The cannabinoid receptor *type 2* as mediator of mesenchymal stromal cell immunosuppressive properties

F. Rossi[1,2], G. Bellini[3], M.E. Bernardo[2], L. Luongo[3], F. Guida[3], A. Conforti[2], J. Pianese[1], M. Coppola[1], B. Nobili[1], V. de Novellis[3], V. Di Marzo[4,5], F. Locatelli[2,6], S. Maione[3,4]

[1] Dept. of Women, Child and General and Specialistic Surgery, Second University of Naples, Naples, Italy
[2] Dept. of Onco-Haematology, IRCCS 'Bambino Gesù' Children Hospital, Rome, Italy
[3] Dept. of Experimental Medicine, Second University of Naples, Naples, Italy
[4] The Endocannabinoid Research Group (ERG), Pozzuoli, Naples, Italy
[5] Institute of Biomolecular Chemistry, National Research Council (CNR), Pozzuoli, Naples, Italy
[6] University of Pavia, Pavia, Italy

Mesenchymal stromal cells are non-hematopoietic, multipotent progenitor cells producing cytokines, chemokines, and extracellular matrix proteins that support hematopoietic stem cell survival and engraftment, influence immune effector cell development, maturation, and function, and inhibit alloreactive T-cell responses. The immunosuppressive properties of human mesenchymal stromal cells (hMSCs) have attracted much attention from immunologists, stem cell biologists and clinicians.

Recently, the presence of the endocannabinoid system in hematopoietic and neural stem cells has been demonstrated. Endocannabinoids, mainly acting through the cannabinoid receptor *subtype 2*, are able to modulate cytokine release and to act as immunosuppressant when added to activated T lymphocytes.

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

We 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 anti-inflammatory properties of human mesenchymal stromal cells, as well as of their survival pathways and their capability to home and migrate towards endocannabinoid sources.

We find that hMSCs express both CB1 and CB2 receptors and that a significant change in endocannabinoid levels, concurrently with a sensible modification of CB2 or CB1 receptor expression, is observed during the different *in vitro* culture passages from P0 to P9. In particular, we show that the expression of the CB2 receptor was barely detectable at the first passage *in vitro* and increased through the following ones, while CB1 expression showed the opposite trend. Moreover we observe a decrease in the levels of AEA and 2-AG, as well as of other endocannabinoid-like molecules (PEA and OEA) from P0 to P9.

We find that CB2 activation counteracts the LPS-induced effects on the extracellular levels of IL-10, IL-1ß, IL-8, IL-17, IL-6, TNF-α, INF-γ.

Accordingly, we find that the phosphorylated isoform of ERK2, known to be involved in CB2 stimulation of IL10 release, increased at P6, when CB2 expression is maximal, and in the JWH-133-treated group.

The highest CB2 expression, as well as its stimulation, corresponds also to an enhancement of the anti-apoptotic BCL-2, to the activation of the AKT-mediated survival pathway and to the phosphorylation of S6K1 the downstream effector of the PI3K-AKT-mTOR pathway activation.

Taken together these data suggest that at P6 the expression of the CB2 receptor is highest and the cells are possibly more resistant to apoptotic signals, more metabolically activated and, finally more responsive to external stimuli.

Finally, live imaging studies by releasing 2-AG from a fixed source show a stimulation of the migration towards a 2-AG source, which is completely blocked by the CB2 receptor selective antagonist AM630, and that S6K1 is a signaling kinase activated during this chemotaxis elicited by 2-AG. These data confirm that endocannabinoids could represent mediators of MSC homing through the CB2 receptor.

In conclusion, we find that selective CB2 stimulation: i) enhances hMSCs viability; ii) exerts anti-inflammatory effects in LPS-challenged cells; and iii) induces cell migration towards a source of 2-AG. Altogether these findings support the hypothesis that extracellular endocannabinoids are important paracrine mediators of cell migration and proliferation.

Bernardo (2009). *Ann N Y Acad Sci.* 1176:101-117
Majumdar (2000). *J Hematother Stem Cell Res*. 9:841-848.
Cencioni (2010). *PLoS One.* 5:e8688.
Compagnucci (2013). *PLoS One.* 8: e54271.
Jiang (2011). *Blood*. 117:827-838.
Bari (2011). *Stem Cells DeV*. 20:139-147.