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COMMENTARY

Cannabinoid Receptor 2 Activation

A Means to Prevent Monocyte—Endothelium Engagement

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The development and maintenance of chronic inflammation appear to be underlying causes of numerous human diseases such as atherosclerosis, obesity, diabetes, arthritis, chronic hepatitis, bone disorders, and neurodegeneration (including Alzheimer disease, multiple sclerosis, and chronic infections of the central nervous system like HIV-1).^{1–4} In all of these diseases, tissue infiltration by monocytes differentiating into macrophages plays a critical role, promoting development of atherosclerotic plaques, liver fibrosis, microglial accumulation around β -amyloid plaques in the brain, and microphage/microglial nodules in HIV-1 encephalitis, to name a few. Pro-inflammatory factors secreted by these cells are the driving force of organ specific injuries like neuronal death, hepatocyte demise and liver fibrosis, destruction of cartilage, or desensitization of insulin receptors. Inflammatory cell engagement of the endothelium leads to endothelial barrier compromise, further exacerbating tissue damage. This is especially important for the blood–brain barrier (BBB), which guards the unique microenvironment in the central nervous system and assures proper function of neuronal circuits.⁵ Despite a significant body of research on this subject, clear understanding of mechanisms, and therefore protective strategies, preventing them are missing. The work published in this issue by Rom et al⁶ addresses an important and an understudied problem of the selective effects of cannabinoid receptor (CB) 2 stimulation in leukocytes versus endothelial cells.

In this regard, the cannabinoid system could offer mechanistic insight into the development, propagation, and resolution

of chronic inflammation. Two types of cannabinoid receptors have been discovered and are relatively conserved in different species. CB1 was originally detected mainly in the central nervous system on certain types of neurons.⁷ Its stimulation mediates the psychoactive effects of marijuana. CB2 is predominantly expressed on immune cells (T and B lymphocytes, monocytes, macrophages, and microglia) and more recently has been found on endothelial cells. B lymphocytes, monocytes, and macrophages are the cell types that most highly express CB2 receptors in both rodents and humans.⁸

Endocannabinoids produced by numerous cell types in the body react with both CB1 and CB2, thereby regulating a number of important functions.⁹ One of the potential roles of CB2 activation is modulation of immune and inflammatory responses. The protective anti-inflammatory effects of CB2 stimulation by highly selective agonists have been documented in animal models for atherosclerosis,¹⁰ chronic neuroinflammation (multiple sclerosis),¹¹ Alzheimer disease,¹² liver and kidney injury,¹³ and arthritis.¹⁴ All factors implicated in inflammation-associated neurodegeneration—like secretion of pro-inflammatory factors, excitotoxicity, oxidative stress,¹⁵ and impairment of the BBB³—could be mitigated by CB2 stimulation. Indeed, CB2 agonists are able to reduce leukocyte migration¹⁶ and cytokine production by immune cells¹⁷ and protect the endothelial barrier by decreasing

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leukocyte–brain endothelial engagement.³ Furthermore, activation of CB2 provides immunomodulation in response to pathogens or transplantation of solid organs. All these data point to the potential clinical benefits of selective CB2 agonists that are devoid of the psychoactive effects of CB1 activation. Yet, such compounds are not being used in clinical practice.

End-organ injury in chronic inflammation is driven by immune cell infiltration. It is known that CB2 agonists limit inflammatory cell migration at the site of acute and chronic injury such as ischemia and reperfusion injury, atherosclerosis, multiple sclerosis, hepatitis, cellular rejection of transplanted solid organs, etc, in rodent models (lymphocytes, neutrophils, monocytes, and macrophages).⁹ However, it is not clear whether endothelial cells, immune and inflammatory cells, or both are responsible for mediating these beneficial properties, and the relative contribution of each cell type is yet unknown. To address this question, Rom et al⁶ used *in vivo* imaging and adoptive transfer of leukocytes treated with CB2 agonists *ex vivo* in an animal model of systemic inflammation on lipopolysaccharide administration. They demonstrated a >90% decrease in leukocyte adhesion to brain endothelium. Furthermore, they demonstrated a 50% increase in adhesion of leukocytes from CB2 knockout animals when they were transferred to wild-type mice injected with lipopolysaccharide. For the first time, they demonstrated the selective effects of CB2 stimulation in leukocytes versus endothelial cells.

Interactions between endothelium and inflammatory cells are critically important in the initiation and propagation of inflammatory injury. Leukocyte rolling, adhesion, and eventual migration across endothelium are tightly regulated by expression of adhesion molecules on endothelium (intercellular adhesion molecule, vascular cell adhesion molecule 1, and others) and their respective ligands [lymphocyte function-associated antigen 1, very late antigen 4 (VLA-4), etc] on lymphocytes, monocytes, and other inflammatory cells. Despite indirect evidence from animal studies, there is very limited data on how CB2 signaling mediates its anti-inflammatory effects in monocytes and macrophages, particularly in human cells. Rom et al⁶ used highly relevant human cellular systems, such as primary brain microvascular endothelial cells and primary human monocytes, to selectively activate CB2 in human monocytes. They showed decreased adhesion to and migration of monocytes across activated brain endothelial monolayers, and also established that inhibition of small GTPases (RhoA and Rac1) was responsible for these effects. Furthermore, these outcomes were associated with specific cytoskeletal changes, diminished lamellipodia formation, and down-regulation of the active form of VLA-4 (but not total VLA-4 expression). These results provide novel mechanistic insights on the protective effects of CB2. Although some effects such as RhoA inhibition and diminished migration have been documented before,¹⁸ none of the previous studies have addressed anti-inflammatory effects of CB2 activation in monocytes in such a comprehensive fashion.

Figure 1 Cannabinoid receptor (CB) 2 stimulation in monocytes: Signaling pathways. CB2 activation in monocytes leads to i) the inhibition of small GTPases, RhoA, and Rac1, ii) cytoskeleton rearrangement via augmented levels of inhibitory sites of the actin binding proteins, cofilin and VASP, upstream regulators of conformational integrin changes, iii) prevention of lamellipodia formation (key factors in monocyte migration), and iv) down-regulation of expression of integrin VLA-4 active form (mediating adhesion to vascular cell adhesion molecule 1 expressing endothelial cells). The combination of these events results in decreased monocyte adhesion to and migration across brain endothelial cells and protects blood–brain barrier.

Nevertheless, the study has some limitations. Specifically, *in vivo* imaging of transferred cells has been limited to 1 hour and BBB permeability was not assessed. On balance, the use of real-time measures of barrier function in primary brain microvascular endothelial cells in this study demonstrated the protective effect of CB2 activation in monocytes. It is worth mentioning that the same group has also demonstrated positive effects of CB2 stimulation in brain endothelium resulting in diminution of the pro-inflammatory phenotype of human brain endothelial cells and shielding of the BBB under inflammatory and physiological conditions confirming these *in vitro* assays by intravital microscopy.³ Figure 1 summarizes the signaling pathways involved in CB2 stimulation in monocytes. Taken together, it appears that CB2 stimulation could be a powerful tool in the treatment of inflammation and immunomodulation. It is critical to further develop a number of these compounds and thoroughly investigate their long term *in vivo* effects.

An important question that remains to be answered is what actual physiological role the endocannabinoid system plays in inflammatory response.¹⁹ It seems to be up-regulated in

inflammation as shown by increased expression of CB2 in Alzheimer disease, multiple sclerosis, and HIV encephalitis with enhanced expression of its synthesizing and degrading enzymes.^{19,20} Furthermore, the endocannabinoid system may be involved in inflammation resolution. This point is critical for chronic inflammation, where exaggerated and poorly controlled inflammatory reactions propagate tissue damage. Additional confirmation comes from a recent study indicating that chronic hepatitis C patients with polymorphism in CB2 are prone to severe necrotizing inflammation.²¹

As mentioned above, there is emerging clinical evidence for the importance of targeting monocytes and macrophages in chronic inflammatory diseases and on the therapeutic potential of selective CB2 agonists in large number of conditions. It is not clear what role CB1 plays in inflammation. There are seemingly opposing regulatory roles of CB1 and CB2 in monocytes and macrophages.²² Several studies have shown that CB1 antagonists are protective in atherosclerosis models, decreasing macrophage inflammatory response,²³ reactive oxygen species generation, and cytokine production.^{24,25} It will be very important to understand these signaling events to design better therapies. These observations recommend the rationale of combining peripherally restricted CB2 agonists with peripherally restricted CB1 antagonists.

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