of nausea and emesis associated with chemo- or radiotherapy, pain relief, mood elevation, and relief from insomnia [10]. Evidence based on: (a) studies evaluating the effects endocannabinoids or cannabinergic ligands in various cancer cell lines



or rodent models of explanted tumors; (b) epidemiological studies investigating the relationship of cannabis smoking and various forms of cancer; and (c) association studies in which CB<sub>1/2</sub> receptor expressions, endocannabinoids and their metabolizing enzyme expressions and/or activities were determined in various human cancers and correlated with pain, survival, and other determinants of progression, yielded inconsistent, often conflicting results suggesting that cannabinoids may both promote or inhibit cancer growth depending on tumor or cancer cell line and/or experimental condition (reviewed in [10,23,24]). The proposed mechanisms of cancer growth inhibition are multifaceted and may comprise of antiproliferative effect, induction of apoptosis in tumor cells, and attenuation of metastatic formation through inhibition of angiogenesis and tumor cell migration [10,23,24]. However, many of these effects can not clearly be linked to cannabinoid receptor activation. Furthermore, often there is no obvious association between expression of cannabinoid receptors in tumors (or tumor endocannabinoid levels) and the disease progression or patients' survival [10,23,24,26]. Instead of detailed discussion of these studies, we will provide only a few characteristic examples reflecting the overall situation in the field, and refer readers to several excellent recent overviews on this subject [23–26]. Casanova et al. [275] reported that human skin carcinomas (e.g. basal cell carcinoma, squamous cell carcinoma) express both CB<sub>1</sub> and CB<sub>2</sub>, and that local administration of synthetic CB<sub>1/2</sub> agonists triggered marked growth inhibition of malignant inoculated skin tumors accompanied by enhanced intra-tumor apoptosis and impaired tumor vascularization (altered blood vessel morphology, decreased expression of pro-angiogenic factors such as VEGF). Cannabinoids were also reported to inhibit the *in vivo* growth of melanomas that express CB<sub>1</sub> and CB<sub>2</sub>, by decreasing growth, proliferation, angiogenesis and metastasis formation, while increasing apoptosis [207]. In contrast, a recent study of Zheng et al. [208] showed that CBs are involved in the promotion of *in vivo* skin carcinogenesis. Using CB<sub>1/2</sub> double gene deficient mice, they demonstrated that an absence of CB<sub>1/2</sub> receptors resulted in a marked decrease in UVB-induced skin carcinogenesis [208].

The levels of CB<sub>2</sub> receptor and its endogenous ligand 2-AG are elevated in endometrial carcinoma [210] and CB<sub>2</sub> receptors are also expressed on malignancies of the immune system [209]. In immune cancer cells CB<sub>2</sub> stimulation triggered apoptosis, suggesting that selective CB<sub>2</sub> ligands may serve as novel anticancer agents to selectively target and kill tumors of immune origin [209].

As the above mentioned examples clearly illustrate that the situation regarding the role of the endocannabinoid system in cancer is very complicated and may largely depend on the tumor type, expression of cannabinoid receptors in the tumor, tumor microenvironment, and several other factors. On the other hand, several studies are very encouraging in support of the use of cannabinoids not only as palliative therapy, but also because of their ability to inhibit the growth and metastasis formation of certain types of tumors (e.g. in gliomas, tumors of immune origin, etc.). Furthermore, recent milestone discoveries suggesting a key role of the local inflammation in cancer progression, growth and metastasis formation provide additional reasons for future optimism regarding the possibility of therapeutic utility of the selective modulation of CB<sub>2</sub> receptors in cancer.

#### 4.9. Metabolic, reproductive and inflammatory eye disorders

The endogenous cannabinoid system through CB<sub>1</sub> receptors plays pivotal role in the regulation of energy homeostasis in multiple organs and at multiple regulatory levels (reviewed in [63,215–217]), however the role of CB<sub>2</sub> receptors in these processes is still very controversial and not supported by any obvious phenotype of CB<sub>2</sub> knockout mice. In endocrine pancreas CB<sub>2</sub> receptors were densely present in somatostatin-secreting delta cells, but absent in glucagon-secreting alpha cells and in insulin-secreting beta cells [100]. In contrast, other studies concluded that CB<sub>1</sub> and CB<sub>2</sub> receptors in beta cells may be present

and/or regulate insulin secretion [283,284]. Agudo et al. [138] found that CB<sub>2</sub> receptor knockout mice had greater age-dependent increases in food intake and body weight, however, even at 12-month age these obese CB<sub>2</sub><sup>-/-</sup> mice did not develop insulin resistance and showed enhanced insulin-stimulated glucose uptake in skeletal muscle. They also showed that adipose tissue hypertrophy was not associated with inflammation [138] and that treatment of wild-type mice with CB<sub>2</sub>R antagonist resulted in improved insulin sensitivity. Moreover, when 2-month-old CB<sub>2</sub><sup>-/-</sup> mice were fed a high-fat diet, reduced body weight gain and normal insulin sensitivity were observed. These results indicated that the lack of CB<sub>2</sub>R-mediated responses protected mice from both age-related and diet-induced insulin resistance, suggesting that these receptors may be a potential therapeutic target in obesity and insulin resistance [138]. In contrast in another study, Deveaux et al. [136] showed in both high fat-fed wide type mice and ob/ob mice (model of obesity), that CB<sub>2</sub> receptor expression underwent a marked induction in the stromal vascular fraction of epididymal adipose tissue that correlated with increased fat accumulation and inflammation. Treatment with the CB<sub>2</sub> agonist JWH-133 potentiated adipose tissue inflammation in high fat diet-fed wild type mice and the high fat diet-induced insulin resistance increased in response to JWH-133 and was reduced in CB<sub>2</sub><sup>-/-</sup> mice. Similarly, JWH-133 also enhanced the high fat diet-induced hepatic steatosis in WT mice which was blunted in CB<sub>2</sub><sup>-/-</sup> mice. This study suggested that CB<sub>2</sub> receptor antagonists may open a new therapeutic approach for the management of obesity-associated metabolic disorders. Nevertheless, further studies are warranted to investigate the role of CB<sub>2</sub> receptors and its peculiar cellular targets in context of metabolic disorders.

The endocannabinoid system and CB<sub>2</sub> receptors have also been implicated in various dysfunctions of the reproductive system [29,30], and selective CB<sub>2</sub> upregulation was reported in women affected by endometrial inflammation [211]. This topic was recently covered by several excellent overviews [29,30].

Xu et al. reported anti-inflammatory property of the CB<sub>2</sub> receptor agonist JWH-133 in a rodent model of autoimmune uveoretinitis (uveitis can be often a consequence of various systemic autoimmune diseases in humans), which was explained via inhibition of the activation and function of autoreactive T cells and prevention of leukocyte trafficking into the inflamed tissue [212].

## 5. Conclusions

Overwhelming evidence has established an important role for endocannabinoid-CB<sub>2</sub> receptor signaling in a large number of the major pathologies affecting humans. The broad spectrum of actions that can be attributed to CB<sub>2</sub> receptor signaling in inflammatory, autoimmune, cardiovascular, gastrointestinal, liver, kidney, neuro-degenerative, psychiatric and many other diseases have been reviewed here in some detail (Fig. 1 and Table 2). Most likely, the primary cellular targets and executors of the CB<sub>2</sub> receptor-mediated effects of endocannabinoids or synthetic agonists are the immune and immune-derived cells (e.g. leukocytes, various populations of T and B lymphocytes, monocytes/macrophages, dendritic cells, mast cells, microglia in the brain, Kupffer cells in the liver, etc.), however the number of other potential cellular targets is also expanding, including now endothelial and smooth muscle cells, fibroblasts of various origin, cardiomyocytes, and certain neuronal elements of the peripheral or central nervous systems (Fig. 2). Interestingly, it appears that in many of the above mentioned pathological conditions the inflammation/tissue injury not only triggers rapid elevation in local endocannabinoid level, which in turn initiates fast signaling processes in immune and perhaps other cell types modulating their critical functions, but may also lead to marked increases in CB<sub>2</sub> receptor expressions both in inflammatory, as well as in some parenchymal cells. However, the drastic elevation in CB<sub>2</sub> receptor expressions

recently reported in various diseased tissues calls for some cautious note until these studies are confirmed using more rigorous positive and negative controls and supported by functional evidence. The latter is extremely important given the known, but often ignored limitations of the recently available CB<sub>2</sub> antibodies and knockout mouse models.

In immune or immune derived cells CB<sub>2</sub> activation generally mediates immunosuppressive effects comprising inhibition of proliferation, induction of apoptosis, suppression of cytokine and chemokine production and migration of stimulated immune cells, and induction of T regulatory cells, with consequent attenuation of autoimmune inflammatory response. These effects limit the tissue injury in large number of the above mentioned pathological conditions, particularly in those associated with sterile inflammatory response. However, in other disease states these immunosuppressive effects of the CB<sub>2</sub> receptor activation may enhance or even inflict tissue damage as discussed in previous parts (e.g. in infections with various live pathogens, certain types of cancers in which the protective role of the immune system is critical to control the cancer growth, etc.). It is also important to keep in mind that even the relatively selective CB<sub>2</sub> agonists may activate CB<sub>1</sub> receptors in target tissues, particularly at higher doses (often resulting in opposite cellular and functional consequences on the disease progression as it became evident in many pathological conditions recently) and when the relative expression of CB<sub>1</sub> over CB<sub>2</sub> is high in the diseased tissue. This can also be one explanation for the bell-shaped dose response often seen with recently available CB<sub>2</sub> agonists in various disease models. Furthermore, in many disease models the CB<sub>2</sub> agonists appear to be most effective if given before the initiation of the insult, and may lose their effect or even promote inflammation when given at later time points. Thorough knowledge of CB<sub>2</sub> receptor signaling in key target immune and/or other cell types is still largely enigmatic; hence understanding of the precise course of the disease and the exact sequel of pathological events behind these changes is extremely important in order to devise meaningful therapeutic approaches based on targeted modulation of CB<sub>2</sub> receptor signaling.

Collectively, multiple lines of evidence discussed above support the view that lipid endocannabinoid signaling through CB<sub>2</sub> receptors represents an aspect of the mammalian protective armamentarium, and its modulation by either selective CB<sub>2</sub> receptor agonists or inverse agonists/antagonists (depending on the disease and its stage) holds unique therapeutic potential in huge number of diseases (the main targets of CB<sub>2</sub> receptor modulation in inflammation and tissue injury are summarized in Fig. 3). Excitingly, large number of novel synthetic [64–69] and natural [285,286] CB<sub>2</sub> receptor ligands has also been intensively investigated, giving hope that some of the above discussed preclinical results could also be translated into clinical treatments in the near future to ease human sufferings.

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## Abbreviations

<b>2-AG</b>	2-arachidonoyl glycerol
<b>AEA</b>	anandamide
<b>ALS</b>	amyotrophic lateral sclerosis
<b>CB<sub>1/2</sub> receptors</b>	CB <sub>1</sub> or CB <sub>2</sub> receptors (also termed CNR1 or 2)

<b>CB<sub>2</sub><sup>-/-</sup> or CB<sub>2</sub><sup>+/+</sup> mice</b>	cannabinoid receptor 2 knockout or wild type mice
<b>CNS</b>	central nervous system
<b>DAGL</b>	diacyl glycerol lipase
<b>FAAH</b>	fatty acid amine hydrolase
<b>HIV</b>	human immunodeficiency virus
<b>ICAM-1</b>	inter-cellular adhesion molecule 1 (also termed CD54)
<b>I/R</b>	ischemia/reperfusion
<b>LPS</b>	lipopolysaccharide (endotoxin)
<b>iNOS</b>	inducible nitric oxide synthase
<b>MAGL</b>	monoacyl glycerol lipase
<b>MAPK</b>	mitogen-activated protein kinase
<b>MCP-1</b>	monocyte chemoattractant protein (CCL2)
<b>MIP-1a2</b>	macrophage inflammatory protein-1alpha/2 (CCL3 or CXCL2, respectively)
<b>MS</b>	multiple sclerosis
<b>MPTP</b>	1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, a neurotoxin that causes permanent symptoms of Parkinson's disease by destroying dopaminergic neurons
<b>NK cells</b>	natural killer cells
<b>NOX4, NOX2, and NOX1</b>	isoforms of reactive oxygen species generating NADPH oxidase enzyme
<b>PMNs cells</b>	polymorphonuclear leukocytes/granulocytes
<b>THC</b>	Δ9-tetrahydrocannabinol
<b>TNF-α</b>	tumor necrosis factor-alpha
<b>NF-κβ</b>	nuclear factor kappaB
<b>RANKL</b>	receptor activator of nuclear factor kappa-B ligand
<b>RhoA</b>	a small GTPase protein
<b>ROS/RNS</b>	reactive oxygen or nitrogen species
<b>VCAM-1</b>	vascular adhesion molecule 1 (also termed CD106)

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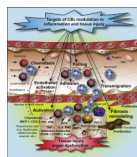
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**Fig. 1.** Role endocannabinoid-CB<sub>2</sub> signaling in various diseases affecting humans. Selected disease conditions are shown in which dysregulation of the endocannabinoid-CB<sub>2</sub> signaling is implicated in the pathology. Conversely, in these conditions pharmacological modulation of CB<sub>2</sub> receptors may represent a novel therapeutically exploitable strategy.



**Fig. 2.** Cannabinoid 2 receptor (CB<sub>2</sub>) expression and its known cellular functions. CB<sub>2</sub> receptors are most abundantly expressed in cells of the immune system and in cells of immune origin. In these cells CB<sub>2</sub> receptors mostly mediate immunosuppressive effects. CB<sub>2</sub> receptor expression was also reported in activated/diseased various other cell types. Importantly, in various pathological conditions CB<sub>2</sub> receptors were reported to be markedly upregulated in most of the shown cell types, and were attributed to play important role in various indicated cellular functions. Some of these effects are context dependent and may largely be determined by the type of the injury/inflammation and its stage.



**Fig. 3.**

Therapeutic targets of cannabinoid 2 receptor ( $CB_2$ ) modulation in inflammation and tissue injury. Endothelial cell activation and endothelial dysfunction is an early event inflicted by any kind of tissue injury/insult. Activated endothelial cells release various pro-inflammatory chemokines (e.g. monocyte chemoattractant protein 1 (MCP-1/CCL2), chemokine (C-C motif) ligand 5/Regulated on Activation Normal T Cell Expressed and Secreted (also CCL5/RANTES), macrophage inflammatory proteins (e.g. MIP-1 $\alpha$ /CCL3, MIP-2/CXCL2s), etc.), which attract inflammatory cells to the site of injury. Activated endothelial cells increase the expression and also release soluble adhesion molecules such as vascular adhesion molecule 1 (VCAM-1/CD106), intercellular adhesion molecule 1 (ICAM-1/CD54), which facilitated the attachment of inflammatory cells. This is followed by transmigration of inflammatory cells through the damaged endothelium and attachment to the parenchyma cells and activation (e.g. release of proinflammatory cytokines (tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), IL-6, IF- $\gamma$ , etc.), chemokines (CCL2, CCL5, CCL3, and CXCL2), reactive oxygen and nitrogen species (ROS/RNS), as well as various factors which promote matrix/tissue remodeling (e.g. matrix metalloproteinases (MMPs), TGF- $\beta$ , etc.). The pathological remodeling is further facilitated by ROS/RNS- and inflammatory mediators-induced activation of vascular smooth muscle cells and fibroblasts leading to their increased proliferation and release of various profibrotic and other pro-inflammatory mediators. The initial phase of inflammation may largely depend on the pathological condition (e.g. during the ischemia/reperfusion injury the initial players are predominantly neutrophils, while in early atherosclerosis mostly monocytes/macrophages) and the presence of residual inflammatory-derived cells (e.g. Kupffer cells in the liver, microglia in the brain, etc.), but later most inflammatory cells are present at various degree.  $CB_2$  receptor agonists (or in certain cases as discussed in this review the inverse agonist) protect against inflammation and tissue injury by attenuating endothelial cell activation/inflammatory response, chemotaxis of inflammatory cells, rolling and adhesion of inflammatory cells to the endothelium, transendothelial migration, adhesion to the parenchymal cells and activation (release of pro-inflammatory cytokines, chemokines, ROS/RNS, MMPs, etc.) and fibrosis.

**Table 1**Prototypical cannabinoid receptor ligands used in overviewed studies with effects on CB<sub>2</sub>.

Cannabinoid ligand	K <sub>i</sub> CB <sub>2</sub> (nM)	K <sub>i</sub> CB <sub>1</sub> (nM)	Origin	Reference
<i>Agonists</i>				
Mixed				
Anandamide	279–1940	61–543	Endocannabinoid	[42,70]
2-AG	145, 1400	58.3, 472	Endocannabinoid	[42,70]
(-)-Δ <sup>9</sup> -THC	3.13–75.3	5.05–80.3	Plant-derived	[42,70]
CP55940	0.69–2.8	0.5–5.0	Synthetic	[42,70]
R-(+)-WIN55212	0.28–16.2	1.89–123	Synthetic	[42,70]
HU-210	0.17–0.52	0.06–0.73	Synthetic	[42,70]
CB <sub>1</sub> > CB <sub>2</sub>				
ACEA	195, >2000	1.4, 5.29	Synthetic	[42,70]
CB <sub>2</sub> > CB <sub>1</sub>				
HU-308	22.7	>10,000	Synthetic	[42,70]
JWH-015	13.8	383	Synthetic	[42,70,71]
JWH-133	3.4	677	Synthetic	[42,70,71]
AM1241	3.4	280	Synthetic	[42,70]
O-1966	23 ± 2.1	5055 ± 984	Synthetic	[72]
O-3853	6.0 ± 2.5	1509 ± 148	Synthetic	[73]
GP1a	0.037	363	Synthetic	[74]
GW-405,833	3.92	4772	Synthetic	[75]
<i>Inverse CB<sub>2</sub> agonist/antagonist</i>				
AM630	31.2	5152	Synthetic	[42,70]
JTE-907	35.9	2370	Synthetic	[42,70]
SR144528	0.28–5.6	50.3–>10,000	Synthetic	[42,70]

K<sub>i</sub> values of cannabinoid CB<sub>1</sub>/CB<sub>2</sub> receptor ligands for the in vitro displacement of a tritiated compound from specific binding sites on rat, mouse, or human CB<sub>1</sub> and CB<sub>2</sub> receptors. Structures of the compounds listed are shown in indicated references. Large number of additional selective CB<sub>2</sub> ligands is under development [64–69,71].

**Table 2**Proposed role of endocannabinoid-CB<sub>2</sub> receptor signaling in selected diseases.

Disease, samples	Increase in endocannabinoid levels	Expression of CB <sub>2</sub> receptors	Effects attributed to CB <sub>2</sub> stimulation	References
Myocardial infarction ischemia/reperfusion injury (R, P, H)	Circulating immune cells	Myocardium	Decrease in inflammation (leukocyte infiltration) and myocardial protection	[106–110]
Atherosclerosis, restenosis (R, H)	Serum, atherosclerotic plaques	Infiltrating and other immune cells	Context dependent attenuation or promotion of vascular inflammation (monocyte chemotaxis, infiltration and activation) and factors of plaque stability; attenuation of endothelial activation and/or vascular smooth muscle proliferation	[95,102,111–117]
Stroke, spinal cord injury (R, H)	Serum, brain	Brain, microglia, infiltrating immune cells	Attenuation of inflammation (endothelial activation, leukocyte infiltration), and tissue injury; attenuation of motor and autonomic function deficits in a mouse model of spinal cord injury	[73,107,118–124]
Heart failure, cardiomyopathy (R, H)	Myocardium, cardiomyocytes	Myocardium, cardiomyocytes, endothelial cells	Attenuation of inflammation, decreased injury	[92,93,109,125]
Septic shock by live bacteria (R, H)	Serum	N.D.	Decrease or increase in inflammation and tissue injury most likely by affecting bacterial load	[126–129]
Hepatic ischemia–reperfusion injury (R, P, H)	Liver, serum, hepatocytes, Kupffer and endothelial cells	Inflammatory immune cells, activated endothelium	Attenuation of inflammation (endothelial activation, leukocyte chemotaxis, infiltration and activation), decrease in oxidative stress and tissue injury	[103,130–132]
Autoimmune hepatitis (R)	Liver	Infiltrating immune cells	Attenuation of T lymphocyte-mediated inflammation	[133,134]
Nonalcoholic fatty liver disease, obesity (R, H)	Liver	Hepatocytes, inflammatory cells	Enhancement of high fat diet-induced steatosis and inflammation or attenuation of obesity associated with age	[135–138]
Liver fibrosis, cirrhosis (R, H)	Liver, serum, inflammatory cells	Activated Stellate cells	Attenuation of fibrosis	[5,96,139–141]
Cirrhotic cardiomyopathy (R, H)	Myocardium, circulating immune cells and platelets	N.D.	Attenuation of hypotension by decreasing liver inflammation	[139,142–144]
Liver injury and regeneration (R)	Liver	Hepatic myofibroblasts	Reduced liver injury, accelerated regeneration	[131,145]
Hepatic encephalopathy (R)	N.D.	N.D.	Improved neurological and cognitive function in experimental models of hepatic encephalopathy	[146–148]
Inflammatory bowel disease, colitis, diverticulitis (R, H)	Inflamed gut	Epithelial cells, infiltrating inflammatory cells, enteric nerves	Attenuation of inflammation and visceral sensitivity	[4,88,149–153]
Pancreatitis (R, H)	Inflamed pancreas	Pancreas	Attenuation of inflammation	[98,99,101]



Disease, samples	Increase in endocannabinoid levels	Expression of CB <sub>2</sub> receptors	Effects attributed to CB <sub>2</sub> stimulation	References
Nephropathy (R)	Kidney	N.D.	Attenuation of inflammation (chemokine signaling and chemotaxis, inflammatory cell infiltration and endothelial activation) and oxidative/nitrosative stress	[8,9]
Bone disorders (e.g. osteoporosis) (R)	N.D.	Osteoblasts, osteoclasts	Attenuation of bone loss by enhancement of endocortical osteoblast number and function and suppression of trabecular osteoclastogenesis	[27,28,91,105,154–156]
Neurodegenerative/ neuroinflammatory disorders (multiple sclerosis, Alzheimer's, Parkinson's and Huntington's disease, spinal cord injury, neuro acquired immune deficiency syndrome) (R, H)	Brain, spinal fluid	Microglia, inflammatory cells, brain lesions, neurons?	Attenuation of inflammation (microglia activation, secondary immune cell infiltration), facilitation of neurogenesis	[10–12,123,157–176]
Pain (R)	Site of induced chronic inflammatory pain	Inflammatory cells, certain neurons	Attenuation of chronic inflammatory pain	[21,22,177–193]
Psychiatric disorders (schizophrenia, anxiety and depression) (R, H)	Blood, cerebrospinal fluid, brain (increased in schizophrenia, but decreased in brain in depression)	Glial, inflammatory cells, neurons?	Largely unexplored, in rodent models of depression/anxiety it may modulate CNS inflammation and either attenuate or promote anxiety-like behavior	[15–20,194–198]
Allergic dermatitis (R)	Inflamed skin	Infiltrating immune cells	Context dependent anti- or pro-inflammatory effects in contact dermatitis	[32,177,199–201]
Scleroderma/dermal fibrosis (R)	N.D.	N.D.	Attenuation of dermal and lung fibrosis/inflammation and leukocyte infiltration	[202,203]
Rheumatoid arthritis (H)	Synovial fluid, synovia	N.D.	Attenuation of the autoimmune inflammatory response	[204]
Allergen-induced airway inflammation (asthma bronchiale) (R)	N.D.	N.D.	Inhibition of antigen-induced plasma extravasation and neurogenic inflammation in airways, modulation of smooth muscle function?	[10,205,206].
Cancer (R, H)	Various tumors	In various tumors or cancer cells, in cells of the tumor microenvironment (e.g. in inflammatory cells, endothelium)	Context dependent attenuation or promotion of tumor growth (apoptosis, angiogenesis, proliferation, migration, etc.)	[10,23,24,26,207–210]
Reproductive diseases/ dysfunctions, endometriosis (R, H)	Reproductive organs/tissues	Testis, ovarium, uterus	Embryo development, induction of spermatogenesis. In endometriosis attenuation of inflammation and proliferation	[29,30,211]
Uveitis (R)	N.D.	N.D.	Decrease in T cell-mediated inflammation	[212]

Abbreviations: H: human; R: rodent; P: pig; N.D.: not determined.