The Role of Cannabinoid System on Immune Modulation: Therapeutic Implications on CNS Inflammation

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Abstract: There is a growing amount of evidence suggesting that cannabinoids may be neuroprotective in CNS inflammatory conditions. Advances in the understanding of the physiology and pharmacology of the cannabinoid system have increased the interest of cannabinoids as potential therapeutic targets. Cannabinoid receptors and their endogenous ligands, the endocannabinoids, have been detected in cells of the immune system, as well as in brain glial cells. In the present review it is summarized the effects of cannabinoids on immune reactivity and on the regulation of neuroinflammatory processes associated with brain disorders with special attention to chronic inflammatory demyelinating diseases such as multiple sclerosis.

Keywords: Cannabinoids, immune function, neuroinflammation, glial cells, multiple sclerosis.

INTRODUCTION

Cannabis has been widely used as a recreational drug and as a therapy for a variety of disorders. The discovery of the psychoactive principle of *Cannabis Sativa L*. plants, delta 9-tetrahydrocannabinol [1] initiated research into the physiological role of cannabinoids. In the last years our understanding has been modified significantly by new discoveries.

Cannabinoids are best known for their effects on CNS functions. They produce euphoria, alterations in cognition and analgesia, have anticonvulsant properties, and affect temperature regulation, sleep and appetite. However, cannabinoids also possess immunomodulatory activity and anti-inflammatory properties. Many diseases of the nervous system, including Alzheimer disease, Parkinson's disease AIDS dementia, and mainly multiple sclerosis (MS) involve inflammation, and cause an upregulation of cytokines and other inflammatory mediators in the CNS. Within the brain, glial cells, microglia and astroglia, participate in immunological responses and surround the brain microvasculature to constitute the blood-brain-barrier. Because of the lack of a lymphatic drainage system, the brain has been considered an immune privileged site. However, under certain inflammatory conditions, such as the case of MS, the blood brain barrier is less restrictive to the migration of activated monocytes, T and B lymphocytes, and other immune cells. Therefore, a bi-directional communication can be established between immune-derived cells and glial cells, through soluble factors (cytokines, chemokines, etc) and by direct cell-cell interactions. This review aims to improve understanding of immunomodulatory effects of cannabinoids, specifically in relation to CNS inflammation and their potential role as therapeutic agents in neurological diseases.

Two cannabinoid receptors have been identified: the cannabinoid CB1 receptor [2] mainly expresed in the CNS, and the CB2 receptor [3] mainly expressed in cells of the immune system. Both receptors are members of the large seven transmembrane G-protein-coupled receptors family. Besides, there is evidence indicating the possible presence of yet uncloned cannabinoid receptors on the basis of pharmacological studies and results obtained using CB1 and CB2 receptor-deficient mice [4-6]. Moreover, two endogenous ligands have been identified and characterized: anandamide and 2-arachydonoylglycerol (2-AG) [7, 8], whereas a number of cannabinoid-like compounds exhibit cannabimimetic activities without activating CB1 and CB2 receptors. Biosynthesis of endocannabinoids occurs via hydrolysis of membrane lipid precursors [9], and are degraded into arachidonic acid through the fatty acid amide hydrolase (FAAH) or the monoglyceride lipase [10]. There is also evidence for the existence of specific transporters for endocannabinoids, but they have not been cloned yet.

CANNABINOID SYSTEM AND IMMUNE FUNCTION

The cannabinoid CB2 receptor is expressed abundantly in various types of inflammatory cells and immune competent cells at levels 10-100 times higher than CB1 receptor mRNA [11, 12]. The rank order of cannabinoid CB2 receptor expression on human blood leukocytes is B cells >NK cells>monocytes>neutrophils> T8 cells >T4 cells. The CB2 receptor has been associated with most of the immunomodulatory activity of cannabinoids [13], but several reports indicate that the CB1 also may be linked to cannabinoid-mediated alterations of immune cell reactivity. Most of the actions of cannabinoids are related to the

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downregulation of immune system. [14, 15]. Cannabinoids exhibit immunosuppressive properties by interfering with humoral immunity, cell-mediated immunity and cellular defenses against infectious agents [16]. A number of *in vitro* studies reported that cannabinoids inhibit T cell mitogenesis and IL-2 production from lymphocyte cell lines [17]. In general, IL-2 regulates both antigen-specific and non-antigen-specific proliferation of T cells, natural killer cells and B cells. One of the most significant observations is that the expression level of CB2 receptors depends on the state of activation of the cells. Thus, differentiation of B cells is followed by decreased expression of CB2 receptor, and activation of B cells by anti-CD40 antibody increases its levels [18]. Peripheral and bone marrow-derived dendritic cells as well as macrophages, which play a key role in the initiation and development of the immune response express CB2 receptors which may be related to the reduction of antigen presentation by cannabinoids described *in vitro* [19]. The possibility that cannabinoids may modulate the TRC-class II MHC interactions which trigger multiple signaling pathways leading to expression of co-stimulatory molecules (CD40, CD40L, B7-1 and B-72) and various chemokines and cytokines, raised important implications on both, innate and acquired immunity (Fig. **1**). Levels of CB2 receptors in cells of macrophage lineage undergo changes depending on cell activation by increasing its expression under inflammatory conditions [20, 21]. Moreover, dendritic cells as well as macrophages generate anandamide and 2-AG in response to inflammatory conditions and express CB receptors and the enzyme responsible for endocannabinoid hydrolysis, the fatty acid amide hydrolase (FAHH), pointing out the existence of a complete endocannabinoid system [21, 22, 23]. The above data suggest a physiological role of the endocannabinoid system in the functions of immune system which may have important implications in pathological inflammatory conditions including brain-immune related disorders.

CANNABINOIDS AND CYTOKINE NETWORK IN GLIAL CELLS: ANTI-INFLAMMATORY PROPERTIES

There are two groups of glial cells in the CNS: the macroglia, including astrocytes, oligodendrocytes, and ependymal cells, and the microglia. Astrocytes are the major glial cells within the CNS and have a critical role in CNS homeostasis. Astrocytes and especially microglia are considered as immunocompetent cells within the brain, due

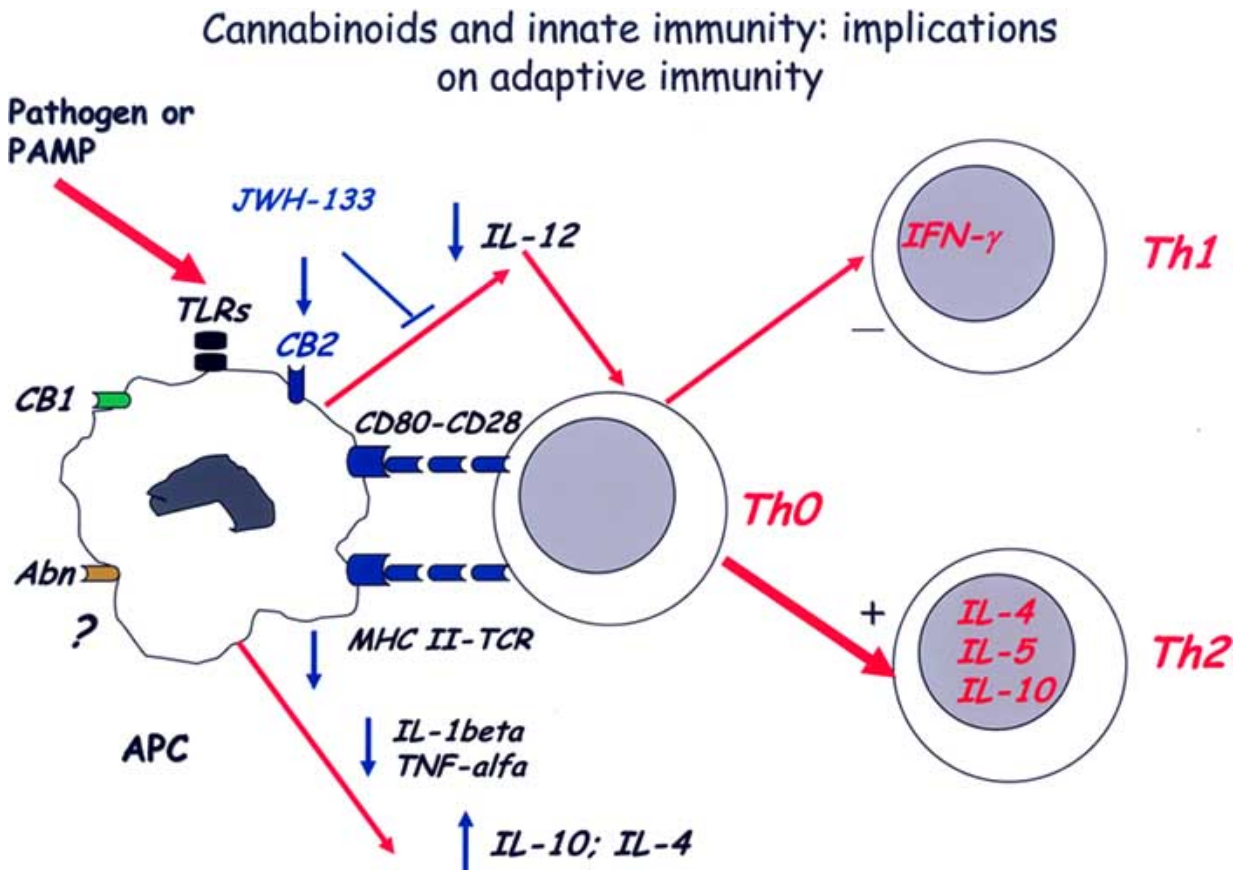

Fig. (1). Proposed actions of cannabinoids upon innate and adaptive immunity: Antigen presenting cells (APC) recognise pathogens or pathogen-associated molecular patterns (PAMP) by toll-like receptors (TLR), leading to the upregulation of cell surface expression of co-stimulatory molecules and major histocompatibility complex class II (MHC class II). APC express CB1, CB2 receptors and putative new receptors (Abn-Cbd) and their activation by selective cannabinoid agonists interfere with MHC class II antigen expression, inhibit the generation of proinflammatory cytokines (IL-1β; TNF-α, IL-12), and increase the production of antiinflamatory cytokines (IL-4, IL-10). Because IL-12 contributes to the differentiation of naïve or activated T-cells into T helper (Th1) cells, cannabinoids could diminish Th1 responses. Cannabinoids could also play a role in the induction of Th2 responses by activating IL-4 generation. Thus, establishment of adaptive immunity may be influenced by cannabinoid compounds.

to their capacity to express class II major histocompatibility complex (MHC) antigens and costimulatory molecules (B7 and CD40) that are critical for antigen presentation and T-cell activation. The ability of astrocytes and microglia to produce a wide array of chemokines and cytokines with the immunological properties of these mediators point to the importance of these cells in neurological diseases with an immunological component. Glial cell function can be modulated by cannabinoid compounds. CB1 as well as CB2 receptors have been described to be present in glial cells such as astrocytes, and astrocytome cells [24, 25], microglial cells [12, 26, 27, 28], and oligodendrocytes [29]. Moreover, astrocytes and microglia produce anandamide and 2-AG under several stimulatory conditions [28, 30, 31], and astrocytes have been shown to express FAAH, the enzyme which catabolizes endocannabinoids [32]. Antigen-presenting cells (APC), dendritic cells and macrophages express mRNA for FAAH [23, 33, 34], then, it would be possible that the main APC within the brain, the microglial cells, also have endocannabinoid mechanisms of inactivation. The role of glial endocannabinoid system in the modulation of neuroinflammation awaits a more complete analysis of glial cannabinoid molecules expression patterns *in vivo* and *in vitro*.

The role of Th1 and Th2 responses in the CNS is important in regulating immune responses, inflammation and ultimately repair during a variety of CNS diseases. The types of Th cells are defined by the profiles of cytokines that they produce. Th1 cells produce IL-2, IFN-γ and TNF-α, leading to macrophage activation, inflammation, and tissue damage. Th1 cells have been implicated in the pathogenesis of CNS autoimmune diseases, such as MS. In contrast Th2 cells produce IL-4, IL-5, IL-6, IL-10 and IL-13, cytokines that mediate humoral immune responses and inhibit numerous macrophage inflammatory functions. Within the CNS Th2 type cytokines play a role in down-regulating Th1 responses and macrophage/microglial activation. Cannabinoids not only modulate Th1 and Th2 cytokine responses [35], but also within the CNS are able to regulate cytokine production in glial cells. In a cell culture preparation of Theiler's virus-infected astrocytes anandamide potentiated the synthesis of IL-6, a potentially anti-inflammatory cytokine [36] and suppressed the production of the pro-inflammatory cytokine TNF-α [37]. Later studies also reported reductions of TNF-α as well as of IL-1β by LPS-stimulated microglia in response to cannabinoids [38, 39]. In mixed glial cultures non selective cannabinoid agonists increase LPS-induced synthesis of IL-1 receptor antagonist, an endogenous blocker of IL-1 actions [39]. Upregulation of inducible nitric oxide synthase (iNOS) in glial cells or invading macrophages or both, is regarded as a source of extensive oxygene radical production, with particular interest in MS, Alzheimer's disease, Parkinson's disease, stroke and inflammatory conditions. Several studies pointed to a suppressive effect of cannabinoids in the generation of nitric oxide (NO) by glial cells, microglia [40, 41] and astrocytes [25, 37]. There is evidence supporting a role of NO in oligodendrocyte injury, demyelination and axonal degeneration, but NO may also affect the clearance of inflammation in the CNS [42]. Taken together, these observations suggest that cannabinoid agonists are able to counteract inflammatory responses by glial cells. Nevertheless, it remains to know whether CB1 and/or CB2 receptors are involved in the above effects, or if other new CB receptors and mechanisms are implicated.

CANNABINOIDS AND MULTIPLE SCLEROSIS

Multiple sclerosis (MS) is the most important chronic inflammatory demyelinating disorder of the CNS. The permanent neurological impairment observed in later phases of the disease is due to axonal loss resulting from recurrent episodes or progressive immune-mediated demyelination. There is growing amount of evidence to suggest that cannabis and cannabinoid agonists may be effective in ameliorating symptomatology of MS, especially spasticity and pain [43]. Results from the first large-scale randomized trial to assess the potential benefits of cannabis in MS indicated some improvement in patients´ mobility and pain perception [44]. Results obtained with animal models of MS also provide support for the beneficial effects of cannabinoids in this disease. The earlier studies about cannabinoids actions on experimental autoimmune encephalomyelitis (EAE) indicate that THC treatment prior to inoculation prevented EAE symptomatology, while THC treatment after inoculation delayed the onset and reduced symptoms severity and inflammation in the spinal cord [45]. In the same line are the results obtained by Wirguin *et al*., [46] which showed that 8 and 9 delta THC decrease clinical signs of EAE. Dexanabinol, a synthetic cannabinoid which blocks NMDA receptors and has antioxidant properties, without acting on CB1 or CB2 receptors, also decreased signs of EAE in rats, perhaps through the inhibition of TNF-α release [47]. Baker *et al*., [48] have investigated the role of cannabinoid receptors in the acute effects of cannabinoids in spasticity and tremor of mice with chronic EAE (CREAE). They described that THC and WIN55212-2, a synthetic, non-specific cannabinoid agonist suppressed the above symptoms and that these effects were attenuated by pretreatment with either the CB1-selective antagonist SR141716A or the CB2 receptor antagonist SR144528. They also reported beneficial effects by treatment with methanandamide, a CB1 agonist; but also with JWH-133, a selective CB2 agonist. Interestingly, spastic CREAE mice have elevated concentrations of the endocannabinoids, anandamide and 2-AG [49], suggesting the possible existence of a tonic control of spasticity by the endocannabinoid system. Changes in CB receptors expression and function have been also described in the CNS of EAE mice [50], supporting the participation of the cannabinoid system in neuroinflammatory disorders.

In other experimental models of MS, such as the Theiler's murine encephalomyelitis virus (TMEV) infection of CNS, which serves as a relevant model for progressive human MS, cannabinoids have been found to affect its pathogenesis. Arévalo-Martín *et al*., [51] described that treatment with CB1 and CB2 agonists once established symptomatology in the infected mice, improved motor function, decreased microglial reactivity, MHC class II antigen expression and CD4 T cell infiltrates. These effects were accompanied by enhanced spinal cord remyelination (Fig. **2**). Evidence for the importance of immunomodulatory activity of cannabinoids in the TMEV model was also provided by the work of Croxford and Miller [52] which

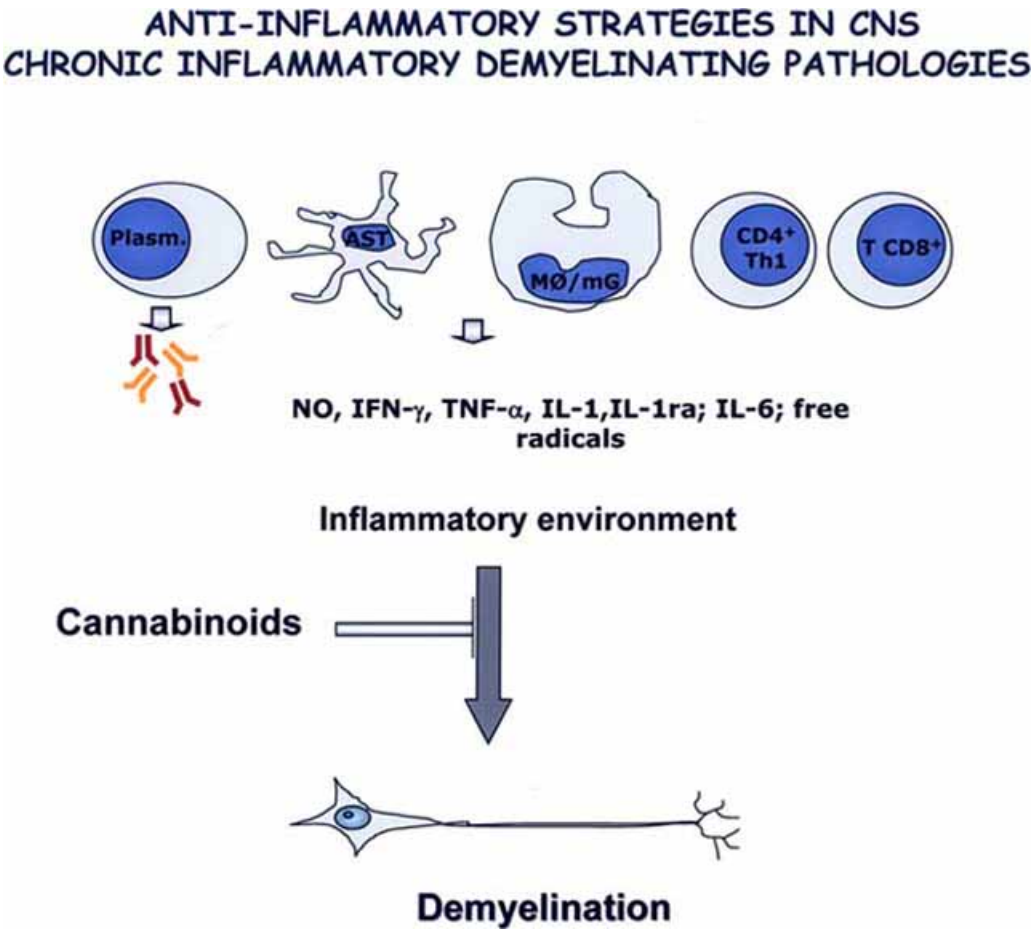

Fig. (2). Summary of potential anti-inflammatory actions of cannabinoids on different cellular components of the immune system and on glial cells: The anti-inflammatory properties of cannabinoid compounds may be important not only to restrain the demyelination process, but also to enhance the endogenous reparative remyelination.

showed that WIN55212-2 under different administration schedules attenuated clinical disease signs and also decreased mRNA expression of several cytokines, like TNF-α, L-1β and IL-6.

It is also noteworthy that induced chronic relapsing EAE in CB1 receptor-deficient mice showed several differences in comparison with wild type mice, as a delayed remission, joined to a more accumulated axonal lost, with a decreased expression of neurofilament and increased caspase 3 activity as index of apoptotic level [53]. This suggested that the presence of CB1 receptors is mediating a degree of neuroprotection during autoimmune attack, which indicates a role of endocannabinoids in neuroprotection. However, the nature and the mechanisms through the endogenous CBs exert this neuroprotective action has yet to be further investigated.

CONCLUSIONS

There is increasing amount of evidence suggesting that cannabinoids may be neuroprotective in several compromised conditions. Regarding the CNS inflammation, the summary of the above studies suggest that cannabinoids are effective against the symptomatology associated with chronic inflammatory demyelinating pathologies. The beneficial effects of cannabinoids may be exerted at multiple levels i) by improving motor function; ii) by limiting neuroinflammation, iii) by promoting remyelination. Therefore, an important area for future research is to find treatment strategies which avoid unwanted effects of these drugs, particularly their psychotropic effects. The endocannabinoid system is a valuable target for drug discovery because it is involved in the regulation of neuroinflammatory responses associated with brain disease as well as brain injury. The use of drugs that activate the endogenous cannabinoid system, increasing extracellular levels of endocannabinoids, through inhibition of their membrane transporter or metabolism may be one possibility for modulation CNS inflammation. Another possibility is to use CB2 selective agonists, expected to lack psychotropic properties, or even combined treatments. However, much basic research is still needed to understand neuroprotective effects of cannabinoids in relation to their immunomodulatory-antiinflammatory properties, in order to delineate, in a valuable manner, the potential use of cannabinoids in brain inflammatory disorders.

REFERENCES

[1] Gaoni Y.; Mechoulam R. *J. Am. Chem. Soc.*, **1964**, *86*, 1646 -1647.

[2] Matsuda, L.A.; Lolait, S.J.; Brownstein, M..J.; Young, A.C.; Bonner, T.I. *Nature*, **1990**, *346*, 561-564.

[3] Munro, S.; Thomas, K.L.; Abu-Shaar, M. *Nature*, **1993,** *365*, 61-65.
[4] Jarai, Z.; Wagner, J.A.; Varga, K.; Lake, K.D.; Compton, D.R.; Martin, B.R.; Wang, L.; Zimmer, A.M.; Bonner, T.I.; Buckely, N.E.; Mezey, E.; Razdan, R.K; Zimmer, A.; Kunos,G. *Proc. Natl. Acad. Sci. USA*, **1999**, *96*, 14136-14141.
[5] Di Marzo, V.; Breivogel, C.S.; Tao, Q.; Bridgen, D.T.; Razdan, R.K.; Zimmer, A.M.; Zimmer, A.; Martin, B.R. *J. Neurochem.*, **2000**, *75*, 2434-2444.
[6] Breivogel, C.S.; Griffin, G.; Di Marzo, V.; Martin, B.R. *Mol. Pharmacol.*, **2001**, *60*, 155-163
[7] Devane, W.A.; Hanus, L.; Breuer, A.; Pertwee, R.G.; Stevenson, L.A.; Griffin, G.; Gibson, D.; Mandelbaum, A.; Etinger, A; Mechoulam, R. *Science*, **1992**, *258*, 1946-1949
[8] Mechoulam, R.; Ben-Shabat, S.; Hanus, L.; Ligumsky, M.; Kaminski, N.E.; Schatz, A.R.; Gopher, A.; Almog, S.; Martin, B.R.; Compton, D.R. *Biochem. Pharmacol.*, **1995**, *50*, 83-90.
[9] Di Marzo, V.; Bisogno, T.; DePetrocellis, L.; Melck D.; Orlando, P.; Wagner, J.A.; Kunos, G. *Eur. J. Biochem*., **1999**, *264*, 258-267.
[10] Cravatt, B.F., Giang, D.K., Mayfeld, S.P., Boger, D.L., Lerner, R.A., Gilula, N.B. *Natur*e, **1996**, *384*, 83-87.
[11] Galiegue, S. ; Mary, S.; Marchand, J. ; Dussossoy, D. ; Carriere, D., Carayon, P. ; Bouaboula, M. *Eur. J. Biochem.*, **1995,** *232*, 54-61.
[12] Carlisle, S.J.; Marciano-Cabral, F.; Staab, A.; Ludwick, C.; Cabral, G.A. *Int. Immunopharmacol.*, **2002**, *2*, 69-82.
[13] Kaminski, N.E.; Abood, M.E.; Kessler, F.K.; Martin, B.T. Schartz, A.R. *Mol. Pharmacol*.,**1992**, *42*, 736-742.
[14] Klein, T.W.; Newton, C.; Friedman, H. *Immunol. Today*, **1998,** *19*, 373-381.
[15] Klein, T.W.; Newton, C.; Larsen, K.; Chou, J.; Perkins, I; Lu, L.; Nong, L.; Friedman, H. *J. Neuroimmunol.*, **2004**, *147*, 91-105.
[16] Berdyshev E.V. *Chem. Phys. Lipids*, **2000**, *108*, 169-190.
[17] Faubert, B.L.; Rockwell C.E.; Kaminski, N.E. *J. Pharmacol. Exp. Therap*., **2003**, *306*, 1077-1085.
[18] Carayon, P.; Marcahnd, J.; Dussossoy, D.; Derocq, J.M.; Jbilo, O.; Bord, A.; Bouaboula, M.; Galiegue, S.; Mondiere, P.; Penarier, G.; Fur, G.L.; Defrance, T.; Casellas, P. *Blood*, **1998**, *92*, 3605-3615.
[19] McCoy, K.L.; Matmeyeva, M.; Cralisle, SJ, Cabral, G.A. *J. Pharmacol. Exp. Therap*., **1999**, *289*, 1620-1625.
[20] Derocq, J.M.; Jbilo, O.; Bouaboula, M.; Segui, M.; Clere, C.; Casellas, P. *J. Biol. Chem*., **2000**, *275*, 15621-15628.
[21] Di Marzo, V.; Fontana, A.; Cadas, H.; Schinelli, S.; Cimino, G.; Schwartz, J.C.; Piomelli, D. *Nature*,**1994**, *372*, 686-691.
[22] Varga, K.; Wagner, J.A.; Bridgen, D.T.; Kunos, G. *FASEB J*., **1998**, *12*, 1035-1044.
[23] Matias, I.; Pochard, P.; Orlando, P.; Salzet, M.; Pestel, J.; DiMarzo, V. *Eur. J. Biochem*., **2002**, *269*, 3771-3778.
[24] Sanchez, C.; Galvé-Roperth, I.; Guzmán, M. *Mol. Pharmacol*., **1998**, *54*, 834-843.
[25] Molina-Holgado, F.; Molina-Holgado, E.; Guaza, C.; Rothwell, N.J. *J. Neurosci. Res*., **2002**, *67*, 829-836.
[26] Facchinetti, F.; Del Giudice, E.; Furegato, S.; Passarotto, M.; Leon, A. *Glia*, **2003**, *41*, 161-168.
[27] Walter, L.; Franklin, A.; Witting, A, Moller, T.; Stella, N. *J Neurosci***. 2003**, *23*, 1398-1405.
[28] Carrier, E.J.; Kearn, C.S.; Barkmeier, A.J.; Breese, N.M.; Yang, W.; Nithipatikom, K.; Pfister, S.L.; Campbell W.B.; Hillar C. J. *Mol. Pharmacol*., **2004**, *65*, 999-1007.
[29] Molina-Holgado, E.; Arévalo-Martín, A.; Vela, J.M.; Almazán, G.; Molina-Holgado, F.; Borrell, J.; Guaza, C. *J. Neurosci*., **2002,** *22*, 9742-9753.
[30] Walter, L.; Franklin, A.; Witting, A.; Wade, C.; Xie, Y.; Kunos, G.; Mackie, K.; Stella, N. *J. Biol. Chem.*, **2002**, *26*, 26-34.
[31] Walter, L.; Stella, N. *Glia*, **2003**, *44*, 85-91.
[32] Romero, J.; Hillard, C.J.; Calero, M.; Rabano, A. *Brain Res. Mol. Brain Res*., **2002**, *100*, 85-90.
[33] Bisogno T.; Maurelli, S.;Melck, D.; De Petrocellis, L.; Di Marzo, V. *J Biol. Chem*., **1997**, *272*, 3315-3323.
[34] Liu, J.; Batkai, S.; Parcher, P.; Harvey-White, J.; Wagner, J.A.; Cravatt, B.J.; Gao, B.; Kunos, G. *J. Biol. Chem*., **2003**, *278*, 45034-45039.
[35] Yuan, M.; Kiertscher, S.M.; Cheng, Q.; Zoumalan, R.; Tashkin, D.P.; Roth, M.D. *J. Neuroimmunol*., **2002**, *133*, 124-126.
[36] Molina-Holgado, F.; Molina-Holgado, E.; Guaza, C. *FEBS Lett*., **1998**, *433*, 139-142.
[37] Molina-Holgado, F.; Lledó, A.; Guaza, C. *NeuroReport*, **1997**, *8*, 1929-1933.
[38] Puffenbarger, R.A.; Boothe, A.C.; Cabral, G.A. *Glia*, **2000**, *29*, 58-62.
[39] Molina-Holgado, F.; Pinteaux, E.; Moore, J.; Molina-Holgado, E.; Guaza, C.; Gibson, R.M.; Rothwell N**.** *J. Neurosci.*, **2003**, *23*, 6470-6474.
[40] Waksman, Y.; Olson, J.M.; Carlisle, S.J.; Cabral, G.A. *J. Pharmacol. Exp. Ther.*, **1999,** *288*, 1357-1366.
[41] Cabral, G.A.; Harmon, K.N.; Carlisle, S.J. *Adv. Exp. Med. Biol.*, **2001** *493*, 207-210.
[42] Smith, K.J.; Lasmann, H. *Lancet*, **2002**, *1*, 232-242.
[43] Pertwee, R.G. *Pharmacol. Therap.*, **2002** *95*, 166-182.
[44] Zajicek, J.; Fox, P.; Sanders, H.; Wright, D.; Vickery, J.; Nunn, A.; Thomson, A. *Lancet*, **2003**, *362*, 1517-1526.
[45] Lyman, W.D.; Sonett, J.R.; Brosnan, C.F.; Elkin, R.; Bornstein, M.B. *J. Neuroimmunol.*, **1989**, *23*, 73-81.
[46] Wirguin, I.; Mechoula, R.; Breuer, A.; Schezen, E.; Weidenfeld, J.; Brenner, T. *Immunopharmacol.*, **1994**, 209-214.
[47] Achiron, A.; Miron, S.; Lavie, V.; Margalit, R.; Biegon, A. *J Neuroimmunol.*, **2000**, *102*, 26-30.
[48] Baker, D., Pryce, G., Croxford, J.L., Brown, P., Pertwee, R.G., Huffman, J.W.; Layward, L. *Nature*, **2000**, *404*, 84-87.
[49] Baker, D.; Pryce, G.; Croxford, J.L.; Brown, P.; Pertwee, R.G.; Makriyannis, A.; Khanolkar, A.; Layward, L.; Fezza, F.; Bisogno, T.; di Marzo, V. *FASEB J*., **2001,** *15*, 300-302.
[50] Berrendero, F.; Sánchez, A.; Cabranes, A.; Puerta, C.; Ramos, J.A.; García-Merino, A Fernandez-Ruiz, J. *Synapse*, **2001**, *41*, 195-199.
[51] Arévalo-Martín, A.; Vela, J.M.; Molina-Holgado, E.; Borrell, J.; Guaza, C. *J. Neuroci.*, **2003,** *23*, 2511-2516.
[52] Croxford, J.L.; Miller, S.D. *J. Clin. Invest.* **2003,** *111*, 1231-1240.
[53] Pryce, G.; Ahmed, Z.; Hankey, D.J.R.; Jackson, S.J.; Croxford, J.L.; Pocok, J.M.; Ledent, C.; Petzold, A.; Thompson, A.J.; Giovannoni, G.; Cuzner, M.L.; Baker, D. *Brain*, **2003**, *126*, 2191-2202.