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Do Cannabinoids have a therapeutic role in transplantation?

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Abstract

Cannabinoids have emerged as powerful drug candidates for the treatment of inflammatory and autoimmune diseases due to their immunosuppressive properties. While significant clinical and experimental data on the use of cannabinoids as anti-inflammatory agents exist in many autoimmune disease settings, virtually no studies have been performed on their potential role in transplant rejection. Here we suggest a theoretical role for the use of cannabinoids in preventing allograft rejection. While the psychotropic properties of CB1 agonists limit their clinical use, CB2 agonists may offer a new avenue to selectively target immune cells involved in allograft rejection. Moreover, development of mixed CB1/CB2 agonists that cannot cross the blood-brain barrier may help prevent their undesired psychotropic properties. In addition, manipulation of endocannabinoids *in vivo* by activating their biosynthesis and inhibiting cellular uptake and metabolism may offer yet another pathway to regulate immune response during allograft rejection.

Introduction

Cannabinoids are a group of terpenophenolic compounds structurally similar to delta-9-tetrahydrocannabinol (THC) from the plant *Cannabis sativa*. The cannabinoid receptor *CB1* gene was first cloned from brain [1] while the *CB2* gene was cloned from immune cells a few years later [2]. CB1 receptors are expressed primarily in brain and to some extent in peripheral tissues such as vasculature [3] and immune tissues [4]. CB2 receptors are expressed at high density on immune cells. Also, CB2 receptors are expressed in other peripheral tissues and some cells in the brain, although the levels are very low when compared to immune cells [5]. Endocannabinoids are the family of lipid transmitters derived from arachidonic acid and they act as endogenous ligands for cannabinoid receptors. Anandamide (AEA), an amide of arachidonic acid and ethanolamine, was the first endogenous cannabinoid ligand discovered [6]. Subsequently, 2-arachidonoylglycerol (2-AG) was discovered and noted to be in much higher concentration in serum and brain than AEA [7,8]. In addition to their actions at cannabinoid receptors, endocannabinoids such as AEA and plant cannabinoids such as cannabidiol (CBD) exert their effect by binding to vanilloid receptors (TRPV) in vasculature as well as immune cells [9,10].

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Statement of Conflicts of Interest

The authors do not have conflict of interest.

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In recent years, cannabinoids have emerged as novel anti-inflammatory agents because of their efficacy in the treatment of many immune-mediated disorders such as multiple sclerosis, rheumatoid arthritis and autoimmune hepatitis [11–15]. Also, some countries have approved their use in clinical settings for chemotherapy-associated nausea, vomiting and pain [16]. Clinically, one of the main drawbacks in using cannabinoids, such as THC, is the psychotropic effects exhibited by these compounds following activation of CB1 receptors. However, recent studies have suggested that CB2-select agonists can also cause immunosuppression, and that such cannabinoids that are devoid of psychotropic effects, may serve as ideal candidates for use as immunosuppressive agents [17,18]. The other advantage in using CB2 select agonists stems from the fact that CB2 is selectively expressed at higher density on immune cells and therefore likely to target primarily such cells.

Transplantation is one critical area of medicine that requires the use of immunosuppressants. When an organ or tissue is exchanged between two genetically non-identical members of the same species, it is called an allograft. Thus, in most instances, tissue or organ transplants in humans are allografts. When the transplanted cells express antigens that are not expressed in the recipient of the graft, such cells are recognized as foreign by the recipient. This leads to activation of the recipient's immune system and transplant rejection. To prevent this, the recipient is treated with immunosuppressive drugs. The immunosuppressive regimen in the recipient has to be carefully monitored so that it is not excessive and make the recipient more vulnerable to infections and cancer. Moreover, there are various complications associated with current use of immunosuppressive drugs, such as nephrotoxic effects, hypertension, hyperlipidemia, and diabetes, which together stress the need for development of better immunomodulatory drugs. Several recent reviews have highlighted these challenges [19,20].

Here we discuss the actions of cannabinoids on immune cells as well as on endothelial cells, which play a crucial role in allograft survival, and argue that these properties make the cannabinoids an ideal candidate for effective immunomodulatory therapy in transplantation.

Transplantation

The main targets for the immune response to transplanted grafts are the major histocompatibility complex (MHC)-encoded molecules [20]. When an organ is transplanted, the donor-derived MHC molecules are recognized as “non-self” antigens by the recipient. This phenomenon is called allorecognition. This activates the immune response in the recipient which destroys the transplanted tissue (Figure 1). Another form of transplantation is haematopoietic cell transplantation (HCT), which is used to treat high-risk haematological malignant disorders or other serious haematological diseases [21]. In contrast to allogeneic solid-organ transplantation, the complications in HCT result from donor-derived immunity against the recipient, referred to as graft-versus-host disease (GVHD). GVHD arises when donor T cells get activated primarily in response foreign MHC antigens expressed on the cells of the host, leading to severe tissue injury [22].

There are two types of immunosuppressive protocols currently in use for transplantation patients: initial and maintenance therapies. Initial therapy employs polyclonal antibodies (anti-thymocyte globulins or anti-lymphocyte globulins) or monoclonal antibodies (anti-CD3 antibody: Muromonab-CD3 (OKT3) or two anti-CD25 antibodies: basiliximab (Simulect), daclizumab (Zenapax) or anti-CD52 antibody: alemtuzumab (Campath-1)) [23]. These antibodies play a role in preparing the recipient's immune system to adapt and create tolerance towards the allograft. However, routine use of these agents increases the risk of serious infection or malignant diseases. Maintenance therapy mostly includes calcineurin inhibitors (tacrolimus and cyclosporine), which block translocation of nuclear factor of

activated T cells (NFAT), resulting in a failure to induce crucial genes such as IL-2 regulated by this transcription factor [24]. The routine use of these medications has dramatically decreased the incidence of rejection and graft loss; however, these approaches also exert a wide range of toxicities [25].

Immune Modulation by Cannabinoids

Induction of cell death in immune cells is one of the major mechanisms by which cannabinoids cause immune suppression [26]. Among various cannabinoids, apoptotic effects of THC on immune cell populations have been studied in detail. Initially, it was observed that THC induced apoptosis via the downregulation of Bcl-2 and upregulation of caspases in murine macrophages and T cells *in vitro* [27]. Later, significant levels of apoptosis in T cells, B cells, dendritic cells and macrophages after *in vivo* administration of THC and its derivative ajulemic acid were demonstrated in mouse models of disease [28,29]. *In vitro* treatment with CBD, which is a major non-psychoactive natural cannabinoid, caused apoptosis in mouse CD4+ and CD8+ T lymphocytes by inducing reactive-oxygen-species as well as caspases 8 and 3 [30]. JWH-015, a potent CB2-select synthetic agonist, induced cell death *in vitro* in murine immune cells, via both death receptor and intrinsic pathways [17]. Because of selective high expression of CB2 on immune cells, CB2 select agonists are likely to induce apoptosis in immune but not in non-immune cells of an allograft.

Cannabinoid receptors are G-protein-linked transmembrane receptors whose activation leads to inhibition of adenylyl cyclase cascade, which leads to a decrease in the production and accumulation of intracellular cyclic adenosine 3'-5'-monophosphate (cAMP) [31]. The demonstration that a decrease in cAMP leads to immunosuppression is contrary to the long held view that cAMP signaling serves as a negative regulator of immune cell activation. However, this notion has been challenged, as discussed in other reviews [32]. In general, agents that elevate cAMP have been shown to inhibit the production of Th1 cytokines such as IFN- γ and IL-2 [33]. However, depending on experimental conditions used, the effects of cAMP elevating agents on Th2 cytokine production appear to vary. Thus, cAMP-elevating agents either inhibited [34] or increased the production of IL-4 [35].

Cannabinoids such as THC, in general, suppress the production of inflammatory Th1 cytokines while promoting Th2 cytokines, although this effect is dependent on the nature of inflammation or the cell type studied *in vitro* [36]. For example, lung alveolar macrophages obtained from marijuana smokers showed impaired production of TNF- α , IL-6 and granulocyte-macrophage colony-stimulating factor (GM-CSF) upon LPS activation [37]. In human T, B, CD8+, NK, and eosinophilic cell lines, addition of THC and CBD to *in vitro* cultures induced suppression of TNF- α , GM-CSF, IFN- γ and IL-10 production, whereas IL-8 levels were increased [38]. While such studies suggest pro- and anti-inflammatory effects of cannabinoids, it should be noted that these studies used transformed cell lines. In an animal model of myocardial ischaemia-reperfusion injury, treatment with WIN55,212-2, a CB1-select agonist, decreased inflammation and tissue injury with decreased levels of IL-1 β and CXC-chemokine ligand 8 (CXCL8) [39]. Recent studies have shown that both synthetic cannabinoids, CP55,940 and WIN55,212-2, decreased IL-6 and IL-8 production from IL-1 β stimulated rheumatoid fibroblast-like synoviocytes by a CB1/CB2 independent mechanism [40]. Using *Legionella pneumophila* infection of mice as a model, it was demonstrated that THC attenuated Th1 immunity in mice and induced a shift to Th2 immunity [41]. In contrast, some studies have also indicated that cannabinoids may increase the production of certain cytokines, including TNF- α , IL-1 and IL-6, when administered together with bacteria or other antigens. For example, THC was shown to enhance secretion of IL-1 from macrophages that were stimulated with endotoxin *in vitro* [42]. Thus, the effect

of cannabinoids on cytokine production may depend on the nature of signal that triggers inflammation as well as the type of cannabinoid used [43]. Among the T helper cells, transplant rejection is believed to be primarily mediated by Th1 cells through production of inflammatory Th1 cytokines. Whether cannabinoids can suppress Th1 response during allogeneic transplantation remains an interesting possibility that needs to be investigated.

Regulatory T cells (Tregs) are an important suppressor cell population and play a significant role in the induction of long-term tolerance after transplantation. These cells specifically express Forkhead helix transcription factor p3 (Foxp3) in addition to CD4 and CD25, and suppress the proliferation of naïve T cells and their differentiation to effector T cells, which has been the subject of recent reviews [44]. In addition, Tregs suppress the effector activities of differentiated CD4⁺ and CD8⁺ T, the function of natural killer cells, natural killer T cells, B cells, macrophages, osteoclasts, and dendritic cells [45]. Tregs can induce and maintain antigen-specific immune tolerance and facilitate allogeneic graft survival successfully in animals [46]. Recent studies from our laboratory demonstrated that THC could protect mice from ConA-mediated acute autoimmune hepatitis [15]. We noted that THC treatment led to significant increase in absolute number of Foxp3⁺ T regulatory cells in liver as well as Foxp3 mRNA expression in splenocytes and liver mononuclear cells. In these studies, pretreatment with anti-CD25 antibodies to deplete Tregs significantly reversed the ability of THC to protect mice from hepatitis, indicating that CD25⁺ regulatory cells were essential for THC-mediated suppression of hepatitis [15]. If cannabinoids can induce Treg expansion in response to alloantigens, this may be beneficial inasmuch as Tregs help in preventing graft rejection [47].

Endocannabinoids have also been shown to regulate cytokine production. Endocannabinoid, 2-AG suppresses IFN- γ expression in murine splenocytes in a CB receptor-independent manner. The mechanism partially involves suppression of intracellular calcium signaling and perturbation of NFAT nuclear translocation [48]. AEA causes a concentration-dependent inhibition of IL-2 secretion in primary murine splenocytes, which is independent of CB1/CB2 and involves the activation of peroxisome proliferator activated receptor (PPAR γ) [49]. AEA also inhibits the TNF- α -induced signals leading to I κ B activation, I κ B α degradation, and NF- κ B activation and that this activity is essentially CB1- and TRPV1-independent [50].

Endothelial cells in Allograft Rejection

Endothelial cells line the blood vessels and they are the “gate-keepers” regulating the interactions between blood and tissues [51]. Endothelial cells are crucially involved in allograft rejection because of their role in the infiltration of immune cells into the engrafted tissue [52]. In addition, endothelial cells themselves serve as antigen presenting cells during allograft recognition [53]. It is known that allograft rejection is mostly T cell mediated; however humoral response directing antibodies against ABO blood group types or human leukocyte antigen (HLA) types is also involved in the rejection process. Non-HLA and Non-ABO antibodies have also been detected in organ transplantation, and some studies identified anti-endothelial cell antibodies (AECAs) as the cause for rejection [54,55].

We propose that cannabinoids may serve as excellent candidates for interfering with the actions of endothelial cells during allograft rejection. A recent study demonstrated, both *in vivo* and *in vitro*, that treatment of human coronary artery endothelial cells (HCAECs) with HU-308 and JWH-133 inhibited adhesion molecule expression, transendothelial migration and expression of chemoattractants [56]. High glucose treatment usually leads to upregulation of adhesion molecules, superoxide generation, increased transendothelial migration, as well as increased vascular permeability. CBD treatment attenuated all such

harmful effects including those on endothelial cells [57]. Hepatic ischemia/reperfusion (I/R) injury is a fatal complication that may occur after transplantation, and several studies showed the beneficial effects of cannabinoids in I/R injury, especially on endothelial cells. Targeting CB2 receptors with JWH-133 and HU-308 showed decreased adhesion of neutrophils to human liver sinusoidal endothelial cells *in vitro*, and the adhesion molecule expression such as ICAM-1 and VCAM-1 was reduced after cannabinoid receptor activation[58].

A graft needs to establish new blood vessels to get accepted and function normally [59]. Currently, there are no studies examining the effects of cannabinoids on angiogenesis during organ transplantation; however in tumor models cannabinoids acted as anti-angiogenic drugs suppressing tumor growth [60]. Such properties may pose a problem in the use of cannabinoids in transplantation. Clearly, additional studies are necessary to determine the role of cannabinoids on angiogenesis in organ transplantation. Also, this drawback can be overcome by the fact that vessel development is critical at early stages of transplantation and therefore cannabinoids can potentially be used after the graft establishment.

Emerging Concepts

Here, we have highlighted the anti-inflammatory properties of purified cannabinoids based on their selective modulation of cannabinoid receptor signaling and their potential use in preventing allograft rejection. This assertion can be interpreted to indicate the potential benefits of marijuana in transplantation. However, this is not our intention. Marijuana has over 480 compounds [61] and the effect of this complex mixture on inflammation and allograft rejection remains unclear. In a recent study, it was noted that patients that were positive for marijuana had similar survival rates when compared to patients that did not test positive [62], thereby suggesting that marijuana was not beneficial for liver transplant acceptance. It should be noted that some liver transplant centers have maintained a policy of marijuana abstinence for any patient to be considered for liver transplantation [63], which has created significant controversy following the death of a patient who was declined a liver transplant for using medical marijuana [62,64]. In contrast to the use of marijuana or cannabinoids that activate CB1 receptors in the brain and thereby exert psychotropic effects, we suggest the use of CB2 select agonists. The selective high density expression of CB2 receptors on immune cells and the lack of psychotropic properties exerted by CB2 select agonists, make CB2 receptor targeting, during transplantation, an exciting new avenue of treatment modality against transplant rejection. The psychotropic side effects of cannabinoids can also be overcome by developing new synthetic CB1/CB2 mixed agonists that do not cross the blood-brain barrier. The non-psychoactive cannabinoid, CBD, which has exhibited significant immunosuppressive potential by acting via vanilloid receptors, is another attractive candidate.

A strong immune reaction, specifically T cell-mediated, against the engrafted organ, is the culprit for rejection, and even though current therapies in transplantation aim at suppressing the immune response, they also exhibit significant toxicity upon chronic use. In rodents, long term exposure to THC did not affect survival, and in some cases, incidence of several types of cancers was reduced upon THC treatment [65]. This property of cannabinoids may be useful in the treatment of transplant recipients who often develop malignancies due to chronic immunosuppressive regimen [66]. However, our studies have also shown that THC treatment may increase the risk of cancers that do not express cannabinoid receptors, which could be a concern [67].

In addition, manipulation of endogenous cannabinoids to maintain chronic low grade immunosuppression may also benefit transplant recipients. For example, inhibitors of fatty

acid amide hydrolase (FAAH), an enzyme that breaks down endogenous cannabinoids such as AEA, have been shown to significantly elevate the levels of endogenous cannabinoids *in vivo* [68]. Thus, regulating the levels of endocannabinoids with the use of FAAH inhibitors, to control the immune response, offers another exciting avenue to prevent graft rejection. To this end, we and others have recently shown that FAAH inhibitor can suppress ConA-induced hepatitis and liver inflammation [15] and FAAH^{-/-} mice that exhibit high levels of endocannabinoids were protected from inflammatory damage during experimental colitis [69] and hepatitis [15].

The role of endocannabinoids in transplantation has not been investigated thus far. Thus, a number of experimental studies could be performed to address their role including whether: 1) inhibitors of endocannabinoid catabolism and transport would suppress immune response against the allograft, 2) FAAH^{-/-} mice exhibit increased tolerance to allografts, 3) levels of endocannabinoids are modulated during allograft rejection and 4) endocannabinoids play a role in endothelial cell function at sites of allograft. Such studies will form the basis of understanding the involvement of the endocannabinoid system in allograft rejection. Inasmuch as, immune cells constitute an important resource of endocannabinoids, it may be easier to manipulate their levels during an immune response, which could have a direct and immediate impact on such cells that determine the fate of the allograft.

In summary, targeting cannabinoid receptors and understanding the role and use of exo- and endocannabinoids in experimental allograft rejection models may provide an exciting new beginning with significant translational impact.

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Glossary

Cannabinoids	are the tetraphenolic compounds found in the plant, <i>Cannabis sativa</i> . Natural cannabinoids discussed in this paper are delta9tetrahydrocannabinol (THC), cannabidiol (CBD), and ajulemic acid (Aja). There are also synthetic compounds, which are JWH-015, JWH-133, HU-308, CP-55,940 and WIN-55,212-2. Finally the two endocannabinoids that are mentioned in this article are anandamide (AEA) and 2-arachidonylglycerol (2-AG)
Cannabidiol (CBD)	is a major non-psychoactive cannabinoid component from <i>Cannabis</i> constituting up to 40% in the extracts. It is found to have potent anti-inflammatory and immunosuppressive effects in addition to its ability to alleviate convulsion, anxiety, nausea, and inhibit tumor growth
Endocannabinoid system	is comprised of the endocannabinoids, the cannabinoid receptors (CB1 and CB2), and the enzymes responsible for synthesis and degradation of endocannabinoids
Allorecognition	When the transplanted organ or tissue is genetically incompatible with the recipient, the donor's immune cells recognize the transplant as "foreign" and mount a reaction leading to transplant rejection

Allograft	is a tissue or organ transplant between members of the same species. In case of an allograft, the donor and the host are not genetically identical. As a result, the recipient rejects the donor transplant, because the recipient's immune system recognizes the transplant as foreign. All human transplants from one person to another, except in the case of identical twins, are allografts
Major Histocompatibility Complex (MHC)	is a large gene family involved in the regulation of the vertebrate immune system. The antigens encoded by the MHC, present on the allograft, play a crucial role in transplant rejection. The human MHC molecules are referred to as Human Leukocyte Antigens (HLA)
Graft versus Host disease	When grafted tissue has mature T cells, they can recognize the recipient's alloantigens as foreign and mount a reaction to destroy the host tissue, a phenomenon called graft-versus-host disease

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Figure 1. Steps of allograft rejection and possible points of interference by cannabinoids

Allograft rejection occurs via three main mechanisms. (a) During direct recognition, donor APC presents donor antigens to recipient's CD4+ or CD8+ T cells. (b) During indirect recognition, recipient APC activates recipient T cells by presenting donor antigens. (c) Alternatively, recipient APCs can acquire intact donor MHC molecules and undertake direct presentation to recipient T cells. After antigen presentation, T cells are activated and they produce a plethora of cytokines and chemokines leading to the amplification of the immune response. Chemotaxis of different immune cells into the engrafted organ occurs via adhesion and transmigration through the endothelial cells that line the blood vessels. Once in the engrafted tissue, graft cells can be destroyed by direct killing (via the actions of NK and CD8+ cells), by Th1 cells that trigger type-IV hypersensitivity and activate macrophages or via complement activation due to the presence of allo-antibodies. Cannabinoids can interfere with the actions of APCs such as macrophages and dendritic cells, T cells, NK cells and B cells, inhibiting cytokine production and cell proliferation. In addition, cannabinoids can induce apoptosis in activated effector cells such as T cells as well as in antigen presenting cells such as macrophages and dendritic cells, eliminating the cells that are responsible for the amplification of the immune response. Cannabinoids can also interfere with the involvement of endothelial cells in the rejection process by downregulating adhesion molecules and inhibiting transmigration of immune cells into the engrafted tissue. Lastly, cannabinoids can induce Tregs that down-regulate the immune response.

✗ Block ⚡ Induce apoptosis