

Cytokines as the Major Mechanism of MSC Clinical Activity: Expanding the Spectrum of Cell Therapy

Edwin M. Horwitz MD PhD¹ and William R. Prather RPh MD²

¹The Children's Hospital of Philadelphia and The University of Pennsylvania School of Medicine, Philadelphia, PA, USA

²Pluristem Therapeutics, Inc., Haifa, Israel

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 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.

IMAJ 2009;11:00-00

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

Mesenchymal 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

“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 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, systemic 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

MSCs = mesenchymal stem cells

ISCT = International Society for Cellular Therapy

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 a 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 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 pro-

cessing 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.

Correspondence:

Dr. E.M. Horwitz

The Children's Hospital of Philadelphia, Abramson Research Center, 1116D, 3615 Civic Center Boulevard, Philadelphia, PA 19104, USA

Phone: (1-215) 590-5476

Fax: (1-215) 590-3770

email: horwitz@email.chop.edu

References

1. Horwitz E, Le Blanc K, Dominici M, et al. Clarification of the nomenclature for MSC: The International Society for Cellular Therapy position statement. *Cytotherapy* 2005; 7: 393-5.
2. Dominici M, Le Blanc K, Mueller I, et al. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. *Cytotherapy* 2006; 8: 315-17.
3. Wagner W, Feldmann RE Jr., Seckinger A, et al. The heterogeneity of human mesenchymal stem cell preparations – evidence from simultaneous analysis of proteomes and transcriptomes. *Exp Hematol* 2006; 34: 536-48.
4. Friedenstein AJ, Petrakova KV, Kurolesova AI, Frolova GP. Heterotopic of bone marrow. Analysis of precursor cells for osteogenic and hematopoietic tissues. *Transplantation* 1968; 6: 230-47.
5. Owen M. Marrow stromal stem cells. *J Cell Sci Suppl* 1988; 10: 63-76.
6. Caplan AI. Mesenchymal stem cells. *J Orthop Res* 1991; 9: 641-50.
7. Caplan AI, Bruder SP. Mesenchymal stem cells: building blocks for molecular medicine in the 21st century. *Trends Mol Med* 2001; 7: 259-64.
8. Prockop DJ. Marrow stromal cells as stem cells for nonhematopoietic tissues. *Science* 1997; 276: 71-4.
9. Lazarus HM, Haynesworth SE, Gerson SL, Rosenthal NS, Caplan AI. Ex vivo expansion and subsequent infusion of human bone marrow-derived stromal progenitor cells (mesenchymal progenitor cells): implications for therapeutic use. *Bone Marrow Transplant* 1995; 16: 557-64.

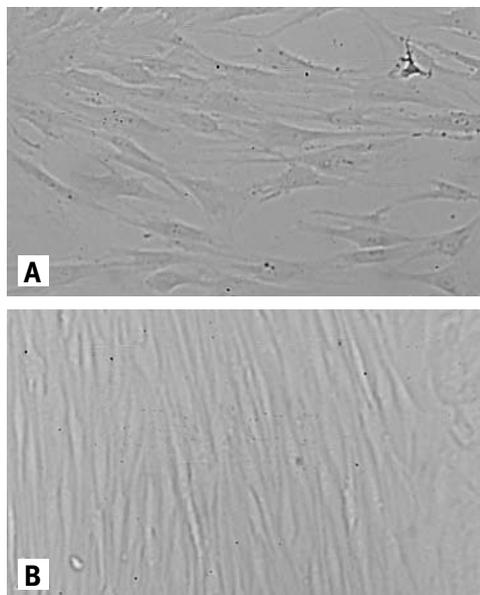


Figure 1. In vitro tissue culture of MSCs. Photomicrographs of human MSCs during ex vivo expansion [A] at 50% confluence and [B] near 100% confluence. Original magnification x 40.

10. Horwitz EM, Gordon PL, Koo WKK, et al. Isolated allogeneic bone marrow-derived mesenchymal cells engraft and stimulate growth in children with osteogenesis imperfecta: implications for cell therapy of bone. *Proc Natl Acad Sci USA* 2002; 99: 8932-7.
11. Le Blanc K, Rasmuson I, Sundberg B, et al. Treatment of severe acute graft-versus-host disease with third party haploidentical mesenchymal stem cells. *Lancet* 2004; 363: 1439-41.
12. Le Blanc K, Frassoni F, Ball L, et al. Mesenchymal stem cells for treatment of steroid-resistant, severe, acute graft-versus-host disease: a phase II study. *Lancet* 2008; 371: 1579-86.
13. Ball LM, Bernardo ME, Roelofs H, et al. Cotransplantation of ex vivo expanded mesenchymal stem cells accelerates lymphocyte recovery and may reduce the risk of graft failure in haploidentical hematopoietic stem-cell transplantation. *Blood* 2007; 110: 2764-7.
14. Prather WR, Toren A, Meiron M, et al. The role of placental-derived adherent stromal cells (PLX-PAD) in the treatment of critical limb ischemia. *Cytherapy* 2009. In press.
15. Zimmet JM, Hare JM. Emerging role for bone marrow derived mesenchymal stem cells in myocardial regenerative therapy. *Basic Res Cardiol* 2005; 100: 471-81.
16. Chopp M, Li Y. Treatment of neural injury with marrow stromal cells. *Lancet Neurol* 2002; 1: 92-100.
17. Zappia E, Casazza S, Pedemonte E, et al. Mesenchymal stem cells ameliorate experimental autoimmune encephalomyelitis inducing T-cell anergy. *Blood* 2005; 106: 1755-61.
18. Deans RJ, Moseley AB. Mesenchymal stem cells: biology and potential clinical uses. *Exp Hematol* 2000; 28: 875-84.
19. Honczarenko M, Le Y, Swierkowski M, Ghiran I, Glodek AM, Silberstein LE. Human bone marrow stromal cells express a distinct set of biologically functional chemokine receptors. *Stem Cells* 2006; 24: 1030-41.
20. Crigler L, Robey RC, Asawachaicharn A, Gaupp D, Phinney DG. Human mesenchymal stem cell subpopulations express a variety of neuro-regulatory molecules and promote neuronal cell survival and neuritegenesis. *Exp Neurol* 2006; 198: 54-64.