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Advances in Flow Cytometry Investigation of Cannbinoid CB2 Receptor Agonists in Multiple Sclerosis: Commentary

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It is commonly accepted that the cannabinoid 2 receptor (CB2R) is expressed by most cell types of the rodent and human immune system, although some studies have also reported its expression in cells of the central nervous system [1-3]. The importance of this receptor in multiple sclerosis (MS) is emerging by increasing number of studies reported in literature, suggesting the protective role of this receptor in several neurological disorders associated with a driving autoimmune component. Recent flow cytometry studies provided evidence of the expression levels of the CB2R in different immune cell subsets. Higher levels of CB2R were detected in NK cells, B-lymphocytes and monocytes than in CD4+ or CD8+ T-lymphocytes, whereas neutrophils expressed a low level of CB2R. Furthermore, taking advantage of a sensitive technique like flow cytometry, it was observed that CB2R are present on resting T-lymphocytes at low abundance in some healthy subjects [4].

This study helped to suggest which blood leukocyte subset from healthy donors could represent a target for CB-mimetic drugs in humans. Following studies were focused on assessing differences between blood leukocytes from healthy donors and those from MS patients. Interestingly, the immune-modulatory effects of novel described CB2R selective agonists, 1,8-naphthyridine, pyridine and quinoline derivatives [5-7] were investigated. Although, the effects of some of these compounds were partially mediated by the CB2R, different T cell activation between leucocytes from healthy donors and those from MS patients was observed by flow cytometry analyses. In particular, on CD4+T cells thought to initiate and drive the inflammatory process in MS, activation markers like CD 69 and the adhesion molecule CD 54 (ICAM-1) were potently down-regulated by naphthyridine and quinoline derivatives, CB 74 and VL 23 respectively. The effects observed were higher in CD4+ T cells isolated from MS patients than in control cells, thus supporting a likely specific effect of these compounds in lymphocytes isolated from patients.

Of note, a compound with lower CB2R affinity [7], a pyridine derivative, AF4 efficiently reduced CD 69 and CD 54 (ICAM-1) in healthy donor cells, whereas did not affect the same markers in cells derived from MS patients, these results suggest that higher CB2R affinity is needed to reduce activation in lymphocytes from MS patients. Previous studies also showed that the down-regulation of adhesion molecule by cannabinoid agonists interferes with the progression of MS [8], thus proposing a therapeutic benefit derived by the use of modulators of these molecules involved in the regulation of transmigration of blood leukocytes across the blood–brain barrier (BBB) in MS. Further flow cytometry studies performed on other compounds belonging to the naphthyridine scaffold, confirmed the efficiency of a particular drug, CB 91 to decrease cell activation and adhesion by down-regulation of the expression of CD 69, the integrin CD 49d (α4 integrin chain or VLA-4α chain) and the adhesion molecule CD 54 (ICAM-1) on human CD4+T cells of healthy donors.

In addition, CB 91 has high CB2R affinity and functions in immune cells by a CB2R mediated mechanism, indeed, the finding that this substance is endowed with medium level of intestinal absorption and BBB permeability [9], may open the way to further investigations aimed to optimize the properties of this promising class of compounds for example enhancing their BBB permeability. It is known that endothelial signaling cascade results in downstream effector mechanisms which influence the progression of neuro-inflammation and that the increase of BBB permeability during neuro-inflammation is one of the contributing factors associated with neuro-degeneration. The next step forward might be the investigation of the effects of these drugs on the migratory capacity of leukocytes across the BBB by using for example brain microvascular endothelial cells (BMVEC).

In fact, an interesting study, based on the consideration of the emerging relevance of CB2R in MS, investigated the role of CB2R and CB2R agonists in human brain BMVEC. Flow cytometry assays established the effects of the selective CB2R agonist, O-1966, a novel resorcinol-based compound on leukocyte adhesion to endothelium showing that the decrease in monocyte adhesion was due to the CB2R mediated diminution of ICAM-1 and VCAM-1 surface expression induced by LPS or TNFα [10]. These findings suggest that the effects on both immune cells and endothelial cells cannot be uncoupled, they are not only one-sided (for example immune cells), but the brain endothelium also responds to CB2R agonists and prevents adhesion molecule induction under inflammatory insult. In conclusion, CB2R may represent a therapeutic target, not only for CB2R agonist effects on attenuating immune cell activation, but also in protecting the BBB during neuro-inflammation. In particular, in course of MS or in distinct phases of the disease it will be of interest for the scientific community to investigate immune cells-endothelium adhesion and explore the role of the CB2R in their interactions.

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