

# Mesenchymal stromal cells: tissue repair and immune modulation

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BM-derived mesenchymal stromal cells (MSC) differentiate along the mesenchymal lineage to bone, fat and cartilage. *In vitro*, MSC induce little, if any, proliferation of allogeneic lymphocytes. MSC inhibit the proliferation of activated T cells and the formation of cytotoxic T cells. *In vivo*, they appear to have anti-inflammatory effects. Preliminary

studies suggest that MSC preferentially home to damaged tissue and therefore have therapeutic potential. Possible clinical indications include therapy-resistant severe acute GVHD, treatment of rejection of organ allografts and autoimmune disorders.

## Are MSC true stem cells?

Stem cell plasticity remains a matter of debate but it is generally agreed that adult BM contains several populations of multipotent stem cells. Stem cells are characterized by a capacity for self-renewal and an ability to differentiate into at least one mature cell type. In addition to hematopoietic stem cells (HSC), BM also contains mesenchymal progenitor cells [1]. They have the capacity to differentiate *in vitro* and *in vivo* into several mesenchymal tissues, including bone, cartilage, tendon, muscle, adipose tissue and possibly BM stroma [2]. Mesenchymal stromal cells (MSC) are capable of differentiating into several lineages under defined conditions but have an expansion limit *in vitro* and have not yet been demonstrated *in vivo* at a single-cell level to be capable of regenerating or maintaining a tissue compartment. For that reason, ISCT has recently encouraged the scientific community to adopt the term multipotent mesenchymal stromal cells to depict these cells [3].

## MSC characteristics

Because of our current inability to isolate MSC prospectively, due to their rarity *in vivo* and lack of characteristic markers, current data are based on studies performed on cells expanded *in vitro*. MSC have the capacity to proliferate extensively and form colonies of fibroblastic cells (defined as colony-forming units-fibroblastic; CFU-F).

Assays of CFU-F indicate that the cells are rare in human BM, yielding *in vitro* colony numbers of between 1 and  $20 \times 10^{-5}$  plated mononuclear cells [4]. Studies on human fetal BM suggest that stromal progenitors capable of generating CFU-F *in vitro* express CD34 but, in contrast to hematopoietic precursors, not CD38, CD50 or HLA-DR. One of the first Ab shown to enrich for CFU-F in fresh marrow aspirates was STRO-1 [5]. Not all cells within the STRO-1<sup>+</sup> fraction have CFU-F capacity but positive STRO-1 selection results in a 10–20-fold increase in CFU-F. Other markers, including CD90, CD49A, CD10 and CD146, may also be employed to enrich for CFU-F. Jones *et al.* used microbeads against D7-FIB, a fibroblast-specific molecule, to select positively for CFU-F [6]. D7-FIB<sup>+</sup> CD45<sup>low</sup> cells capable of chondrogenic, adipogenic and osteogenic differentiation comprise 0.01% of BM cells and are uniformly positive for CD105, LNGFR, HLA-DR, CD10, CD13, CD90 and STRO-1.

Isolated from BM cells by their adherence to the tissue culture dish and consecutive passage, MSC proliferate as fibroblastic spindle-shaped cells. MSC produce a vast array of matrix molecules, including fibronectin, laminin and collagen, review in [7]. They also express various integrin  $\alpha$ - and  $\beta$ -subunits that constitute receptors for extracellular matrix components. Characterization of surface molecules by flow cytometry has determined that MSC express ligands for surface molecules present on cells of

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the hematopoietic lineage, including intracellular adhesion molecule (ICAM-1, ICAM-2), vascular cell adhesion molecule-1, etc. No specific marker or combination of markers specifically identifies MSC and the cells have therefore been defined by using a combination of phenotypic markers and functional properties. *Ex vivo*-expanded MSC do not express the hematopoietic markers CD34, CD45 or CD14 but can be identified as cells that stain positive for CD29, CD73, CD90, CD105 and CD166 by flow cytometry. Under defined conditions *in vitro*, the cells should also be capable of differentiating into osteoblasts, chondrocytes and adipocytes.

### **Immunomodulatory properties of MSC**

In contrast to the relative lack of information concerning the *in vivo* role of MSCs in health and disease, the ability of MSCs to exert an anti-proliferative, immunomodulatory and anti-inflammatory effect has recently focused attention on them as potential therapeutic agents in various clinical settings. Human MSC fail to induce proliferation of allogeneic lymphocytes *in vitro*, even though they express HLA class I and, after they have been induced by IFN- $\gamma$  to express HLA class II, or are transfected with B7-1 or B7-2 co-stimulatory molecules [8,9]. Similarly, MSC are not lysed by either cytotoxic lymphocytes nor KIR ligand-mismatched NK cells [10]. Transplanted allogeneic mismatched MSC engraft in experimental animal models and human MSC persist after infusion *in utero* to fetal sheep [11]. It is clear, however, that species-specific differences exist that may prevent cross-species engraftment, as human MSC were rejected in a xenogeneic model after infusion into immunocompetent Sprague–Dawley rats [12]. Alloreactivity also exists after transplantation between different strains of mice. We recently reported on fully mismatched allogeneic fetal liver-derived MSC transplanted into an immunocompetent fetus with osteogenesis imperfecta in the third trimester of gestation [13]. No immunoreactivity was observed when patient lymphocytes were re-exposed to the graft *in vitro*, indicating that MSC can be tolerated when transplanted across MHC barriers in humans. *In vitro*, human MSC suppress proliferation of lymphocytes activated by allogeneic cells or mitogens review in [7]. The suppression appears to be mediated, at least in part, by soluble factors and affects several types of immune cells. Several factors have been proposed to mediate the suppressive effect, including TGF- $\beta$ , hepatocyte growth factor (HGF), prostaglandin

E<sub>2</sub> and indoleamine 2,3-dioxygenase (IDO)-mediated tryptophan depletion.

*In vivo*, MSCs prolong the time to rejection of histoincompatible skin grafts in baboons and improve the outcome of renal, neural and lung injury in experimental animal models, mainly through paracrine effects, shifting the environment at the site of injury from pro-inflammatory to anti-inflammatory [14–17]. In humans, more than 100 patients have received MSCs, review in [7]. In most cases, the MSC were derived from an allogeneic HLA-matched donor and the cells given in the context of HSCT for malignancy or inborn errors of metabolism. Infusion of MSC was tolerated well and no side-effects were noted. Protection from an immunologic reaction by MSC infusion has been reported in a 9-year-old boy who received a matched unrelated donor HSCT for leukemia [18]. Severe acute steroid-resistant GvHD of the gut and liver was reversed by infusion of haplo-identical MSC derived from the patient's mother. Future studies will further evaluate the potential of MSC to both prevent and treat steroid-resistant GvHD and reduce the incidence and severity of GvHD.

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