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The potential of mesenchymal stromal cells in immunotherapy

“... a detailed understanding of the pathological processes (local environment) accompanying different diseases is a prerequisite before applying mesenchymal stromal cell therapy...”

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• MSC derivative products

Over the past years, extensive studies have been conducted to develop safe and effective therapeutic strategies to treat human diseases. Immunomodulation refers to the manipulation of the immune system either by enhancing (immunopotentialization strategy), or suppressing (immunosuppression strategy) the immune responses. Immunomodulation can be achieved by different agents, referred to as immunomodulators, including monoclonal antibodies, cytokines, glucocorticoids, adjuvants, ultraviolet light, plasmapheresis and many others [1]. Mesenchymal stromal cells (MSCs) are immunomodulators of special interest and have been suggested as promising therapeutics for several autoimmune diseases and transplantation complications [2]. According to the International Society for Cellular Therapy, MSCs are defined as plastic-adherent cells with a specific surface antigen expression profile (CD73⁺, CD90⁺, CD105⁺, CD34⁻, CD45⁻, CD11b⁻, CD14⁻, CD19⁻, CD79a⁻ and HLA DR⁻) and have the potential to differentiate, under standard *in vitro* differentiation conditions, into adipocytes, chondroblasts and osteoblasts [3]. Being a major component of the hematopoietic stem cell (HSC) niche, MSCs were first isolated from the bone marrow and identified for their capacities to support hematopoiesis [4], thus being of special importance for treating hematological disorders. Later, MSC-like cells were found and isolated from a variety of adult

and fetal tissues including adipose tissue, umbilical cord and placenta [5]. This wide distribution in different parts of the organism highlighted a potential role for MSCs in tissue repair, and regeneration throughout individual's life.

The discovery of the superior immunoregulatory capacities of MSCs over different immune cells, raised their medical importance as they are used, nowadays, in preclinical and clinical studies as a potential therapy for treating autoimmune diseases, and modulating inflammatory responses [6]. The rationale of MSC-based therapy, is derived from their special immunologic profile and potent immunomodulatory capacity. Although MSCs isolated from different tissues exhibit differences in their expression profiles of some endothelial- and stromal-associated markers, all MSCs show low expression levels of different surface antigens, including MHC class I and the co-stimulatory molecules (CD40, CD80 and CD86) as well as no MHC class II expression [7]. Thanks to this special immunologic profile, MSCs can evade being rejected by host immune responses, thus contributing for their hypoimmunogenicity. Besides not being recognized as alloantigens, MSCs can actively sense the surrounding inflammatory microenvironment and modulate, accordingly, the function of different immune cells within the host [8]. In fact, different parameters including the concentration

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and type of cytokines present in the surrounding microenvironment can influence the immunologic phenotype and behavior of MSCs, demonstrating the plasticity of their immunomodulatory capacity [9]. When present in an inflammatory milieu, proinflammatory cytokines (such as TNF- α and INF- γ) will activate, via TLR3, MSCs which in turn adopt an immunosuppressive phenotype [8,9]. However, when inflammation is absent, activation of TLR4 renders MSCs in a proinflammatory phenotype [8,9]. The potent immunosuppressive and anti-inflammatory capacities of MSCs involves interaction with various innate and adaptive immune cells via different mechanisms. Paracrine signaling is the primarily mechanism for MSCs-dependent immunosuppression and involves different soluble molecules including TGF- β , HGF, IL-10, SEMA-3A, PGE2, GAL, IDO, HO, TSG-6 [10,11]. Cell-to-cell direct contact is another mechanism for MSC-mediated immunosuppression that involves physical interaction between different MSC membrane-attached antigens such as CD200 ligand and ephrin (Eph) receptor with their respective targets, CD200 receptor (CD200R) and Eph ligand, expressed on immune cells [11].

Within the innate immune system, dendritic cells, serving as professional antigen-presenting cells (APCs), macrophages, monocytes and natural killer (NK) cells are major targets for MSC-mediated immunomodulation. In fact, MSCs can alter the generation of DCs by impairing their differentiation and maturation [12]. These immature DCs have tolerogenic features as they exhibited reduced ability to activate T cells, secreted the anti-inflammatory cytokine IL-10 and inhibited the secretion of pro-inflammatory factors such as TNF- α and IL-12 [12]. According to their environmental signals, macrophages can adopt the M1 (pro-inflammatory) or M2 (anti-inflammatory) phenotype. In the presence of MSCs, M1 macrophages are shifted into M2 cells, being characterized by a high production of IL-10 and low levels of TNF and INF- γ [13]. When co-cultured with MSCs, NK cells, serving important role in allograft rejection since they can discriminate between self and non-self, became less activated with a reduced INF- γ secretion and an impaired cytotoxic capacity [14].

In the adaptive immune system, MSCs are able to impair T cells activation, proliferation and differentiation [15]. During inflammatory conditions, MSCs can shift the differentiation of naive CD4⁺ from cells producing proinflammatory Th1 cytokines into cells secreting anti-inflammatory Th2 molecules [16]. Besides suppressing the differentiation of CD4⁺ Th0 into proinflammatory Th17 cells, MSCs can stimulate the differentiation of Th0 cells into regulatory

T cells (Tregs) [17] that are important for conditioning immune responses. The effect of MSCs on B lymphocytes is contradictory and poorly understood. Some reports describe an inhibitory effect of MSCs on B cells proliferation, terminal differentiation and immunoglobulin production [18]. Currently, it is also reported that MSCs stimulate the differentiation of B cells into regulatory B cells (Bregs) that participate in ensuring tolerance upon suppressing the immune responses [19].

The *in vitro* and *in vivo* MSCs-immunosuppressive capacity inspired the development of MSC-based therapy. *In vitro* preclinical and clinical studies were performed to assess the MSCs-therapeutic efficacy in treating different immune-based diseases including allograft rejection, graft-versus-host disease (GVHD), myocardial inflammation, Crohn's disease, Multiple Sclerosis, Systemic Lupus Erythematosus and Rheumatoid Arthritis. The outcome was not totally satisfactory due to the critical discrepancy between expected and actual results. These discrepancies could be attributed to many causes where some important variables are currently highlighted in literature [20]. The procedures involving isolation, culture, expansion and cryopreservation of MSCs are all critical parameters. The age of the donor, the source of MSCs, the dose and frequency of injected cells as well as the transfusion method significantly impact the curative efficacy of MSCs. Nowadays, attention is focused towards the *in vitro* manipulation of MSCs. As described above, MSCs-immunosuppressive capacity is not constitutive but rather needs to be triggered either by potent inflammatory cytokines such as TNF- α , INF- γ , IL- α and/or IL-1 β or by activating TLR3 signaling pathway. Hence, a detailed understanding of the pathological processes (local environment) accompanying different diseases is a prerequisite before applying MSC therapy. Besides, the timing of MSCs administration is critical since the inflammatory environment can change during the course of disease, thus the plasticity of MSCs can be affected. Cell therapy based on the use of MSCs and/or and their derivatives as an alternate source of biological products for therapeutic purposes, particularly for the management of immunological disorders, is gaining more and more notoriety. Nowadays, extensive studies are concerned with dissecting the molecular mechanisms by which different MSCs sense the surrounding inflammatory milieu and translate these messages into immunosuppressive responses. In parallel, much effort is dedicated for the exogenous and endogenous manipulation of MSCs in order to improve their immunotherapeutic efficacy.

Currently, several challenges are being addressed and have to be well investigated:

- As MSCs are present in all tissues, can we specifically identify and mobilize *in vivo* a type of ‘immunosuppressive MSCs’ to avoid allogeneic transplantation?
- As MSCs harbor different immunoregulatory pathways, can we target a relevant active regulatory factor that will ensure their therapeutic effects?
- As MSCs-immunomodulatory capacities are enhanced by inflammatory priming, can we develop therapeutically relevant cells for clinical uses (with increased homing potential to damaged tissues and/or increased production of immunoregulatory molecules)?
- As MSCs could readily be genetically engineered, can they be used as cellular therapeutic vehicles to selectively deliver potent immunoregulatory factors?
- As soluble factors play critical roles in mediating MSCs-immunomodulatory effects, can those cells be replaced by their immunosuppressive supernatant?
- As MSCs can mediate, in part, their immunomodulatory effects through secreted exosomes

carrying different bioactive molecules, could these microvesicles be selectively isolated and infused instead of the cells?

Due to their specific immunobiology, and in particular their immunomodulatory properties, the use of MSCs and/or and their derivatives in the field of immunotherapy is a major concern. As immunotherapeutic products, their use is becoming highly interesting and promising for the management of inflammatory and immunological disorders. However, before proceeding with clinical applications, the aims and challenges emerging from such field have to be addressed in order to improve the immunotherapeutic efficacy as well as ensure the safety of MSC-based immunotherapy.

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