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Comparative analysis of the immunomodulatory properties of different mesenchymal stromal cells and their extracellular vesicles

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Background. In recent years, mesenchymal stromal cells (MSC) have been an attractive target for their translation into clinical research applications. This is mainly due to their paracrine, regenerative, multi-lineage differentiation and immunomodulatory properties. However, MSC from different sources possess subtle differences that could affect the immunomodulatory molecular mechanisms used to exert their effects. MSC interact with a broad range of immune cells such as B and T cells and seem to exert their immunomodulation by synergic cell contact-dependent mechanism and soluble factors, including Interleukin-10 (IL-10), Prostaglandin E-2 (PGE-2), Nitric oxide (NO) and Indoleamine 2,3-dioxygenase (IDO) amongst others. MSC have been increasingly used in a variety of therapeutic fields such as transplantation, kidney injury, graft versus host disease (GvHD) or autoimmune diseases. Many preclinical murine studies involve administration of not only autologous and allogeneic, but also xenogeneic MSC. In fact, human MSC application in animal models is performed to assess their therapeutic potency and verify their safety and efficacy, as required by regulatory authorities. Nevertheless, the use of hMSC in murine models raises numerous concerns and the lack of homogeneous results still limit the translation to clinical research. MSC-derived conditioned media (CM) and extracellular vesicles (EV) have been portrayed by many as a strong alternative to cell-therapy, overcoming many of the regulatory challenges faced by MSC clinical translation. However, as the EV field is quite recent, there is contradictory data concerning EV immune potency. Further studies are needed to clarify their modulatory prospect and the mechanisms involved.
Aim. The main goal of this study was to evaluate different human MSC modulatory properties and elucidate their mechanisms while comparing the impact of MSC-secretome (CM and EV) on immunomodulation *in vitro.*
Methods. In particular, we assessed: (1) Different human MSC and (2) MSC-secretome immunomodulatory potential; and (3) evaluated UC-based EV isolation protocol.
To assess their immunosuppression capacities, we first set up direct cocultures with human PBMC and CD4+ T cells. We investigated IDO as a candidate involved in the inhibition of PBMC. To verify whether MSC modulatory strength are affected by potential interspecies incompatibilities, rat-human allogeneic and xenogeneic cocultures were set. Supernatants were analyzed for factors involved in their mechanisms of action.
MSC-secretome potential was investigated first through MSC:PBMC direct and indirect cocultures. Second, we established immunosuppression assays with human PBMC and CM or isolated MSC-EV. PBMC proliferation and analysis of coculture supernatants were performed.
A comparative study evaluating an ultracentrifugation (UC)-based EV isolation protocol and its reproducibility in different laboratories was performed. EV basic characterization methods (Nanoparticle tracking analysis (NTA), Western blot (WB), Transmission electron microscopy (TEM), Fluorescence-activated cell sorting (FACS)) were assessed and technical variations induced by equipment and/or operators were evaluated.
Results. Certainly, ASC portrayed to be the most immunosuppressive MSC source inhibiting both PBMC and CD4 T cell proliferation equally, supported by elevated IDO levels. MSC strongly inhibited PBMC proliferation via an IDO-kynurenine immunosuppressive mechanism. This system was verified by the addition of IDO inhibitor, Epacadostat, which completely abolished MSC inhibitory action.
ASC immunomodulation capacities in xenocultures demonstrated incapability to inhibit rat peripheral blood and spleen mononuclear cells (PBMC and SMC, respectively) proliferation, however, ABCB5 inhibited proliferation of both rat MC. On the other hand, rat MSC succeeded in suppressing human PBMC proliferation. We were able to corroborate that murine MSC rely mostly on NO secretion as their

main immunosuppressive mechanism, whilst human MSC rely on IDO. This not only confirmed the different species-dependent modulatory system but also indicated a possible use of ABCB5 cells for research in murine models.
MSC secretome-product immunomodulatory potency confirmed suppression of T cell proliferation in both direct and indirect cocultures. IFNγ-primed MSC-CM successfully inhibited T cell proliferation. In contrast to CM, MSC-EV failed to suppress PBMC proliferation indifferent of IFNγ priming.
Finally, when assessing an UC-EV isolation protocol in an inter-laboratory study we found obvious quantitative differences in the EV yield. We observed that operator and handling time variations impacted EV yield. Thus, to achieve maximal reproducibility, accurate and precise reporting of EV workflow is needed.
Conclusions. In regards to MSC immunomodulation, our study provides important evidence that ASC are the most immunosuppressive source of MSC. Furthermore, despite the lack of suppressive effect of MSC-EV, this project has also elucidated the strong immune strength of MSC-derived CM+IFNγ in inhibiting PBMC proliferation. We strongly recommend further *in vivo* research with CM+IFNγ, which may further clarify the mechanisms involved and represent a potential novel cell-free treatment.