

# Cytokines as the Major Mechanism of Mesenchymal Stem Cell Clinical Activity: Expanding the Spectrum of Cell Therapy

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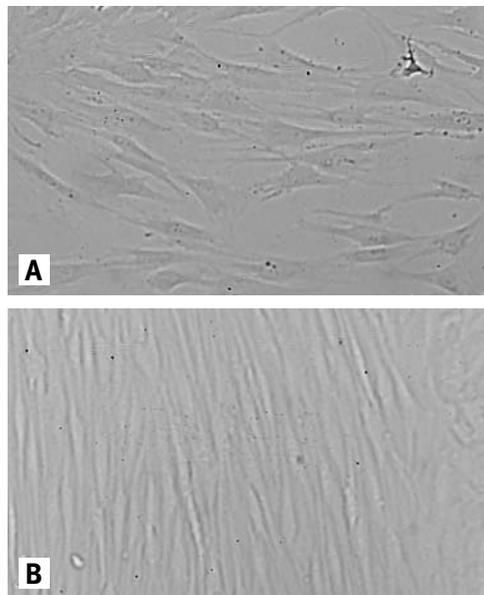
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**ABSTRACT:** Mesenchymal stem cells, or mesenchymal stromal cells, have emerged as a major new cell technology with a diverse spectrum of potential clinical applications. MSCs were originally conceived as stem/progenitor cells to rebuild diseased or damaged tissues. Over the last 14 years, since the first report of MSC infusions in patients, the cells have been shown to suppress graft vs. host disease, stimulate linear growth in a genetic disorder of bone, and foster engraftment of haplo-identical hematopoietic stem cells. In all cases, few, if any, MSCs were identified at the site of clinical activity. This experience suggests a remarkable clinical potential, but a different general mechanism of action. Systemically infused MSCs seem to exert a therapeutic effect through the release of cytokines that act on local, or perhaps distant, target tissues. Rather than serving as stem cells to repair tissues, they serve as cellular factories that secrete mediators to stimulate the repair of tissues or elicit other beneficial effects. Since both the tissue source of MSCs and the *ex vivo* expansion system may significantly impact the cytokine expression profile, these parameters may be critically important determinants of clinical activity. Furthermore, cell processing protocols may be developed to optimize the cell product for a specific clinical indication. For example, MSC-like cells isolated from placenta and expanded in a three-dimensional bioreactor have recently been shown to increase blood flow in critical limb ischemia. Future efforts to understand the cytokine expression profile will undoubtedly expand the range of MSC clinical applications.

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**KEY WORDS:** mesenchymal stem cells, cell therapy, cytokine cell processing, mechanism of action

**M**esenchymal stromal cells are spindle-shaped, plastic-adherent cells isolated from bone marrow, adipose tissue and many other tissue sources [Figure 1] [1,2]. Termed “mesenchymal stem cells” by investigators, the International Society for Cellular Therapy suggested that, in the absence of convincing data to support the notion of “stemness,” the term



**Figure 1.** *In vitro* tissue culture of MSCs. Photomicrographs of human MSCs during *ex vivo* expansion [A] at 50% confluence and [B] near 100% confluence. Original magnification x 40.

“stromal cell” is more scientifically appropriate [1]. A variety of potentially different cells carry the moniker “MSC.” Indeed, isolated populations of cells appear to be genetically heterogeneous [3] despite their uniform morphology. Moreover, unique antigenic markers to distinguish MSCs have not yet been identified. To resolve this latter issue, the ISCT has suggested reasonable criteria to define the heterogeneous populations of cells, recognizing that modifications will be required as our understanding of the basic biology of MSCs increases [2].

MSCs were originally identified by Friedenstein and colleagues [4] as the cells of the marrow microenvironment supporting hematopoiesis and were quickly shown to have a vast *ex vivo* expansion potential as well as the capacity to differentiate to bone [4]. After identification of the high proliferative potential cells that were designated CFU-F (colony-forming

MSC = mesenchymal stem cell

ISCT = International Society for Cellular Therapy

unit-fibroblast) and the report that MSCs could also readily differentiate to fat and cartilage in addition to bone, investigators suggested that MSCs fulfilled the criteria of a stem cell. Indeed, Maureen Owen [5] proposed that the CFU-F may be a stromal stem cell, and Caplan et al. [6] proposed the idea that these cells were actually mesenchymal stem cells with the capacity to differentiate to a wide variety of mesenchymal tissues. According to this concept, MSCs could serve as a broadly applicable stem cell source for regenerative medicine repopulating injured tissues or clinically ablated diseased tissues with healthy, terminally differentiated, tissue-specific cells [7,8]. However, systemically infused MSCs have not been shown to differentiate to non-homologous somatic tissue in animal models or human subjects.

Autologous MSCs were first infused into humans in 1995 demonstrating the safety of cell infusions [9]. Allogeneic MSCs were first infused into patients in 2002; however, these patients had previously undergone bone marrow transplantation so that their immune system was syngeneic with the MSCs [10]. With the safety of MSCs clearly documented, many medical centers around the world are investigating these cells. There are currently two disorders in which MSCs, after intravenous infusion, seem to have a measurable therapeutic effect in humans: osteogenesis imperfecta [10] and graft vs. host disease [11,12]. MSCs have also been reported to reduce the risk of graft failure after haplo-identical transplant [13]. Additionally, preclinical animal models of MSC-based cell therapy for critical limb ischemia [14], acute myocardial infarction [15], neuronal disease and injury such as stroke [16], and autoimmune disorders [17] are quite promising.

Now, over 300 patients have received systemically infused MSCs for various indications, and many studies have assessed localization of the infused cells at the presumed site of activity. In all such cases, few if any MSCs were identified; thus, either very few cells can exert a substantial biologic effect or localization of the cells is not required for biologic activity.

Given that there is usually low or no evidence of engraftment yet often substantial effects, what molecular and cellular mechanism may account for the striking biologic activity? An emerging body of data suggests that soluble factors released by the MSCs are the key elements in the mechanism of action for most, if not all, of the systemic effects. MSCs have been shown to secrete a wide variety of lymphohematopoietic cytokines [18,19] as well as mediators of the central nervous system [20]. Collectively, the vast cytokine secretory potential and the lack of substantial MSC localization suggests that systemically infused MSCs exert a therapeutic effect through the release of cytokines that act on local, or perhaps distant, target tissues. Rather than serving as stem cells to regenerate tissues as originally proposed, they serve as cellular factories that secrete mediators to stimulate the repair of tissues or modulate the local environment to foster other beneficial effects.

There are two key implications to the idea that the principle mechanism of biologic activity after systemic infusion, in virtually all applications, is the secretion of soluble mediators. First, the tissue source of the MSCs may be critically important in determining biologic activity. MSCs have been isolated from many tissue sources and the gene expression seems to reflect the tissue from where the cells were isolated. These data suggest biologically relevant heterogeneity among MSCs in different tissues, possibly including varying cytokine expression profiles. It follows that different tissue sources may be especially well suited for specific clinical applications.

Second, isolation and culture expansion conditions, such as seeding density, culture media, serum supplementation, and extent of *ex vivo* expansion, may significantly impact gene expression, including cytokine expression. Moreover, three-dimensional bioreactors, in contrast to conventional plastic culture flasks, may generate MSCs with unique cytokine expression profiles. These observations suggest that the cell processing protocols can be modified to enhance or repress expression of specific genes in order to optimize the cytokine profile for a given clinical indication.

In review, after systemic infusion, secretion of soluble mediators seems to be the principal mechanism of MSC action for most, if not all, biological effects. We can surmise that unique tissue sources will be identified and novel processing protocols developed to specifically generate MSCs most suited for a given clinical indication. Future efforts to understand the cytokine expression profile will undoubtedly expand the range of MSC clinical applications.

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