The role of Cannabinoid Receptor 2 in human Mesenchymal Stromal Cells

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Background

Mesenchymal stromal cells (MSCs) are non-hematopoietic, multipotent progenitor cells able to differentiate into bone marrow (BM) stroma, as well as into adipocytes, chondrocytes, and
osteocytes.

MSCs produce cytokines, chemokines, and extracellular matrix proteins that support hematopoietic stem cell (HSC) survival and engraftment, influence immune effector cell development, maturation,
and function, and inhibit alloreactive T-cell responses.

hMSC immunoregulatory ability is independent of the major histocompatibility complex. Based on this evidence, administration of hMSCs has been suggested as an alternative strategy for treating
immune-mediated diseases, and their immunosuppressive properties have been explored in a number of experimental autoimmune diseases, as well as in organ transplantation.

The Cannabinoid Receptor type 2 (CB2) is involved in immune regulation by suppressing immune cell activation, through modulation of Thelper cell types 1 and 2 (Th1 and Th2), inhibition of proinflammatory cytokine production, and nuclear factor-B dependent apoptosis. Indeed, a CB2 functional variant has been associated with several inflammatory/immune-based disease. Moreover, recent studies highlighted the presence of the endocannabinoid system in hematopoietic and neural precursor stem cells.

In the present study, we have investigated, through a multidisciplinary approach, the involvement of the endocannabinoids in migration, viability and cytokine release of hMSCs.

Materials and Methods

MSCs were isolated from BM of 12 healthy donors (8 males, 4 females, median age 36.5616 years, range 20–53). MSCs were characterized by flow cytometry. After cell cultures were trypsinized, mRNA extraction, retrotranscription, semiquantitative PCR, Real-Time PCR were performed. Total lysates from MSC cultures obtained through RIPA buffer lysis were analyzed by western blot (WB) experiment at some of the different passage from P1 to P9 and after pharmacological challenge with the CB2 agonist JWH-133 or the CB2 antagonist AM630. The hMSC culture supernatant levels of IL-1b, IL-6, IL-8, IL-10, IL-12, IL-17, TNF-a and INF-c were measured using a commercially available Human Inflammatory Cytokines Multi-Analyte ELISArray Kit. The attractive properties of 2-AG were analyzed in a modified chemiotaxis plate (iuvo Chemotaxis Assay plate, Thermo Scientific Inc., Germany). All the experiments were at least in triplicate. A t-test was performed according to the number of experiments. A p value less than 0.05 was considered statistically significant.

Results:

1)hMSCs in culture express both CB1 and CB2 receptors (Fig. 1, 2), as well as the biochemical enzymatic machinery for the synthesis and degradation of the endocannabinoids anandamide (AEA) and 2 arachidonoylglycerol (2-AG);
2)CB2 expression level in hMSCs increases with cell maturation
3)Stimulation of the CB2 receptor partially reverses the LPS-induced modulation of pro- and anti- inflammatory cytokines in hMSCs
4)Stimulation of CB2 directly stimulates cell survival Pathways
5)Stimulation of CB2 directly stimulates the ERK2 pathway
6)CB2 selective stimulation is associated with mTOR pathway activation
7)2-AG is Chemoattractant for hMSCs through CB2 Receptor
8)mTOR/S6K1 pathway is involved in the hMSCs migration 2AG-mediated

Conclusion:

In conclusion, our data show, for the first time, that cultures of hMSCs express all of the components of the endocannabinoid system and suggest a potential role for the cannabinoid CB2
receptor as a mediator of MSC anti-inflammatory properties, as well as for their survival pathways and their capability to home and migrate towards endocannabinoid sources.

References

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