Current status and prospects for the application of cannabinoids in organ transplantation

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Abstract

Graft versus host disease and allograft rejection are frequent complications of allogeneic hematopoietic stem cell transplantation and solid organ transplantation, respectively. The probability of developing either condition is dependent upon the magnitude of genetic disparity between donor and recipient. In both contexts, alloimmune-mediated processes are responsible for disease pathogenesis and the subsequent complications that are associated with significant morbidity and mortality. Existing prophylactic regimens consisting of intensive immunosuppression are limited by high incidences of graft failure, infection, and toxicity. Cannabinoids are a diverse family of natural and synthetic molecules that, through interaction with the endocannabinoid system, have potent immunoregulatory properties. However, the applicability of cannabinoids to the prevention of graft-versus-host disease and allograft rejection has not been established. This article offers insight into our current understanding of the immunopathophysiology of graft-versus-host disease and allograft rejection, relevant cannabinoid-mediated immune modulation, and emerging evidence on the role of cannabinoids in transplant immunology. With the need for more effective prophylactic strategies and the concordant interest in cannabinoid-based therapeutics, it is pertinent to determine whether the endocannabinoid system can be therapeutically targeted in the post-transplant setting.

Introduction

There is a palpable high and growing interest for the potential medicinal applications of cannabinoids amongst the general population, exacerbated by the recent Canadian legalization of recreational cannabis. This fervor is fuelled by media outlets that promote cannabinoids as future "wonder drugs" and endorse them for the treatment of an array of illnesses and ailments, with an emphasis on chronic pain management.

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Accordingly, medical marijuana use continues to increase, as indicated by the 342,103 Canadians registered as medical marijuana users as of September 2018.[1] The possible applicability of cannabinoids for managing inflammation and associated chronic inflammatory diseases has received less attention. In this light, the field of transplant, which heavily relies upon managing pathologic immune reactions, may represent a future direction for cannabinoid-derived therapeutics. This paper reviews the current state of evidence on the role of cannabinoids in transplant-relevant immune processes as well as studies that investigated their direct application in transplantation.

Cannabinoids and the Endocannabinoid System

Interest in the utility of cannabis is by no means a modern phenomenon. The plant *Cannabis Sativa*, from which marijuana and other preparations are derived, has been used for millennia to treat myriad illnesses as well as for recreation, the latter due to its potent psychotropic effects.[2] These effects are mediated by a large number of biologically active constituents, with greater than 100 isolated structurally related cannabinoids, referred to as phytocannabinoids, in recognition of their exogenous and natural origin.[3] The two most well studied cannabinoid molecules are non-psychotropic (−)-cannabidiol (CBD) and psychotropic (−)-trans-Δ9-tetrahydrocannabinol (THC), which orchestrate a complex array of physiologic responses by acting on the endocannabinoid system (ECS), which is present in various cellular targets. The ECS comprises non-cannabinoid and cannabinoid receptors, predominately type-1 (CB1) and type-2 (CB2) cannabinoid receptors, their endogenous ligands known as endocannabinoids, most classically 2-arachidonyglycerol (2-AG) and anandamide (AEA), as well as associated regulatory and signaling complexes.[4] In accordance with this diversity, the ECS is perceived to be present in and contribute to the regulation of physiologic processes in virtually all body systems. Current knowledge is that cannabinoid receptors are broadly distributed, with CB1 receptors ubiquitously expressed throughout the central nervous system and to a lesser extent in the periphery, and CB2 receptors being largely expressed in immune and hematologic cells.[5] With the presence of the ECS within essentially all immune cells, the system is appropriately positioned to function in immune system homeostasis.

The first evidence supporting an immunomodulatory role for cannabinoids in humans was noted over 40 years ago when two separate groups identified that immune cells isolated from chronic marijuana smokers had suppressed responsiveness and functionality in various in vitro immune assays.[6,7] These seminal observations were substantiated by evidence that the

extent of cannabis consumption in recreational cannabis users positively correlated with several anti-inflammatory measures after controlling for potential covariates.[8] These observational studies acted as a framework for the flurry of discovery that has proceeded to uncover the mechanisms through which these agents participate in immune processes. It is now appreciated that the principle phytocannabinoids CBD and THC have a propensity for suppressing quintessential pro-inflammatory mediators and thereby modulate disease outcomes in pre-clinical models of autoimmune and inflammatory conditions.[9–11] A principal impediment for the clinical translation of cannabinoids such as THC is their therapeutically undesirable psychoactive effects. However, these unwanted effects are uniquely mediated through CB1 receptor activation. Studies employing CB2 genetic knockout models and selective agonists support the CB2 receptor for predominately mediating the immunoregulatory effects, developing interest for CB2-specific targeting of the ECS.[12] In support of this, a conserved CB2 genetic polymorphism, known to decrease the receptor's activity, is implicated in several chronic inflammatory conditions including immune thrombocytopenic purpura, rheumatoid arthritis, and celiac disease.[13–15] Growing evidence supports the utility of cannabinoid-based therapeutics directed at the CB2 pathway as a novel approach for immunosuppression.

Pathophysiology of GVHD and Allograft Rejection

The immunoregulatory properties of cannabinoids could prove particularly beneficial in solid-organ transplantation (SOT) and allogeneic hematopoietic stem cell transplantation (aHSCT), where control of alloreactive immune responses is important. SOT involves the replacement of the diseased organ or tissue with a healthy equivalent, frequently from a genetically non-identical donor, known as an allograft. Alternatively, aHSCT is a potentially life-saving intervention for benign and malignant hematologic conditions that involves eradication of the diseased hematopoietic system with a cytotoxic conditioning regimen and subsequent replacement with donor-derived hematopoietic stem and progenitor cells.[16] The principal limiting factors for achieving favorable outcomes in SOT and aHSCT are allograft rejection and graft-versus-host disease (GvHD), respectively. These two processes largely manifest due to incompatibility between the donor and recipient major and minor human leukocyte antigens (HLA), known as allorecognition, which incites tissue destruction. In allograft rejection, recipient T-cells recognize foreign HLAs expressed by the transplanted organ through either direct interaction with donor antigen presenting cells (APCs) or indirectly through recipient APCs that have engulfed these antigens. This process is potentiated by a concordant onslaught of pro-inflammatory cytokines produced in response to ischemia-reperfusion injury of the allograft,[17] the consequence of which is targeted destruction of the transplanted allograft. In aHSCT, substantial tissue damage caused by the conditioning regimen results in the release of danger signals, which activate APCs that then prime donor alloreactive cytotoxic T (Tc)-cells with the potential to result in widespread tissue damage.[18] Considering the mutual importance of T-cells to the pathogenesis of GvHD and allograft rejection, existing prophylaxis strategies have primarily targeted these effectors using methotrexate, cyclophosphamide, prednisone, cell-cycle inhibitors (e.g., mycophenolic acid and azathioprine), or calcineurin inhibitors (e.g., tacrolimus), as well as alemtuzumab and anti-thymocyte globulin for T-cell depletion, in GvHD prevention. These strategies are only partial effective with graft failure, infection and toxic side effects such as nephrotoxicity and cardiotoxicity remaining long-term sequelae of therapy.[19,20] Accordingly, refined immunomodulatory strategies are needed to achieve sustained tolerance in either transplant context.

Brief Primer on Transplant Immunology

The specific immune mechanisms that mediate GvHD and allograft rejection are similar. CD4+ or T-helper (Th) cells are integral regulators of inflammatory responses that have the capacity to differentiate into diverse lineages, denoted by their unique transcription factor and cytokine profiles, which can potentiate or attenuate inflammation. Experimental models and patients that develop acute GvHD and allograft rejection both present with a skewing towards pro-inflammatory Th1 and classically autoimmune-implicated Th17 cell lineages that are associated with disease initiation.[21–23] This is complemented by observations that regulatory T-cells (Tregs), which are the cellular antagonists to these responses, are found in higher proportions in donor grafts associated with a reduced risk of GvHD.[24] Furthermore, the more robust reconstitution of these cells and their adoptive transfer to patients undergoing aHSCT are associated with a reduced incidence of GvHD.[24] Accordingly, lower frequencies of Tregs have also been identified in the circulation of patients suffering from acute liver or heart transplant rejection.[25] Moreover, myeloid-derived suppressor cells (MDSCs) are a heterogeneous population of innate immune cells with the shared ability to actively oppose alloreactive T-cells. Consistent with the role of Tregs, MDSCs are postulated to play a supportive role in limiting the evolution of GvHD and rejection.[26,27] These observations support the notion that strategies that can promote the expansion of Tregs and MDSCs while suppressing differentiation towards pro-inflammatory T-cell lineages may be effective in inhibiting the onset of GvHD and rejection post-transplant. These represent only a handful of the diverse immune mechanisms postulated to be associated with the pathogenesis of GvHD and allograft rejection, and highlight the challenge in studying cannabinoid effect on the allogeneic response.

Cannabinoid-mediated Immune Modulation

Consistent with the function of alloreactive T-cells as the common effector pathway for eliciting tissue damage in both GvHD and allograft rejection, consideration of the effects of cannabinoids on T-cell function is imperative to their potential application in transplantation. Indeed, in an in vitro system of cultured, antibody-stimulated human T-cells, supplementation with THC, the selective CB2-agonist JWH015 and the selective CB1 agonist MAEA all significantly inhibited the production of interleukin (IL)-2, considered the canonical mediator of non-directional T-cell expansion.[28] This subsequently translated into a pronounced reduction in T-cell proliferation. These effects occurred through the CB2 receptor during both basal and stimulated states. However, the CB1 receptor was only capable of eliciting these effects following cell stimulation.[28] Yuan et al. also identified the CB2-mediated anti-proliferative effects of THC on naïve human CD3+ T-cells.[29] In the same experiments, pro-inflammatory tumor-necrosis factor α (TNF-α) and interferon-gamma (IFN-γ) secretion was decreased in

favour of increased IL-4, representative of a relative expansion of colloquially anti-inflammatory Th2 rather than Th1 cells.[29] In an in vivo setting, treatment of a Legionella pneumophila infection mouse model with THC robustly attenuated expression of the canonical transcription factor for Th1 differentiation, T-bet, while also diminishing dendritic cells with surface ligands specific for Th1 differentiation.[30] Moreover, using mouse models of multiple sclerosis and delayed-type hypersensitivity, administration of the selective CB2 agonist Gp1a and THC, respectively, dramatically reduced the quantity of Th1 and Th17 cells.[31,32] Furthermore, both CBD and THC are known to alter the gene expression signatures in naïve Th cells to promote differentiation of functional Tregs capable of both suppressing the proliferation of naïve T-cells in culture and mitigating enterotoxin B-induced inflammatory lung injury in mice.[33,34] Collectively, these observations highlight the capacity for members of the cannabinoid family to act as homeostatic regulators of T-cell activation relevant to reducing transplant-associated alloimmunity.

Cannabinoids, in general, also suppress the actions of myeloid cells, particularly APCs such as dendritic cells (DCs), which are integral for initiating an inflammatory cascade and priming immunocompetent donor T-cells in GvHD and graft rejection. This is demonstrated by limiting human monocyte differentiation in vitro into phenotypic DCs following THC exposure.[35] Further, human DCs cultured with THC had poor antigen uptake and a reduced capacity for antigen presentation and transformation of naïve T-cells into active effector cells.[35] This was influenced by a reduction of the DC cytokines IL-7, 12, and 15 that are important for the development of activated T-cells.[35] In mouse bone marrow-derived DCs (BMDCs) treated with THC, there was a similar inability to appropriately express the co-stimulatory molecules required for effective naïve T-cell priming.[36] A principle quality of DCs and other innate immune cells is their ability to migrate to sites of inflammation and to secondary lymphoid organs to initiate adaptive immune responses. Adhikary et al. (2012), demonstrated that CB2 receptor activation reduced the migration of BMDCs to popliteal lymph nodes in mice, consistent with a similar observation of impaired murine macrophage migration to potent chemokines.[37,38] Considering the integral role of both DCs and other myeloid cells in the activation of alloreactive T-cells in both GvHD and graft rejection, the capacity for cannabinoids to interrupt antigen presentation and T-cell transformation is highly topical. Moreover, cannabinoids not only restrict many pro-inflammatory processes of myeloid cells but also promote anti-inflammatory functions. A dose-dependent induction of increased MDSC frequency has been observed following THC injection into healthy mice, with these MDSCs being capable of suppressing inflammation in a model of T-cell-mediated liver disease following their adoptive transfer.[39] Therefore, the regulation of innate immunity represents another mechanism of action for cannabinoids.

Cannabinoids and Allograft Rejection

The demonstrated role for positive modulation of cannabinoid signaling in regulating various immune mechanisms relevant to transplantation has incited their direct study in this context. The mixed lymphocyte reaction is often employed as an in vitro surrogate of allograft rejection. Using this model, treatment of murine spleen T-cells with THC or selective agonists of the CB2 receptor induced a dose-dependent inhibition of their capability to proliferate in response to co-culture with genetically distinct splenocytes, which was entirely mediated by CB2-dependent signaling. This observation was corroborated by the simultaneous suppression of IL-2 secretion.[40] Under similar experimental conditions the selective CB2 agonist, O-1966, not only suppressed the proliferation of naïve Th and Tc cells but also further induced the differentiation of Tregs and secretion of their predominant anti-inflammatory cytokine IL-10, which is responsible for suppressing antigen presentation and Th1 cytokine production.[41] This demonstrates that cannabinoids are effective at inhibiting processes quintessential to allograft rejection and promoting those implicated in the establishment of immune tolerance. Functionally, in vivo level evidence demonstrated that CB2-/- mice developed more rapid allograft rejection in a model of fully mismatched cardiac transplantation in comparison to wildtype recipients.[42] A mechanistic explanation for this result is that BMDCs isolated from CB2-/- mice following cardiac allograft rejection were found to secrete substantial quantities of the pro-inflammatory cytokines IL-12 and 23, which is concordant with observed increases in the prevalence of pro-inflammatory Th1 and Th17 cells.[42] Although only pre-clinical, these studies affirm the ability for cannabinoids, primarily acting through CB2, to regulate transplant-relevant immune mechanisms that are capable of suppressing rejection.

Moreover, THC treatment has been shown to promote graft survival in a model of skin allograft rejection through CB1 receptor activation, with contribution, in part, by the induction of MDSCs.[32] Despite positioning CB1 as the principal receptor for coordinating the tolerizing effects of cannabinoids in SOT, identifying which cannabinoid receptor is the most influential may be context or ligand-dependent. Regardless, the expanding body of literature supporting a role for cannabinoids in managing allograft rejection is encouraging for their therapeutic consideration. It should also be noted that with the added immunosuppression imparted by cannabinoids, there is a corresponding increased risk for infection that must be accounted for should these agents be incorporated in allograft rejection prophylactic regimens.

Cannabinoids and GvHD

Cannabinoids may also have activity in the setting of GvHD. Pandey et al. (2011), were the first to identify a GvHD-suppressing capacity for cannabinoids in vivo that was mediated through CB2 receptor activation. They observed reduced severity of GvHD sequelae including weight loss, splenomegaly, and pathologic inflammation of the colon and liver, as well as prolonged survival following THC treatment in a murine model of GvHD.[43] These effects were mediated through the CB2 receptor. Consistent with observations made in models of SOT, this treatment resulted in the suppression of donor Tc cells and their production of pro-inflammatory cytokines such as IFN-γ, yet induced proliferation of donor Tregs. In conjunction with data from SOT experiments, a paradigm is developing whereby cannabinoids are capable of suppressing post-transplant alloimmune reactions by dampening pro-inflammatory cytokine release, skewing T-cell differentiation towards a regulatory phenotype and, in some circumstances, elevating levels of MDSCs.

Notably, a recently conducted phase II clinical trial investigated

the safety and efficacy of CBD addition to a GvHD prophylaxis regimen consisting of cyclosporine and a short course of methotrexate for adult patients undergoing aHSCT for malignant indications. Patients receiving CBD, compared to historical controls, had a significantly prolonged median time to onset of GvHD with a significantly lower risk of developing grades II to IV GvHD by day 100 post-transplant. A lower risk of developing grades III to IV GvHD by day 100 was also noted, but this did not achieve statistical significance. CBD administration was well tolerated with no adverse consequences. Despite these positive outcomes, paradoxically, there was no difference in non-relapse mortality.[44] This study presents promising findings but is limited by its single-arm design that compared outcomes to historical control patients. Ideally, a randomized double-blind placebo-controlled trial would more conclusively support the role of CBD in GvHD prevention. Rapid immune recovery post-aHSCT is important for limiting adverse events. Indeed, a study of a murine model of aHSCT observed that both THC and CBD slowed lymphocyte recovery, but not myeloid recovery, 3 weeks following transplant.[45] This is a clinically relevant observation considering that delayed lymphocyte recovery is associated with increased susceptibility to opportunistic infections. Since cannabinoids are known to heighten the risk of infection, both incidence of infection and lymphocyte recovery post-aHSCT should therefore be closely monitored in future human studies.

Cannabinoid-Mediated Drug Interactions

The potential inclusion of cannabinoids into therapeutic regimens for preventing GvHD or allograft rejection is complicated by potential drug-drug interactions with existing immunosuppressants that are the standard of care. Exogenous cannabinoids are substantially metabolized by, and capable of inhibiting, certain cytochrome P-450 (CYP) enzymes in the 3A, 1A and 2C families as well as UDP-glucuronyltransferase (UGT) enzymes.[46,47] Furthermore, cannabinoids from marijuana also demonstrate inhibition of the P-glycoprotein (P-gp) drug transporter, which influences the tissue distribution of various drugs.[48] Many of these metabolic enzymes and transporters are critical mediators of cannabinoid function and also regulate the pharmacokinetics of various immunosuppressants, such as calcineurin inhibitors.

Accordingly, a male patient receiving the calcineurin inhibitor tacrolimus for GvHD prophylaxis, and concurrently using recreational edible marijuana, was identified with symptoms of tacrolimus toxicity and a 4–5-fold elevated blood tacrolimus concentration, in accordance with a urine toxicology screen positive for THC.[49] A subsequent case was reported of a female patient enrolled in a CBD clinical trial for refractory epilepsy simultaneously receiving a stable dose of tacrolimus, who experienced a 3-fold rise in the dose-normalized blood concentration of tacrolimus and corresponding toxicity.[50] This adverse effect was temporally associated with increasing doses of CBD. Tacrolimus metabolism and distribution is regulated by CYP3A members and P-gp, thus offering the possibility of a cannabinoid-mediated drug-drug interaction. However, causation cannot yet be established. Notably, other post-transplant immunosuppressants such as prednisone, mycophenolic acid, cyclophosphamide, and other calcineurin inhibitors have

contributory metabolisms by CYP and UGT enzymes that have shared affinity for exogenous cannabinoids.[51–54] This posits a theoretical risk for these agents to manifest clinically significant drug-drug interactions, mediating toxicity or graft rejection when co-administered with exogenous cannabinoids. These observations necessitate the design of large, controlled studies to ascertain the incidence and magnitude of the adverse effects associated with the addition of exogenous cannabinoids to existing immunosuppressant regimens.

Understandably, cannabinoids should only be incorporated into existing protocols with recognition for potential drug interactions, under close clinical supervision, and with consideration for the route of administration. In particular, inhaled marijuana is associated with macrovascular disease, obstructive lung disease, membranous glomerulonephritis, and other renal disease in the post-transplant period, as well as potential ramifications for other organ systems.[55–57] These consequences underscore the importance of utilizing less harmful modes of administration and careful monitoring of predicted drug interactions when considering cannabinoids as a therapeutic option in transplantation.

Concluding Perspectives

Effective prevention and management of GvHD and allograft rejection after aHSCT and SOT, respectively, have been perpetually challenging and may be regarded as the "Holy Grail" in transplant medicine. Appropriate prophylaxis must incorporate a fine balance of immunosuppression with proper engraftment of the transplanted organ, sufficient immunity from infection, and mitigation of toxicity. In recent years, the cellular and molecular mediators underlying the alloreactive immune response that orchestrate these conditions have become better understood. As research evolves, it is possible that more selective therapies satisfying this fine balance will emerge. Currently, newer targeted therapies are demonstrating promise and several multi-centre, randomized trials are being undertaken.[58] Moreover, plasma biomarkers are being investigated to identify patients at high risk for GvHD and allograft rejection to enable more appropriate and timely interventions.[59] Targeted approaches have been proven as efficacious, low toxicity approaches in other disease states. However, it is unclear whether targeting single entities is optimal for GvHD or allograft rejection prevention.

For this reason, cannabinoids and therapeutics targeting the ECS may offer innovative immunoregulatory strategies with relatively limited side effect profiles. Numerous studies demonstrate the powerful immunosuppressive effects of the cannabinoids and their derivatives in various model systems, including those that recapitulate transplant. Despite expanding evidence that supports a role for these agents in mitigating GvHD and allograft rejection, available data is almost entirely restricted to preclinical studies that may not recapitulate their activity in humans. Robust studies will also need to be conducted that compare the efficacy, magnitude, and adverse effects of the immunosuppression elicited by cannabinoids to that accomplished by existing immunosuppressants such as calcineurin inhibitors. Importantly, monitoring the frequency of opportunistic infections following the incorporation of cannabinoids into existing immunosuppressant regimens should be made a focus. Furthermore, opposition to the theory that the CB2 receptor is principally responsible for cannabinoid-mediated

immunomodulation should be further investigated to determine whether CB2 selective agents may represent viable therapeutics.

The first-in-man study on the addition of CBD to standard GvHD prophylaxis produced promising results but also raised important considerations. The potential for other cannabinoids to offer similar benefits in humans is unknown, and the long-term effects of CBD and other cannabinoids on immune responses, protection from infection, and appropriate hematopoietic recovery after aHSCT have yet to be determined. A subsequent open-label, phase 2a multi-centre study (clinicaltrials.gov: NCT03840512) is currently under way to investigate the efficacy of longer-term CBD administration for GvHD prevention, which may provide additional data to begin addressing some of these considerations. Similar trials in the context of allograft rejection prevention have not been carried out, but perhaps these studies in GvHD will provide a suitable foundation for future investigation. Ultimately, the recent legalization of cannabis may represent the necessary impetus to improve funding allocation and resources to propel further human studies and randomized clinical trials that can ascertain the role of cannabinoids in transplantation.

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