Do endogenous cannabinoids contribute to HIV-mediated immune failure?

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The failure of the immune system to mount a successful attack on the human immunodeficiency virus (HIV) is an old enigma for AIDS research. The high mutational capacity of HIV, which unremittingly confuses the immune system, is a major factor in immune failure. But this alone cannot fully explain the certain and inescapable failure of the immune system, leading to full-blown AIDS. Here, we propose the hypothesis that endogenous cannabinoids, derived mostly from macrophages, might participate in the general failure of the immune system in HIV-infected individuals.

Cannabinoids, the active ingredients of cannabis (marijuana, hashish), have been known for a long time to act as immunosuppressors[1–10]. This activity is mediated via specific cannabinoid receptors expressed by both B and T cells. Two genes coding for cannabinoid receptors, *CB1* and *CB2*, have been cloned. They are ubiquitously expressed in central and peripheral neurons, astrocytes, smooth muscle, endothelium and lymphocytes, and are involved in numerous physiological functions (Box 1). Cells of the immune system express mostly the CB2 subtype receptor[8–10], and the presence of cannabinoid receptors in invertebrate immunocytes[8] attests to their primordial role in immune regulation. CB1 receptors are also expressed at low levels in human T cells[10]. In addition to the cloned CB1 and CB2 receptors, other cannabinoid receptor subtypes might exist.

Cannabinoid receptor signalling and the immune system

Activation of B- and T-cell CB2 receptors by Δ-9-tetrahydrocannabinol (THC, the major active ingredient of cannabis), by synthetic cannabinoids, or by the endogenous cannabinoids anandamide (arachidonylethanolamide), palmitoylethanolamide and 2-arachidonylglycerol (2-AG) leads to inhibition of adenylate cyclase in these cells and a subsequent reduced response to immune challenge (Fig. 1)[7,9,11]. This signal is transduced by a G_i protein, coupled to inhibition of adenylate cyclase. It is not clear how inhibition of adenylate cyclase leads to immune suppression; one possible mechanism is downregulation of expression of cytokine genes, such as the gene for interleukin 2 (IL-2), by T cells[9]. The role of the cyclic AMP (cAMP) cascade in the regulation of immune responses remains controversial, with some opposing reports on relationships between cAMP levels, regulation of lymphocyte mitogenesis and expression of genes involved in immune function. In numerous cell types, elevations of cAMP levels are linked to cell-cycle arrest, so it is unclear how reduced cAMP levels lead to inhibition of mitogenic events in immune cells. However, brief (30–60 min) elevations of cAMP levels are observed in T cells following antigen stimulation, and are probably important for subsequent activation of cytokine genes that activate the immune system[7]. Apparently, activation of lymphocyte CB2 receptors interferes with the correct timing and exact magnitude of cAMP signalling during the cell cycle, thereby baffling the intricate interplay of the cytokines regulating immune function (see Ref. 12 for a comprehensive review on cannabinoids, cAMP and immune regulation).

Box 1. The physiological effects of cannabinoids

Central effects

Working-memory impairment; hallucinations; analgesia; hypothermia; reduced motor activity; catalepsy; inhibition of pituitary prolactin and luteinizing hormone secretion.

Peripheral effects

Vasorelaxation and hypotension; lowered intraocular pressure; bradycardia; anti-emesis; inhibition of lymphocyte mitogenesis; inhibition of phagocytosis.

Other putative effects have been reported for: fertilization (preventing polyspermy?); development of the preimplantation embryo; uterine receptivity for embryo implantation; delay in the onset of labor.

Known receptors

CB1 (mostly in brain, heart, endothelia); CB2 (mostly in the periphery, including lymphocytes). Both subtypes are G-protein-coupled receptors; additional receptor subtypes might exist.

Known post-receptor events

Inhibition of adenylate cyclase; transient elevation of intracellular free Ca^{2+}; activation of phospholipase A_2 and arachidonic acid mobilization; activation of NO synthase.

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Highlighting the complexity of immune regulation by cannabinoids are recent intriguing observations on immune enhancement by extremely low THC concentrations[13]. In addition, at extremely low doses, anandamide stimulates open-field activity and aggressive behaviour in mice, as well as *in vitro* phagocytosis by leukocytes, effects that were quite the opposite of those observed in mice treated with higher doses[14]. This suggests that, at very low concentrations, anandamide might activate other biochemical pathways that are obscured by massive activation of G_i at higher cannabinoid receptor occupancy. A precedent has been set for such signalling duality by a single receptor subtype: the M_1 acetylcholine receptor can couple to both G_q and G_s proteins[15].

Despite the mechanistic enigma of cannabinoid-mediated immune suppression, it is clear that exogenous cannabinoids reduce immune protection against infective agents. For example, THC administration increases mortality in mice challenged with Friend leukaemia virus or *Legionella pneumophila*[3–5]. The soluble HIV envelope proteins gp120 and its precursor gp160, which are constantly being released into the blood of HIV-infected individuals, are also well-documented immunosuppressive agents. However, the exact mechanism for this effect is not completely understood, and might involve various mechanisms[16,17]. Induction of endogenous transforming growth factor β (TGF-β) might be involved[17] but cannot entirely explain the effects of soluble gp120 on immune cells. Here, we propose that HIV envelope protein-mediated immune suppression might occur relatively early in HIV pathogenesis. According to our model, HIV-mediated immune suppression involves increased production and release of endogenous cannabinoids by macrophages (and possibly other cell types) following activation of the specific macrophage receptors CCR5 and CXCR4 by soluble HIV envelope proteins (Fig. 2).

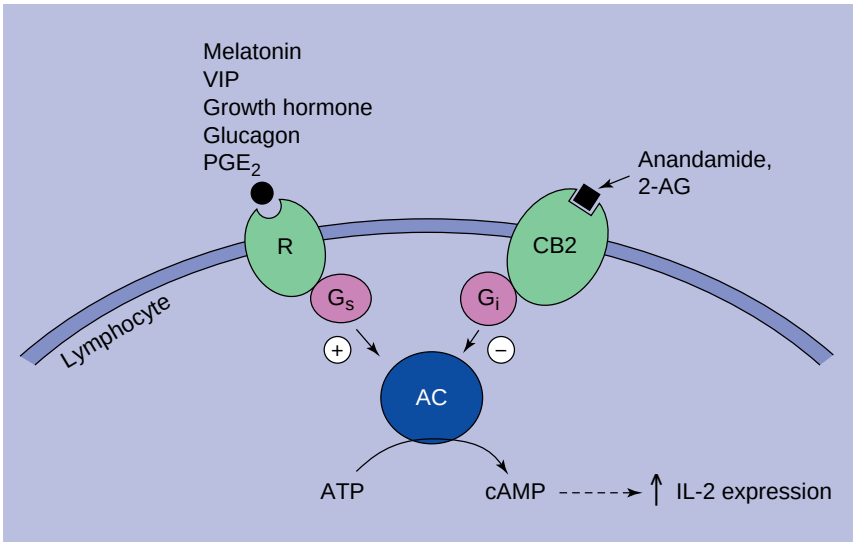

Figure 1. Cannabinoid receptor signalling in lymphocytes. Several agonists, including melatonin, vasointestinal peptide (VIP), growth hormone, glucagon and prostaglandin E_2 (PGE_2), bind to receptors that, via the stimulatory G protein G_s, activate adenylate cyclase. The resulting increase in cyclic AMP (cAMP) can increase the expression of certain cytokine genes, including that for interleukin 2 (IL-2). The endogenous cannabinoids anandamide and 2-arachidonylglycerol (2-AG) bind to CB2 cannabinoid receptors. These are coupled to the inhibitory G protein G_i, thereby opposing the effects of G_s-coupled receptor activation.

Binding of HIV envelope proteins might stimulate endogenous cannabinoid synthesis

Anadamide and 2-AG, the two most ubiquitous endogenous cannabinoids, are formed from membrane phospholipids following G-protein-coupled, receptor-mediated activation of phospholipase D and phospholipase C, respectively; both enzymes are Ca^{2+} dependent[18,19]. The recently identified second HIV receptor, the chemokine receptor CCR5, is a seven-transmembrane, G-protein-coupled receptor whose activation by its natural ligand, macrophage inflammatory protein 1β (MIP-1β), results in elevated cytosolic free Ca^{2+} levels[20]. HIV envelope proteins gp120 and gp160 might mimic this Ca^{2+} signalling upon binding to macrophage CCR5 (Ref. 21). Studies in other cell types, including intestinal epithelial cells and neurons, also demonstrated elevated intracellular Ca^{2+} following interaction with soluble HIV envelope proteins[22–24]. A third macrophage HIV receptor, CXCR4, is also expressed by other cell types, including endothelia[25]. Activation of CXCR4 following binding of its natural ligand, stromal cell-derived factor 1 (SDF-1), or soluble gp120, also leads to elevated cytosolic Ca^{2+} levels[26]. Most notably, immature, nonglycosylated gp120 or gp160 exhibit more potent binding to CXCR4 than their mature counterparts with intact carbohydrate residues[27]. Thus, immature HIV envelope proteins liberated into the bloodstream from infected macrophages or T cells, rather than mature HIV virion particles, seem to play a crucial role in immunosuppression.

Ca^{2+} signals are not observed in $CD4^+$ T cells following interaction with soluble gp120 (Ref. 28), so it is unlikely that a signal originating in this cell population directly mediates immune failure in HIV-positive individuals. In addition to elevated macrophage intracellular Ca^{2+}, soluble gp160 and gp120-activated CCR5 signalling also mediate chemotaxis of activated $CD4^+$ T cells towards the activated macrophages[21]. This might contribute to HIV pathogenesis by promoting migration of activated T cells to sites of viral replication (that is, sites where soluble envelope proteins are likely to be released from infected cells), thereby promoting viral spreading to T-cell populations[21]. Macrophages are the major blood-borne cells capable of producing anandamide. In addition, several other cannabinoid *N*-acyl-ethanolamines (such as palmitoylethanolamide) have been identified in resting mouse peritoneal macrophages[29]. The massive anandamide production capacity of macrophages was recently evidenced by their amazing capacity to transmit haemorrhagic shock-associated hypotension to naive animals[30,31]. Local production and release of endogenous cannabinoids by activated macrophages at the sites of viral replication might thus contribute to viral spread by inhibiting immune function in the T-cell populations recruited to these sites by soluble HIV envelope protein-mediated chemotaxis. Levels of soluble envelope proteins found in HIV-positive individuals[32] are in the same range as those required for CCR5 and CXCR4-mediated Ca^{2+} signalling and chemotaxis, so it is plausible that similar effects of such proteins on macrophage Ca^{2+} signalling occur *in vivo*. Notably, blocking Ca^{2+} signalling might attenuate gp120 toxicity and slow AIDS progression[33–35], in agreement with the hypothesis that gp120-mediated, Ca^{2+}-driven cannabinoid production might play a role in immune failure.

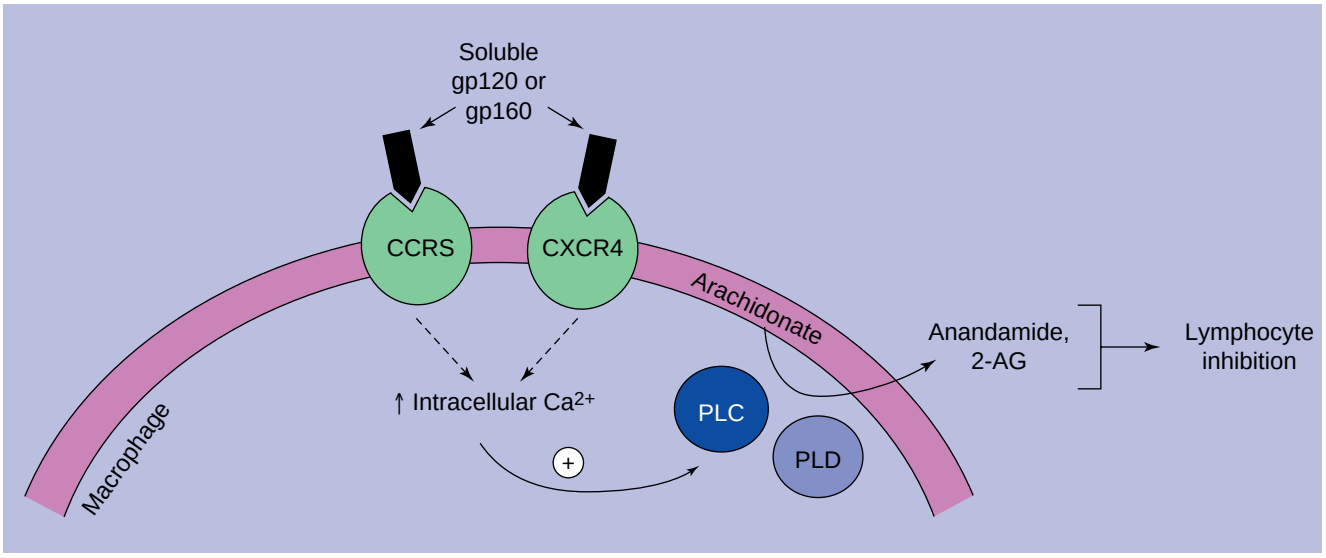

Figure 2. The hypothesis that the soluble HIV envelope proteins gp120 and gp160 might drive the synthesis of endogenous cannabinoids in macrophages. Soluble gp120 and its precursor, gp160, are released into the bloodstream of HIV-positive individuals and can bind to, and activate, the chemokine receptors CCR5 and CXCR4. This leads to an increase in intracellular Ca^{2+} that helps to activate Ca^{2+}-dependent phospholipases (PLC and PLD). These enzymes produce the precursors for the synthesis of anandamide and 2-arachidonylglycerol (2-AG), which might cause immunosuppression by the mechanism shown in Figure 1.

Endogenous cannabinoids in AIDS dementia

HIV-associated cognitive deficiency, designated AIDS dementia, appears in a large proportion of advanced AIDS patients. AIDS dementia is associated with several neuropathological abnormalities, including remarkable gliosis and neuronal apoptotic death[36]. An old puzzle for AIDS researchers is how HIV causes neuronal damage when neurons themselves are rarely infected by the virus. Rather, HIV infects brain microglia and penetrating macrophages[37]. Recent evidence suggests that elevated serum and cerebrospinal fluid (CSF) levels of the excitatory amino acids glutamate and aspartate might be involved in the progression of AIDS dementia[38–40], similarly to their well-documented role in stroke-associated neuronal loss and gliosis. Soluble gp120 can cause the release of glutamate from neurons and inhibit the astrocyte glutamate transporter – a major route for clearance of CSF glutamate[41]. Glutamate, via activation of NMDA and quisqualate-type receptors, elevates neuronal Ca^{2+} levels, thereby promoting production and release of endogenous cannabinoids by neuronal cells[42,43]. Soluble gp120 is detectable in CSF from AIDS dementia patients[44]. Recent data suggest that anandamide might directly reinforce the action of glutamate at neuronal NMDA receptors[45]. Thus, a 'vicious cycle' might be established in AIDS dementia, where soluble gp120 might both directly promote cannabinoid production in brain-penetrating macrophages via activation of their surface CCR5 and CXCR4, as well as indirectly increase cannabinoid production in neurons by elevating CSF glutamate. The locally produced cannabinoids might act as a double-edged sword: they could boost the capacity of glutamate to elevate neuronal Ca^{2+} (Ref. 45), thereby further refueling their own vicious cycle. At the same time, increased production of brain cannabinoids might directly affect cognition: anandamide and 2-AG were shown to attenuate long-term potentiation, a basic process in memory consolidation, similarly to the effect of THC in hippocampal neurons[46,47]. Clearly, our model is over-simplified, as numerous additional brain pathways are affected by HIV and its envelope proteins[44]. However, we propose that cannabinoid-mediated processes might contribute to AIDS dementia, and further complicate other neuron-damaging effects of gp120.

Implications for the medicinal use of marijuana?

Recently, there has been a lot of media hype around suggestions that marijuana should be legalized for certain medicinal uses, such as cancer, multiple sclerosis (MS) and AIDS[48]. This drive is fueled mostly by anecdotal reports on improved overall well-being by patients using 'recreational' marijuana. Some reported beneficial effects of marijuana in cancer and AIDS patients might reflect improved weight gain, owing to the well-documented anti-emetic and appetite-stimulating effects of cannabinoids. This might be a major advantage for cancer patients undergoing rigorous chemotherapy, or advanced AIDS patients. But in view of their potent immunosuppressive effects, and the possibility that endogenous cannabinoids might participate in inducing immunodeficiency in AIDS, great caution is required when considering the use of marijuana, or even synthetic cannabinoids, for treating AIDS, particularly during the early stages of disease. Similar caution seems reasonable for evaluating cannabinoid use in early-stage cancer patients: macrophages play an active role in the anti-tumour immune response; perhaps the failure of the immune system to fight malignancy is also related to overproduction of endogenous cannabinoids.

On the other hand, the same considerations would suggest that cannabinoid use might have certain benefits for MS patients. So far, there have been no large controlled clinical trials of THC or other cannabinoids, and the medical establishment is hesitant to promote such trials. Trials of marijuana or THC are likely to remain controversial, as they are associated with the on-going public debate on the legal status of marijuana and its recreational use. Maybe the solution lies in developing non-psychotropic, receptor-subtype-selective cannabinoids, possibly with reduced brain penetration and fewer behavioural effects. Alternatively, drugs could be

The outstanding questions

- Are endogenous cannabinoids generated and liberated by macrophages *in vivo* following binding of soluble gp120 to CCR5 and CXCR4 in HIV-positive individuals?
- Are such macrophage-derived endogenous cannabinoids capable of inhibiting immune function? To what extent is such inhibition significant for the general immune failure in HIV-infected individuals?
- Is the cyclic AMP signalling pathway directly responsible for inhibition of immune function by endogenous cannabinoids, and what is the physiological role of such inhibition? Is it related to protection from autoimmunity?
- Could removal or neutralization of soluble gp160 and gp120 proteins from blood of HIV-carrying individuals slow down AIDS progression?
- Does smoking marijuana contribute to progression of HIV-positive individuals to full-blown AIDS? Could synthetic cannabinoid antagonists, or inhibitors of cannabinoid synthesis, be employed to slow down AIDS progression?

developed to promote or block the degradation of endogenous cananbinoids. Indeed, several drug companies are now active in this direction. Remarkably, some of us might already, unknowingly, be using a cannabinoid-targeted drug: one of the major non-prescription drugs in the USA, ibuprofen, might relieve pain by inhibiting degradation of anandamide at pharmacologically relevant concentrations, thereby prolonging its analgesic effects[49].

Future directions

Our hypothesis implies that elevated intracellular macrophage Ca^{2+}, resulting from interaction of soluble gp120 and gp160 with macrophage CCR5 and CXCR4, and the resulting increased production of endogenous cannabinoids from membrane phospholipids, might contribute to the immune failure in HIV-positive individuals (Fig. 2). Such notions highlight the central role of macrophages in HIV transmission, and the caution needed when considering marijuana for treating AIDS patients. The availability of new selective inhibitors of anandamide degradation, as well as the introduction of novel, highly selective CB2 receptor agonists and antagonists[50], will help decipher the role of endogenous cannabinoids in immune function. Experiments in animal models of AIDS will be required to test these notions and to study possible beneficial effects of using selective CB2 cannabinoid antagonists or blockers of endogenous cannabinoid synthesis in the early stages of HIV infection.

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