COMMENTARY: Phytocannabinoids as therapeutic agents to combat chronic gingival disease.

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Abstract

The therapeutic potential of cannabis has been harnessed for thousands of years yet the United States Food and Drug Administration (FDA) has still not approved cannabis as a safe or effective drug. The FDA has, however, approved the manufacturing of pharmaceutical drugs, which contain a "synthetic version of a substance that is present in the marijuana" and other compounds that mimic its action. A search of the US National Library of Medicine and National Institute of Health for keywords "CBD" and "Periodontitis" together yields only 2 publications. Although the current State and Federal regulations on the use of cannabis for research and medicinal purposes seem to contradict one another, there is much room for optimism as the number of States allowing its use increases. This manuscript highlights the recent advances made in cannabinoid research as it pertains to oral health and gingival inflammatory disease. With a brief overview of the endocannabinoid system and its network of receptors and ligands, such as CBD, this manuscript provides the reader with the foundational knowledge necessary to answer common patient questions in a clinical setting.

Introduction

The therapeutic potential of cannabis has been harnessed for thousands of years, referenced by Pen Ts'ao Ching in a 2700BC Chinese manuscript describing it as a medicinal herb. Similarly, India in 1000BC classified cannabis as an analgesic and anticonvulsant. Later exported to the West by Irish physician Dr. O'Shaughnessy and French Psychiatrist Dr. Moreau in the late 1800's, cannabis was a part of US pharmacopeia until 1942.[11] What sealed its fate as a controlled substance started with the Marihuana Tax Act of 1937, passed to increase taxes on the use of hemp, a variety of cannabis used primarily in the textile industry. The American Medical Association and clinical pharmacists strongly opposed the bill, but it passed into law regardless. Forty years later, President Richard Nixon appointed a group of politicians and doctors to work on a bill titled "The Controlled Substance Act," which provides the framework on drug classification we still use today. The President simultaneously created the "National

Commission on Marihuana and Drug Abuse"(Shafer Commission), a team of scientists tasked with investigating and reporting the validity of cannabis for use in pharmacopoeia. As a result the Controlled Substance Act chairman temporarily placed cannabis on the highest level of control, pending findings from the Shafer Commission. Two years later, the Chairman and Pennsylvania Governor Raymond P. Shafer presented a report to Congress and the public entitled "Marihuana, A Signal of Misunderstanding." Written by medical doctors, psychiatrists and pharmacists of the Shafer Commission, it favored ending marijuana prohibition. The recommendation was ignored by the White House which in an effort to bolster its approval ratings, declared an outright "War on Drugs," placing cannabis at its epicenter. President Nixon's approval ratings were at their highest until the infamous Watergate Scandal leaked. Thanks to years of grassroots movements, the FDA approved the manufacturing of pharmaceutical drugs, which contain a "synthetic version of a substance that is present in the marijuana" and other compounds that mimic its action.

Although the current State and Federal regulations on the use of cannabis for research or medicinal purposes seem to contradict one another, there is much room for optimism, as the number of States allowing its use increases. Some of the concentrated extracts of the cannabis plant are widely available as over-the-counter (OTC) remedies, as well as for recreational use; claiming to heal sores, burns, inflammation, arthritis, and even cancer, most are marketed for topical or oral consumption. A review that underlines the importance of broad spectrum extracts for medicinal purposes as opposed to isolates of single compounds such as CBD, claiming "the entourage effect" is how cannabinoids and terpenes (compounds in the plant that give its characteristic scent and taste) may be working synergistically in the body.[1] In fact, Dr. Ethan B. Russo MD., published in the British Journal of Pharmacology that the "Entourage Effect" "increases the likelihood that an extensive pipeline of new therapeutic products is possible from this venerable plant."[1] Although some of these therapeutic claims remain to be investigated, there is no arguing that transient changes in cell signaling account for the effects of cannabinoid compounds. A brief look into the signaling cascade of the human endocannabinoid systems reveals the vast complexity of its functions and effects.

The endocannabinoid system is an important signaling system that existed before the evolution of vertebrates and is well preserved across most species. With over 100 active "cannabinoids" the cannabis plant has many cannabinoid compounds with therapeutic potentials yet to be investigated. The most commonly known cannabinoid of the cannabis plant is tetrahydrocannabinol, or THC, which is known to elicit psychoactive effects. Cannabidiol on the other hand, or CBD, along with others such as CBC, CBG, and CBN are non-psychoactive compounds of the plant that are currently being studied.[6] This cell

signaling process takes place within the human endocannabinoid system, a vast array of receptors scattered throughout the body that respond to the binding of a ligand, in this case CBD (Figure 1).

CBD binds loosely and has shown to have an indirect effect, causing downstream signaling cascades that lead to the modulation of hormone release, cytokine and chemokine production such as IL-1, IL-10, IL-6, IL-8, and dopamine. Two forms of the endocannabinoid receptor exist, CB1 receptors (CB1R), which modulate signal transduction primarily in the central nervous system with some receptors also found in the cardiovascular system and the CB2 receptors, (CB2R) which primarily have an effect on peripheral tissues and organs including but not limited to the immune and musculoskeletal systems. The ECS activates the internal signal transduction sequence via ligand binding to G-protein coupled transmembrane receptors.[10] Interestingly, the cannabinoid system shares the same heterotrimeric G-protein alpha subunits as the opioid, adrenergic and GABA pathways. The binding of CBD to CB1R and CB2R is poorly understood and remains an area of interest for immunologists and phytocannabinoid advocates. The many functions of the endocannabinoid system are yet to be fully understood but it is apparent that the lack of both CB1R/CB2R in a mouse model didn't create a notable phenotype, indicating that the ECS may potentiate homeostasis and immune response primarily during a pathologic challenge.[5] In order to activate the ECS pathway, there exists three sources of cannabinoids that can bind to the aforementioned receptors: endocannabinoids, phytocannabinoids, and synthetic cannabinoids.

Cannabinoids

Endocannabinoids, such as anandamide or AEA, are endogenous cannabinoid compounds produced in the CNS which elicit a weak agonistic activity on downstream signaling that affects homeostasis without any underlying psychoactive phenomenon. These downstream byproducts of cell signaling act only locally and instantly, and are metabolized immediately after their action. Figure 2 shows how lipid precursors of AEA activate the production/release of downstream inflammatory products such as prostaglandins, leukotrienes that can result in activation of the assonantic fundamental aspects of inflammation, calor, rubor, and tumor. Drugs that inhibit the enzymatic catalysis of AEA have already been developed with marked results. Their short half-life and dynamic levels of activation make them exciting targets as potential targets for therapeutic drugs for inflammatory disease processes.[11]

The first synthetic cannabinoid compound approved by the FDA for use was Nabilone, which was used to treat comorbidities associated with the treatment of cancer in 1985. In the treatment of cancer, the

therapy can significantly increase the morbidity of the disease and add additional symptoms such as nausea and vomiting. As a result a cocktail of medications are needed to provide an adequate quality of life for the patient. The market need for antiemetic medications with low drug interaction portfolios led to the development of Nabilone. Approved in 2014, Epidiolex catapulted GW Pharmaceuticals to the forefront of cannabinoid pharma with over a 2100% increase in its companies stock price one year after its release. Epidiolex is an FDA approved prescription medicine that is used to treat seizures associated with Lennox-Gastaut syndrome or Dravet syndrome. Interestingly, Lennox-Gastaut most often occurs secondary to brain damage. The brain damage can also occur from perinatal insults, encephalitis, meningitis, tumor, and brain malformation.[3] The resulting pathologic challenge is likely remedied and modulated by the endocannabinoid system of the central nervous system however the exact mechanism in which the cannabidiol contained in Epidiolex creates anticonvulsant effects is not known. Clinical data suggests that the cannabidiol does not create anticonvulsant effects through direct interaction with known CBRs. This data shows that there may be other cannabinoid receptors or upstream binding proteins that modulate the host response.

Finally, phytocannabinoids are cannabinoids produced by photosynthesis in plants. The most common cannabinoid producing plants are hemp and cannabis. Found in high concentrations of the viscous resin that is produced by glandular structures in the cannabis plant called trichomes. This resin is also rich in terpenes, which are responsible for the aroma and taste associated with various strains of the plant. The phytocannabinoids are mostly lipophilic but are also soluble in alcohol and other non-polar organic solvents. In alkaline conditions they can form water-soluble phenolate salts, being essentially phenols. THC is one of the phytocannabinoids found in cannabis known to stimulate cells in the brain to release dopamine creating euphoria. THC has shown to have analgesic, muscle relaxation, antipruritic and bronchodilatory effects. CBD, the other more commonly known phytocannabinoid has been used as an anticonvulsant, anxiolytic and has shown efficacy as an anti-inflammatory treatment. CBD differs structurally from THC mainly by hydrogenation of its central cyclic ether to a hydroxyl group. Figure 3 displays the structural heterogeneity of phytocannabinoids isolated from cannabis. CBG, or cannabigerol, is present in very low concentrations in the cannabis plant and is thought to be a minor cannabinoid. Enzymatic breakdown of CBG followed by UV exposure in the plant leaves leads to its biotransformation into more common subtypes such as THC and CBD.

Current Literature

Our understanding of the innate and adaptive immune systems and their role in the realm of inflammatory disease processes of the mouth has made many advancements thanks to the practice of evidence based dentistry. For years, the idea that periodontitis was a ubiquitous condition with its main etiologic factor being bacterial plaque formation has been replaced with the understanding of the influence of host susceptibility and epigenetic control contributing to individual risk. Major determinants of disease susceptibility are the immune-inflammatory response to antigens found in gingival crevicular fluid. Interestingly, it is these very defense measures that result in the majority of the tissue damage leading to the clinical manifestations of disease. The unique anatomy of the periodontium adds complexity to the pro- and anti- inflammatory signaling that dominates during a pathologic challenge such as periodontal disease.

A search of the US National Library of Medicine and National Institute of Health for keywords "CBD" and "Periodontitis" together yields only 2 publications. Of those publications, researchers from the University of Louisville School of Dentistry Department of Oral Immunology and Infectious Diseases examined the influence of physiologically relevant doses of CBD, CBN, and THC on the interactions of three ultrastructurally variant oral pathogens, *Porphyromonas gingivalis, Filifactor alocis*, and *Treponema denticola* in the presence of human immune cells. CBD, CBN, and THC each suppressed *P. gingivalis*-induced IL-12, IL-6, IL-8, and TNF release while enhancing the anti-inflammatory cytokine, IL-10, from human innate cells (Figure 4). It is known that the downregulation of the inflammatory response is mediated through the central regulator, which is the constitutively active phosphorylatively inactivated inflammatory gatekeeper, GSK3β. GSK3β targeting leads to suppression of TLR-initiated pro-inflammatory cytokines under NF-κB p65 transcriptional control and augmentation of IL-10 via promotion of CREB-dependent gene activation.[12] In order to find out whether the immune modulation witnessed after exposure to CBD was mediated through the TLR-GSK3β axis of activation, two established GSK3β inhibitors, SB216763 and LiCl were employed and neither pharmacological antagonist of GSK3β influenced the efficacy of CBD in repressing the pro-inflammatory cytokine response to LPS. This indicates that modulation of the cytokine profile observed is not mediated through TLR-GSK3β. Instead the investigators found that either pharmacological inhibition or siRNA-mediated silencing of PI3K (Figure 5) rescued the pro-inflammatory response of CBD-exposed, LPS-stimulated monocytes. They were able to conclude that phytocannabinoid mediated immune suppression is propagated through a CB2-PI3K signaling axis. However, the authors of this publication quite interestingly infer that phytocannabinoids may enhance periodontitis via direct toxic effects by compromising innate cell vitality and/or through a suppressed innate response to periodontal pathogens. It is important to note that they also contradict themselves by providing evidence from Nakajima et. al. who

used synthetic anandamide analog, methanandamide which was shown to suppress local TNF (tumor necrosis factor) production and reduce alveolar bone loss and in an LPS-injection model of periodontitis. Nakajima in another publication showed how endogenous cannabinoids suppressed activation of NF-κB activation, the main upstream regulator of the pro-inflammatory cascade.[13] The chronic nature of most periodontal disease underlines the importance of a balanced inflammatory response to not only aid in diapedesis and extravasation of immune cells to the region, but also with resolution and repair of damaged tissues. Resolution of inflammation by altering the host immune response may be a potential therapy to augment the body's ability to clear pathogens. Studies have concluded the important role phytocannabinoids play with their antimicrobial properties and their cytotoxic and immunosuppressive influences on oral bacteria-exposed human monocytes and epithelial cells.[8]

Research on the use of cannabidiol in the realm of inflammatory disease and other extracts of the cannabis plant are emerging rapidly. A review published in Nature, concluded that although AEA is widely accepted as an endocannabinoid due to its interactions with CB1R and CB2R, it is also known to stimulate other receptor types. A large body of evidence demonstrated that lipid-based molecules, such as CBD, but in particular AEA its endogenous counterpart, “can bind TRPV1 vanilloid receptors, the molecular target of capsaicin.” Zygmunt et al. reported that AEA induces vasodilation by activating TRPV1 receptors on perivascular sensory nerves. Additionally the article states that a growing body of evidence suggests that cannabinoids alter the PPAR nuclear receptor family axis of regulation through transcription and expression of genes that controls numerous physiological functions such as inflammation, cell differentiation and homeostasis. Finally, Gianluigi Tanga et. al. conclude that AEA, through potentiation of PPARγ, has anti-inflammatory effects inhibiting the release of the pro-inflammatory cytokine IL-2 in a CB1R/CB2R-independent manner. IL-2 is a known activating cytokine, which can lead to proliferation and activation of monocyte/macrophages and other immune cells of lymphoid origin.[4] These preliminary studies and reviews demonstrate the vast complexity of the endocannabinoid system and underline indications for the use of full or broad spectrum phytocannabinoid extracts for therapeutic uses. Due to Federal and State regulations, cannabinoid pharmaceuticals have been slow to enter the market, however, for families with children battling debilitating seizures, or the families of the 130 Americans that die from opioid abuse, hope remains that researchers will find new ways to reign in the touted benefits of the cannabis plant.[7] Long term clinical studies on the effects of CBD mediated immune-suppression on gingival inflammation is strongly encouraged. The use of UCLA’s huBLT humanized mice with reconstituted human immune systems with $CD34^+$ progenitor cells, fetal thymus and splenic tissue, have been shown to be a superior model to accurately predict the human immune response to a pathologic challenge.[15] The study would utilize in-vivo huBLT mice with the

presence or absence of oral administration of broad spectrum and/or isolate phytocannabinoids and oral microbiome inoculation using the oral gavage method.[14] We hope this will provide valuable translational evidence of the reported efficacy of the Entourage Effect of cannabinoid mediated immune-suppression to a bacterial challenge. Alveolar bone level measurements utilizing microCT scans as well as ELISA assays of overall cytokine secretion profiles like those presented in Figure 4 would help us understand the level of activation of competent immune cells in an in-vivo model closely resembling the human immune system.

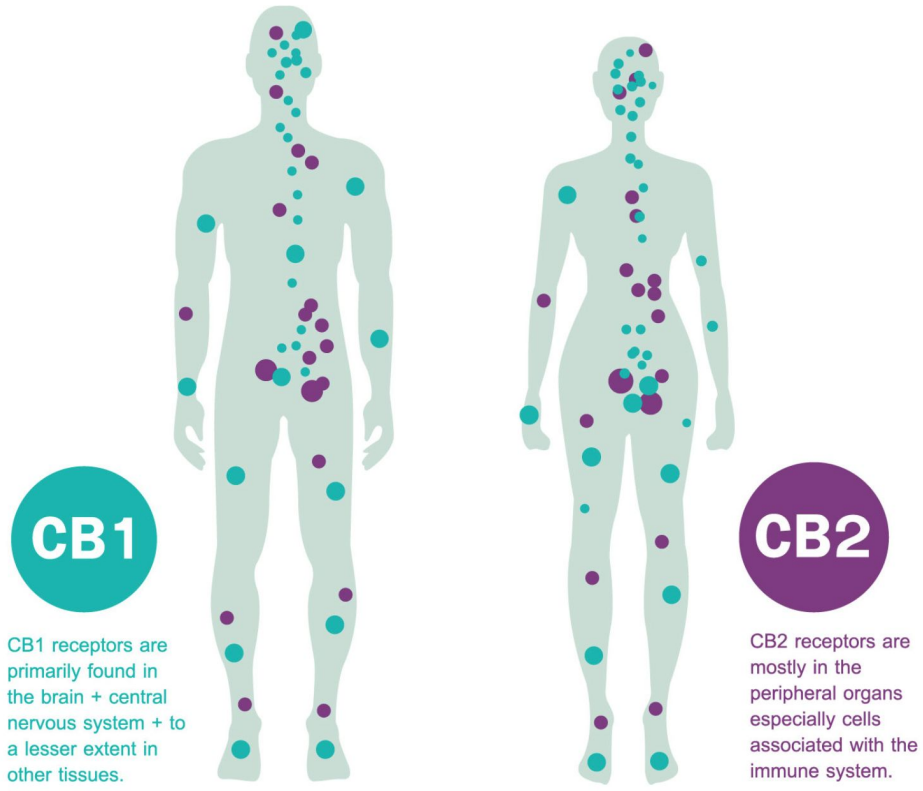

Figure 1. Map of CB1 and CB2 receptors scattered throughout the body. (Source: www.PloverOralHealth.com)

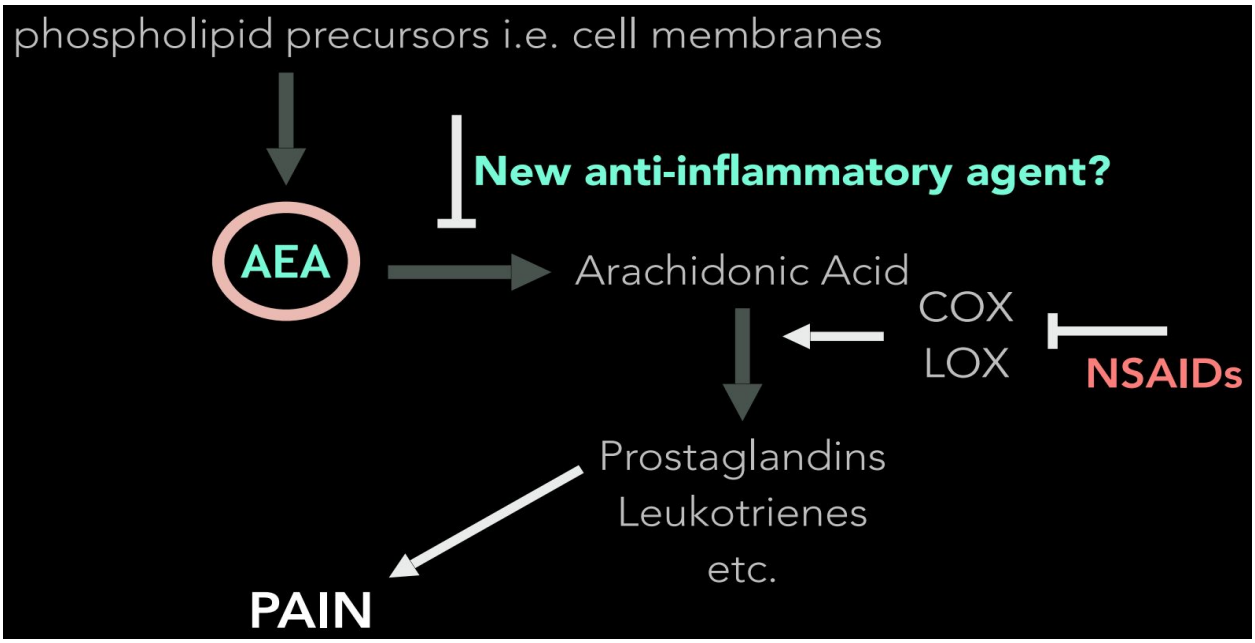

Figure 2: Phospholipid precursors lead to the formation of AEA, whose byproducts can lead to modulation of pain.[11]

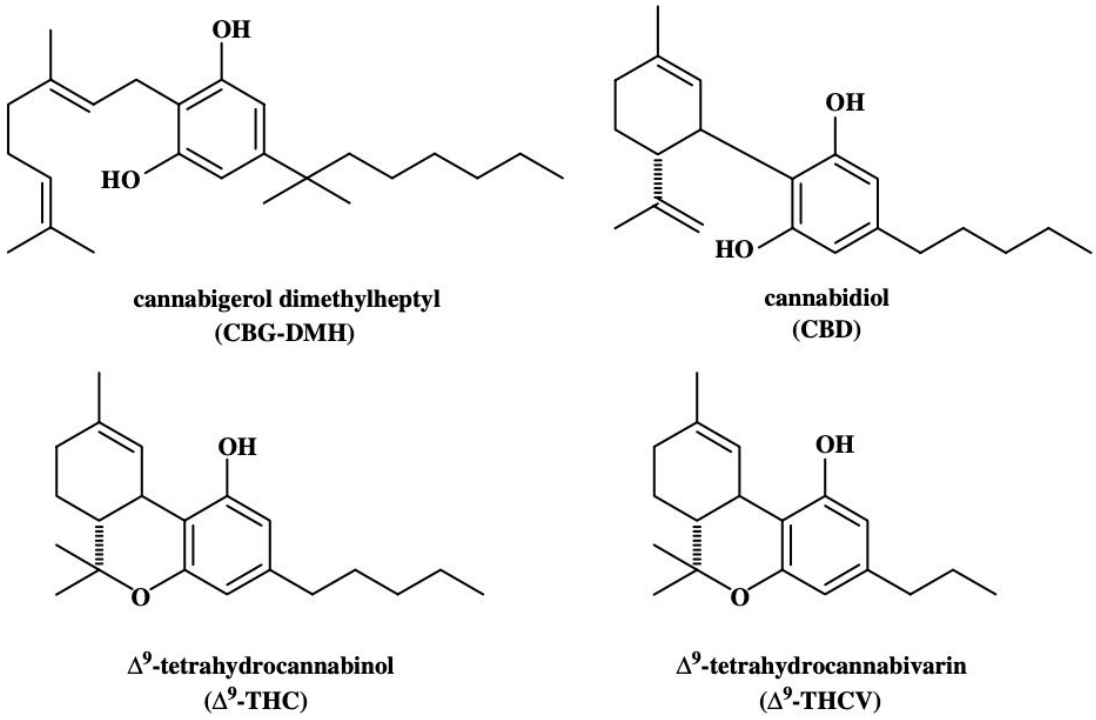

Figure 3: THC, CBD, CBG and THCV and their structural differences.[6]

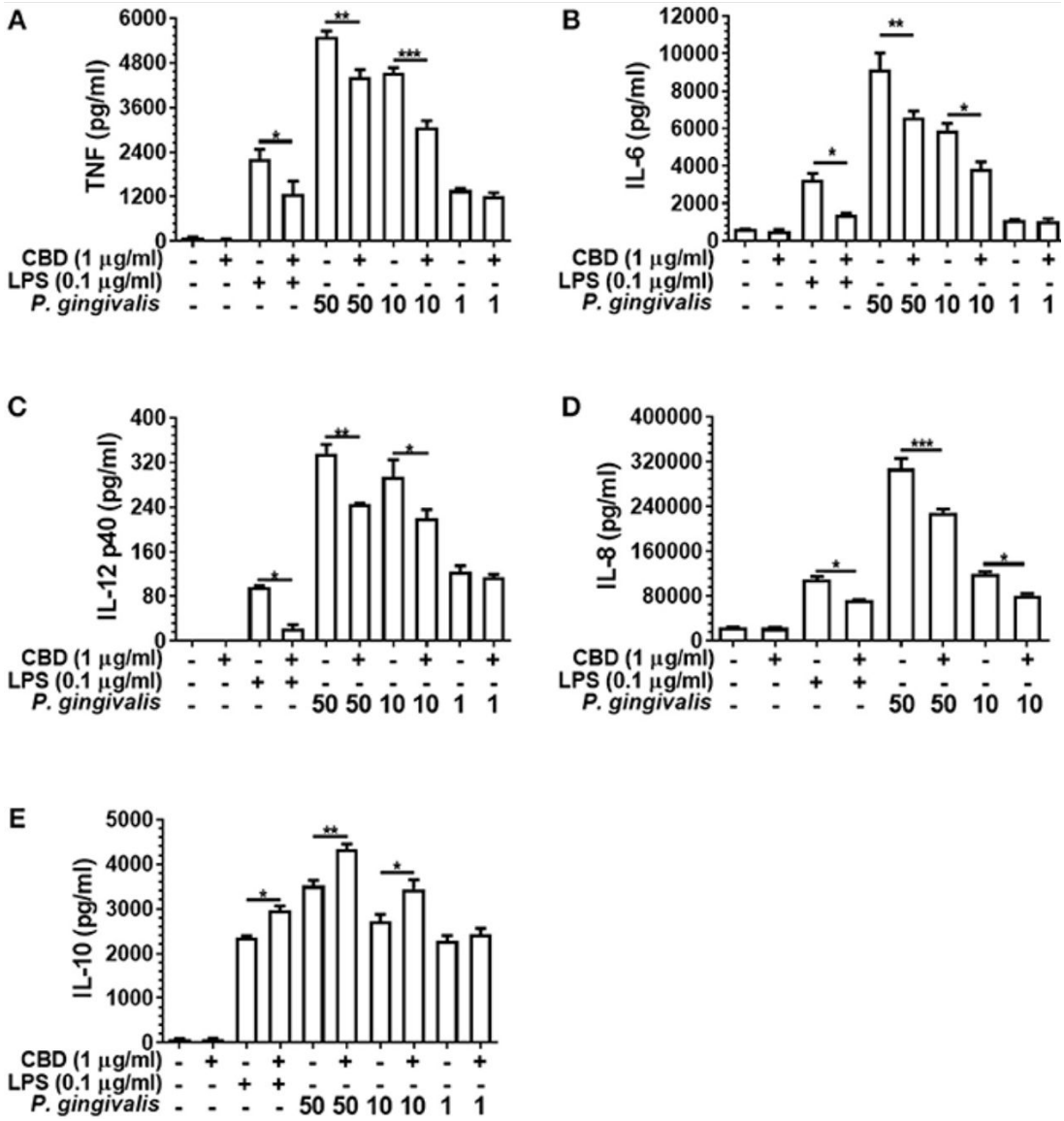

Figure 4: Phytocannabinoids alter the monocytic cytokine release profile in response to oral bacteria. Control and CBD (2 h) pre-exposed monocytes were stimulated, or not, with LPS or *P. gingivalis* and cytokine.[4]

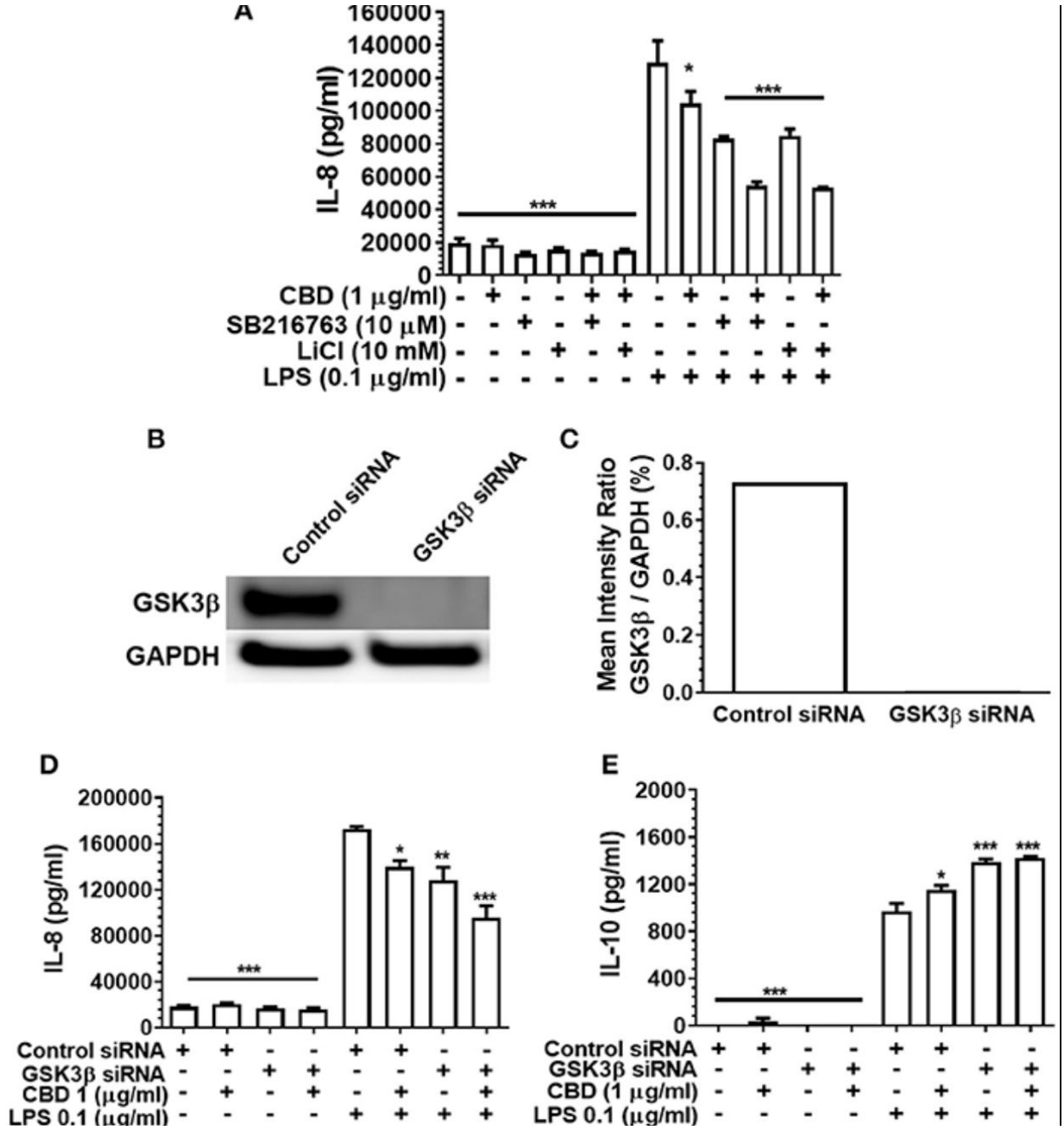

Figure 5: Inhibition or silencing of PI3K abrogates CBD-mediated innate suppression.[4]

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