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Medical Therapies with Adult Stem/Progenitor Cells (MSCs): A Backward Journey From Dramatic Results In vivo to the Cellular and Molecular Explanations

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ABSTRACT

There is currently great interest in the use of mesenchymal stem/stromal cells (MSCs) for the therapy of many diseases of animals and humans. However, we are still left with the serious challenges in explaining the beneficial effects of the cells. Hence, it is essential to work backward from dramatic results obtained in vivo to the cellular and molecular explanations in order to discover the secrets of MSCs. This review will focus on recent data that have changed the paradigms for understanding the therapeutic potentials of MSCs. J. Cell. Biochem. 113: 1460–1469, 2012. © 2011 Wiley Periodicals, Inc.

KEY WORDS: ADULT STEM/PROGENITOR CELL; MESENCHYMAL STEM CELL; MULTIPOTENT MESENCHYMAL STROMAL CELL; STEM CELL

We have known for a long time that evolution has endowed the human body with a formidable series of systems to protect it against a hostile environment. A major challenge of medicine is to improve these systems. One of the most interesting strategies is to understand and harness the secrets of the cells that protect tissues from damage or enhance their repair. Hence, the tremendous excitement continues to be generated in exploring the therapeutic potentials of embryonic stem cells (ES cells), induced pluripotent stem cells (iPS cells), and similar cells from adult tissue that share some of the properties of ES cells. The excitement is readily measured by the observation that PubMed as of this writing cites 24,879 publications on ES cells, 3,119 on iPS cells, and 15,412 on adult stem/progenitor cells referred to as mesenchymal stem/stromal cells (MSCs). It is essential that research on all three kinds of cells continues as rapidly as possible, because each offers different opportunities for experimental exploration. ES and iPS cells provide the spectacular possibility of defining how the genome is programmed and re-programmed during development. They may be useful for some medical therapies in the future. However, their use in patients is severely limited by the fact that their genomes are unleashed and uncontrolled. As a result, they are immortal cells, and they cannot be used in patients without the serious danger of causing tumors and malignancies. There are at the moment no experimental or even theoretic strategies for overcoming this problem. Moreover, the technical barriers are daunting. The technologies for sequencing genomes are rapidly improving, but none have even the theoretical capacity of detecting the presence of a few hundred malignant cells in the large preparations required for most therapeutic applications. Also, tests for tumorigenicity in mice are notoriously insensitive. The potential dangers posed by the uncontrolled and unstable genomes of both ES and iPS cells were recently emphasized by a recent analysis of several lines of each [Laurent et al., 2011] that demonstrated the large number of mutations the cells had acquired. MSCs have more limited plasticity for differentiation than either ES or iPS cells. However, they have a limited lifespan in culture, and therefore their use in patients presents limited risks of tumorigenicity [Prockop et al., 2010a]. These and other features of MSCs have prompted a large number of clinical trials (see www.clinicaltrials.gov). Also, MSCs are a class of cells that normally serve as guardians of excessive responses by the body to tissue injury [Prockop and Oh, 2011]. Therefore, discovering their secrets can provide us with new strategies for improving the natural systems whereby the body can limit the destruction of tissues and initiate regeneration.

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Research to discover the secrets of MSCs has led scientists to a fascinating journey in which new data have repeatedly made them change the assumptions or paradigms on which they design their experiments. The review will focus on some recent data that have changed the paradigms.

THE UNUSUAL CHALLENGE OF MSCs: THE NEED TO WORK BACKWARD

Surprisingly, it has been difficult to achieve a consensus on naming the cells that are the subject of this review. They were originally called fibroblastic colony forming units, then mesenchymal stem cells, and more recently mesenchymal stromal cells (MSCs) [Friedenstein et al., 1976, Owen and Friedenstein, 1988; Caplan, 1991; Prockop, 1997; Pittenger et al., 1999; Dominici et al., 2006; Prockop et al., 2010b; Bianco et al., 2011]. Each of the names reflects a different property or apparent function of the cells, and none has satisfied all investigators in the field. Regardless of the name assigned to the cells, research in the field has faced a major challenge: Dramatic results have been observed following administration of the cells in animal models for multiple diseases and a few patients. But we are left with the serious challenge in trying to explain the beneficial effects of the cells. In effect, we have had to work backward from dramatic in vivo results to the cellular and molecular explanations.

THE ATTRACTIVE PROPERTIES OF THE CELL

MSCs captured the imagination of many scientists soon after they were discovered by Friedenstein et al. [1976] over 40 years ago. Their attractive features were that they were readily isolated from bone marrow by their adherence to tissue culture surfaces, they rapidly expanded in culture, they were highly clonogenic in that they efficiently generated single-cell derived colonies, and they were readily seen to differentiate in culture or in vivo into several cellular phenotypes such as mineralizing cells, adipocytes, and chondrocytes. These remarkable properties are retained as the cells were expanded through 20 or so population doublings, particularly if the cells were plated at low density and passed before they reach confluency (Fig. 1) [Gregory et al., 2005]. As the cells expand in culture, they begin to become more differentiated. However, remarkably, if sub-confluent cultures are re-plated at low density after the first three or four passages, they re-program their genomes to their initial state, and they again generate single-cell derived colonies. For reasons that are not apparent, the single-cell generated colonies vary in size, confluency, and their potential for differentiation. These features suggested that MSCs created their own ''niches'' as they formed colonies. The suggestion was supported by experiments demonstrating that the cells in the inner regions of single-cell derived colonies expressed markedly different patterns of genes from the cells in the outer regions, but that on re-plating, the cells from each region generated single-cell derived colonies with similar different inner and outer regions [Ylöstalo et al., 2008]. The plasticity of MSCs was further illustrated by experiments in which MSCs were cultured without fetal calf serum [Pochampally et al., 2004] or, even more dramatically, when the MSCs were subjected to environmental stress in culture to generate multi-lineage-differentiating stress-enduring MSCs or Muse cells [Wakao et al., 2011]. Under such circumstances, the MSCs reverted to a more primitive phenotype and expressed genes characteristic of embryonic genes.

EVOLVING PARADIGMS IN RESEARCH ON MSCs

The multi-faceted features of MSCs prompted dramatic shifts in the hypotheses or paradigms for the research as studies on the cells progressed [Prockop et al., 2010b]. Initially, the cells were explored as feeder layers that provided a niche for culture of hematopoietic cells (Paradigm I). The paradigm was supported by recent studies that demonstrated that MSCs provide a vital link between the sympathetic innervation of bone that regulates release of hematopoietic precursors in the circulation (Méndez-Ferrer et al., 2010). Subsequently, the cells were explored as reparative cells that can engraft in injured tissues and differentiate to replace damaged cells (Paradigm II). Engraftment and differentiation was observed in rapidly grown embryos with extreme tissue injury, or after local administrations of large concentrations of the cells. One of the clearest demonstrations of engraftment and differentiation was observed [Koga et al., 2008] by injection of MSCs obtained from bone marrow or synovial membranes in the knees of rabbits with surgically induced defects in cartilage. More recent observations demonstrate that under many conditions the cells only transiently appear in injured tissues, but during their brief appearance they respond to cross-talk with injured cells to limit tissue destruction or enhance repair by a variety of mechanisms (Paradigm III). The mechanisms include (a) providing a niche to enhance proliferation and differentiation of tissue-endogenous stem/progenitor cells (as in Paradigm I); (b) up-regulation of genes that modulate excessive immune reactions; (c) transfer of vesicular components that contain mitochondria and microRNAs; and (d) up-regulation of genes that modulate inflammation.

A NICHE FUNCTION OF MSCs IN TISSUE REPAIR

A niche function for MSCs in the central nervous system was illustrated through experiments in which human MSCs (hMSCs) were injected directly into the hippocampus of immune-deficient rats [Munoz et al., 2005]. Contrary to initial expectations, the hMSCs engrafted only briefly. Instead, the cells enhanced proliferation of the endogenous neural stem cells found in the hippocampus. In addition, the neural stem cells increased their migration and their differentiation into neural cells.

A niche function for MSCs was also illustrated by a series of experiments with cancer cells in culture and in vivo [Kidd et al., 2009]. Some of the observations suggested that the MSCs inhibited growth of the cancer cells. Others indicated they enhanced propagation and even metastases of cancer cells [Karnoub et al., 2007]. The discrepancy not been resolved but may be explained by the heterogeneity of cancers. They may also be explained by

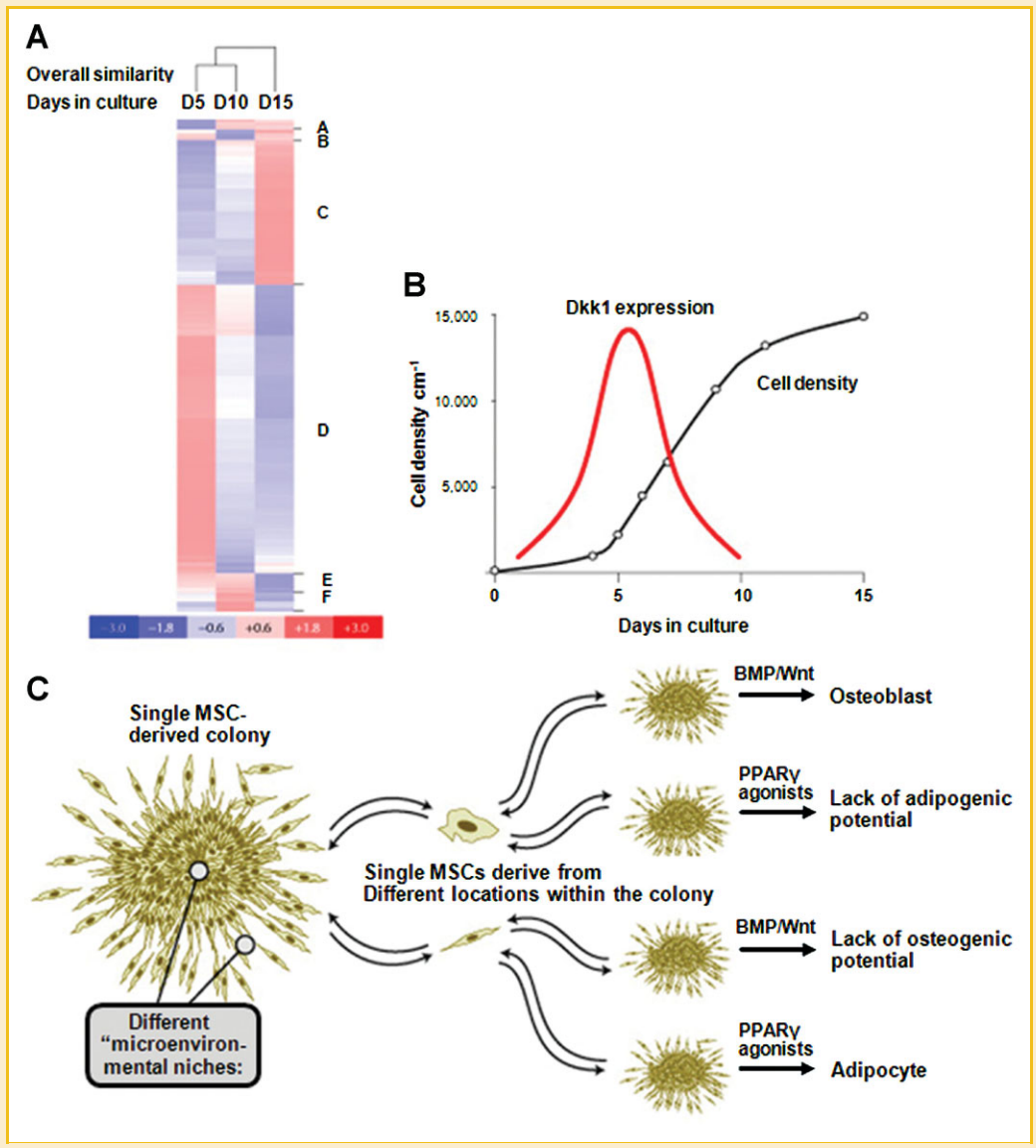

Fig. 1. Summary of some of the unusual properties of MSCs in culture. A: Heat map representation of Affymetrix microarray analysis of MSCs in culture at the log phase (at day 5, column D5), late log phase (at day 10, column D10), and stationary phase of growth (at day 15, column D15). Only the most differentially expressed genes between the conditions are represented, and they appear on the heat map as single-colored segments arranged within each column. The color coding represents gene expression, where red is the highest amount of expression and blue is the lowest. Red segments represent gene expression that is three standard deviations over the mean of the three conditions (D5, D10, and D15), whereas blue segments represent gene expression that is three standard deviations under the mean of the three conditions. White segments indicate that the gene expression values are equal to the mean value of the three conditions, and lighter shades of red or blue represent less than three standard deviations above or below the mean, depending on the color intensity (see legend below map). Log-phase MSCs express the greatest number of cell cycle and dedifferentiation-related genes (cluster labeled D), whereas more confluent, stationary-phase MSCs express genes related to conditioning the microenvironment, such as proteins of the extracellular matrix, cell surface adhesion molecules, and developmentally related genes (clusters labeled A–C). B: The Wnt-inhibitor Dkk-1 is transiently expressed during the rapidly proliferating phase of MSC growth, probably to inhibit inappropriate differentiation. C: A model to explain conditioning of MSCs by "microenvironmental niches" in vitro. MSCs are exposed to different cell densities within a single cell–derived colony (left). The heritable conditioning that occurs depends on the position of a given cell within the colony. When transferred to a new culture (center), the clonally derived MSCs behave differently in response to a given cocktail of soluble factors because of their microenvironmental preconditioning (right). The example here is a representation of a study assaying differentiation into osteoblasts and adipocytes, but the phenomenon is likely to apply to differentiation into other tissues as well. PPARγ, peroxide proliferator-activated receptorγ. Reprinted with permission from the American Association for the Advancement of Science [Gregory et al., 2005].

heterogeneity of different sources and protocols for preparing MSCs. In fact, MSCs from mice have proven difficult to deal with because initial cultures are heavily contaminated by hematopoietic cells and the cells become transformed and tumorigenic as they are expanded [Tolar et al., 2007] much like mouse fibroblasts [Rubin, 2001]. The hMSCs are more readily isolated and expanded, but properties vary with culture conditions, such as cell density and passage number. Unfortunately, many investigators have prepared hMSCs under different conditions, and therefore their data are difficult to compare to results obtained by other investigators.

THE IMMUNE MODULATORY EFFECTS OF MSCs

Some of the most intriguing features of MSCs are their immune modulatory effects. These were first discovered in clinical trials to improve bone marrow transplants with MSCs: In a few patients, the MSCs improved the manifestations of graft-versus-host disease (GVHD) [Le Blanc et al., 2004]. The observations were supported by reports that MSCs inhibited the mixed lymphocyte reaction. These observations in turn prompted experiments that demonstrated intravenous (IV) infusions of MSCs reduced neurological deficits in the experimental autoimmune encephalitis (EAE) model for multiple sclerosis [Uccelli et al., 2008]. Extensive efforts have been made to explain the immune modulatory effects of MSCs, but the field remains controversial and several different scenarios have been advanced by leaders in the field. Some observations suggest that the immune modulatory effects may be explained by the MSCs secreting one or more of a variety of factors that include inducible nitric oxide synthase (iNOS), indoleamine dioxygenase (IDO), chemokine (C–C motif) ligand 2 (CCL2), and prostaglandin E_2 (PGE_2). Other observations suggest that the effects may require cell-to-cell contact with dendritic cells or other components of the immune system. [For more complete reviews, see Uccelli et al., 2008; Ren et al., 2008; Romieu-Mourez et al., 2009; Bernardo et al., 2009; Ben-Ami et al., 2011].

RESCUE OF INJURED TISSUES BY TRANSFER OF MITOCHONDRIA AND MICROVESICLES

In the course of carrying out co-culture experiments, we made the unexpected observation that MSCs can rescue cells with non-functional mitochondria by the transfer of either mitochondria or mitochondrial DNA [Spees et al., 2006]. The observation had broad implications for the therapeutic potentials of MSCs because failure of mitochondria is an initial event in many diseases, particularly with ischemia and reperfusion of tissues. The mitochondria are damaged by the ischemia and then fail to provide adequate electrons to reduce oxygen when the tissue is re-perfused. The result is generation of highly destructive reactive oxygen species (ROS). The transfer of mitochondria we observed, therefore, provided a rationale for use of MSCs as therapy for stroke, myocardial infarction, and other diseases. The observations we made, however, were all in tissue culture, and we were unable to devise an adequate experiment to prove transfer of mitochondria in vivo. Fortunately, this problem has recently been addressed with an ingenious series of observations on relatively benign genital tumors of dogs that were transmitted as allografts over many generations [Rebbeck et al., 2011]. Sequencing of two informative regions in mitochondria in 37 samples of the tumors in dogs from four continents indicated extensive capture of host mitochondrial DNA in most of the samples. The results do not conclusively establish that functional mitochondria were transferred, but they do establish the transfer of mitochondrial DNA.

Recently, there have been multiple reports that MSCs engage in extensive exchange of vesicles containing micro-RNAs during differentiation of the cells [Crobu et al., 2011; Zhang et al., 2011] and in their interaction with malignant cells [Gregory et al., 2011]. [For recent review, see Guo et al., 2011].

MSCs AS GUARDIANS OF INFLAMMATION

Recent reports demonstrated that MSCs can play a role as modulators or guardians of excessive inflammatory responses. Excessive or non-resolving inflammation is now recognized to make a major contribution to the damage caused by diseases such as obesity, diabetes, myocardial infarction, stroke, parkinsonism, and Alzheimer's disease [Nathan and Ding, 2010; Chen and Nuñez, 2010]. The systems for modulating inflammation include small molecules such as prostaglandins, lipoxins, protectins, and resolvins [Serhan et al., 2008]. They also include cells that serve as guardians of excessive inflammation such as alternatively-activated M2 macrophages [Gordon and Martinez, 2010] and regulatory T cells [Izcue et al., 2009; Wan, 2010]. Several recent reports have demonstrated that MSCs also can participate in the modulation of excessive inflammation.

Experiments in a model of bleomycin-induced lung injury indicated that IV administration of MSCs decreased inflammation by being activated to secrete interleukin-1 receptor antagonist (IL-1ra) [Ortiz et al., 2003, 2007]. Experiments with LPS-induced peritonitis in mice [Yagi et al., 2010] indicated that the anti-inflammatory effects were explained by the MSCs secreting a soluble receptor 1 for tumor necrosis factor (sTNFR1). Experiments in which hMSCs were infused into mice with induced myocardial infarcts explained the beneficial effects by a sequence in which the MSCs were trapped in the lung as micro-emboli [Lee et al., 2009]. As a result, the cells were activated by signals from the injured heart to synthesize and secrete the anti-inflammatory protein TSG-6 [Wisniewski and Vilcek, 2004; Milner et al., 2007]. The TSG-6 then suppressed the excessive inflammatory response to ischemia of the heart and thereby decreased the damage to cardiomyocytes by proteases released by neutrophils and macrophages. As a result, there was an improvement in cardiac function and a decrease in scarring of the left ventricle of the heart (Fig. 2).

Secretion of TSG-6 by MSCs was also demonstrated in a model of sterile injury to the cornea in rats. The corneas were injured by brief exposure to alcohol followed by mechanical scraping that removed the epithelium of the cornea and the stem cells found in the limbus. IV infused hMSCs markedly decreased neutrophil infiltration, production of pro-inflammatory cytokines, and development of the opacity in the cornea [Roddy et al., 2011]. Intraperitoneal (IP) infusion of the hMSCs was also effective in suppressing inflammation and preventing the opacity in the cornea. However, the hMSCs with an siRNA knockdown of the TSG-6 gene were not effective. A quantitative assay for human mRNA for GAPDH demonstrated that less than 10 human MSCs were present in the corneas of rats 1 day and 3 days after IV or IP administration of 1×10^7 hMSCs. Also, the beneficial effects of hMSCs were largely duplicated by IV administration of recombinant human (rh) TSG-6. Therefore, the data demonstrated that systemically administered hMSCs reduced inflammatory damage to the cornea without engraftment in the tissue and that the anti-inflammatory effects of the cells were probably explained by their secretion of TSG-6 into the circulation. A related series of experiments demonstrated that direct injection of rhTSG-6 into the anterior chamber of the rat eye also decreased excessive inflammation in injured cornea [Oh et al., 2010]. The anti-inflammatory effects of the rhTSG-6 were dose-dependent (Fig. 3).

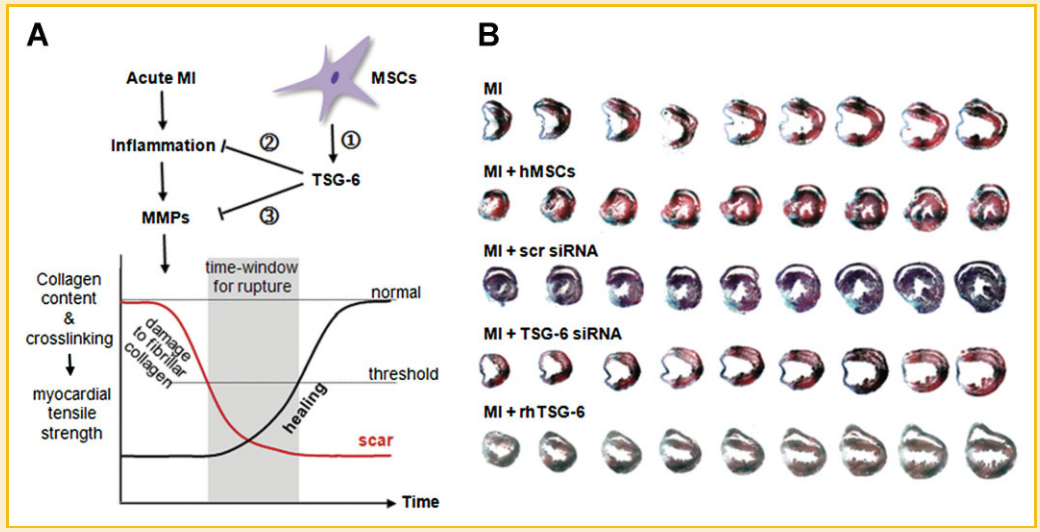

Fig. 2. Anti-inflammatory effects of hMSCs activated to secrete TSG-6 in a mouse model of myocardial infarction. A: Schematic diagram. (1) Human MSCs (hMSCs) injected intravenously were trapped in the lungs and activated to secrete TSG-6 (TNF-α stimulated gene/protein 6). (2) The TSG-6 decreased the normal but excessive inflammatory response that damages the heart. (3) The TSG-6 probably further decreased proteolytic damage to the heart by inhibiting matrix metalloproteinases (MMPs). B: Selected sections through heart. Each heart was cut from apex to base into over 400 sequential 5 μm sections. Every twentieth section is shown. Either hMSCs or hMSCs transduced with the scrambled siRNA (scr siRNA) decreased the size of myocardial infarction examined 3 weeks later. However, hMSCs with a siRNA knockdown of the TSG-6 gene (TSG-6 siRNA) had no effect on infarct size. Intravenous infusion of 100 μg of recombinant human (rh) TSG-6 immediately following the surgery and at 24 h also decreased infarct size. Panel (a) reproduced with modifications and with permission from Elsevier [Fang et al., 2007]. Panel (b) reprinted with permission from Elsevier [Lee et al., 2009].

The suppression of excessive inflammation in the early phase of injury subsequently led to a marked decrease in development of blinding opacity and neovascularization of the cornea at day 21 after injury.

A novel mechanism for the anti-inflammatory effects of MSCs and TSG-6 was demonstrated with experiments in a mouse model for zymosan-induced peritonitis [Choi et al., 2011]. The effects centered on their interaction with resident macrophages, the sentinel cell for inflammatory responses in most tissues [Eigenbrod et al., 2008; Chen and Nuñez, 2010; Rock et al., 2010]. The TSG-6 decreased activation of NF-κB in the resident macrophages by a direct binding to or through a hyaluronan-mediated binding to CD44. The binding to CD44 caused dissociation from TLR2. As a result, there was a decrease in zymosan-TLR2 activation of NF-κB. The overall effect was that the hMSCs introduced a negative feedback loop into the inflammatory response (Fig. 4) in which MSCs and TSG-6 suppressed the initial production of pro-inflammatory cytokines from zymosan-activated macrophages. They thereby inhibited the amplification of the pro-inflammatory signals by mesothelial cells that produce high levels of IL-6 and CXCL1 to recruit neutrophils [Choi et al., 2011]. The negative feedback loop introduced by MSCs and TSG-6 on the TLR2/NF-κB pathway in macrophages may account for the beneficial effects of MSCs in other disease models in which excessive inflammatory responses contribute to tissue damage. However, extensive previous reports demonstrated that TSG-6 had several other anti-inflammatory actions, including binding to pro-inflammatory fragments of hyaluronan, interacting with inter-α-inhibitor to increase its inhibition of the protease cascade released by inflammation, and inhibiting neutrophil migration [Wisniewski and Vilcek, 2004; Milner et al., 2007; Mahoney et al., 2008]. The modulation of NF-κB signaling in macrophages by TSG-6 may precede in time its other anti-inflammatory actions.

A different explanation for the anti-inflammatory effects of MSCs was demonstrated in a mouse model of sepsis produced by cecal ligation and puncture [Németh et al., 2009]. A series of in vivo and in vitro experiments demonstrated a complex series of events (Fig. 5): (a) Toxins released by the sepsis produced TLR4 and TNFR-1-mediated activation of NF-κB in the mouse MSCs that were trapped in the lung after IV infusion; (b) the activation of NF-κB signaling increased expression of cyclooxygenase-2 and thereby increased secretion of PGE_2; (c) PGE_2 bound to EP2 and EP4 receptors on macrophages and changed macrophages to the alternatively activated phenotype that secretes IL-10; (d) the IL-10 produced by host macrophages reduced inflammation in mice with sepsis. IL-10 is an essential component of a negative feedback loop in inflammation, since it typically inhibits the response that initiated its own production and acts on macrophages and other cells that produce inflammatory mediators [Medzhitov, 2010]. The observations by Németh et al. [2009] suggested, therefore, that MSCs can create another negative feedback loop of inflammation, one that apparently does not involve TSG-6. A subsequent publication on the same model of cecal ligation and puncture [Mei et al., 2010] did not find evidence for alternative activation of macrophages by MSCs but the suggestion was supported by three reports in different experimental systems [Kim and Hematti, 2009; Maggini et al., 2010; Zhang et al., 2010].

Therefore, MSCs can suppress inflammation by a variety of mechanisms (Fig. 6).

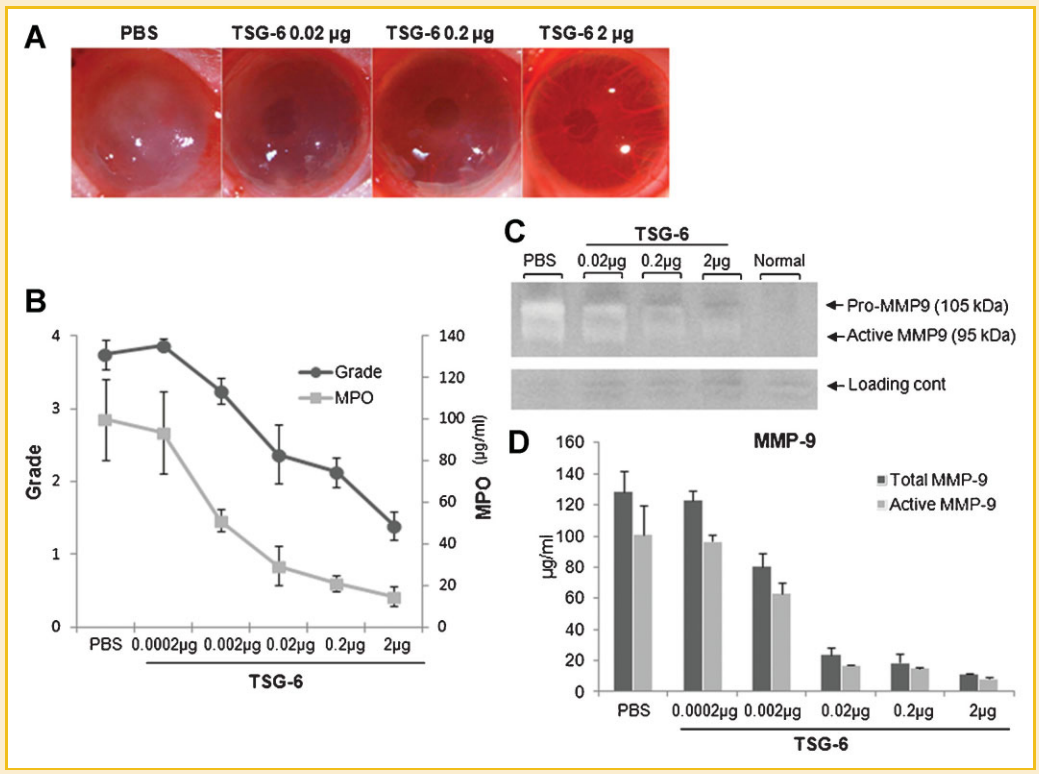

Fig. 3. Dose-dependent effects of TSG-6 in reducing corneal inflammation and opacity. Sterile inflammation was produced in corneas of Lewis rats by brief exposure to 100% ethanol followed by mechanical debridement of the cornea and limbal epithelium that removed the stem cells located in the limbus. A: Representative corneal photographs on day 3 post-injury demonstrated that TSG-6 suppressed development of corneal opacity after chemical injury in a dose-dependent manner. B: The anti-inflammatory effects of TSG-6 were dose-dependent as reflected in clinical grade of corneal opacity and myeloperoxidase (MPO) concentration as a semi-quantitative assay of neutrophil infiltration. Values are mean ± SD; n = 3 for each group. C: Gelatin zymography of corneas for pro-MMP-9 and active MMP-9. D: Total and active MMP-9 concentration in the cornea as assayed by ELISA. Values are mean + SD; n = 5 for each group. Significant improvements were observed with dose of 0.002 μg but maximal effects were obtained with 2 μg. Reprinted with permission from National Academy of Sciences, USA [Oh et al., 2010].

MSCs AS MODULATORS OF APOPTOSIS

In an effort to identify additional factors that might explain the therapeutic benefits of MSCs, we co-cultured MSCs with previously UV irradiated fibroblasts in a transwell system [Block et al., 2009]. The MSCs reduced apoptosis of the irradiated cells. Comparative microarray analysis of MSCs grown in the presence or absence of UV irradiated fibroblasts demonstrated that the MSCs were activated by the apoptotic cells to increase synthesis and secretion of

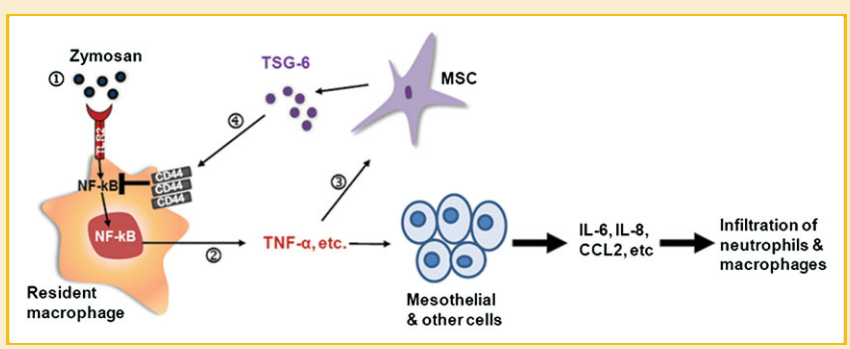

Fig. 4. The anti-inflammatory effects of hMSCs and TSG-6 in a mouse model of zymosan-induced peritonitis. (1) Zymosan activated NF-κB signaling in resident macrophages via toll-like receptor 2 (TLR2). (2) Activation of the NF-κB signaling pathway increased the production of pro-inflammatory cytokines to initiate the cascade of pro-inflammatory cytokines that was amplified by mesothelial cells and other cells of the peritoneum. (3) The pro-inflammatory cytokines also activated the hMSCs to secrete TSG-6. (4) TSG-6 decreased TLR2/NF-κB signaling in the resident macrophages through a direct interaction with CD44 or in a complex with hyaluronan. The amplification of the pro-inflammatory signals by mesothelial cells to recruit neutrophils was modulated by a negative feedback loop introduced by hMSCs and TSG-6. Reprinted with permission from the American Society of Hematology [Choi et al., 2011].

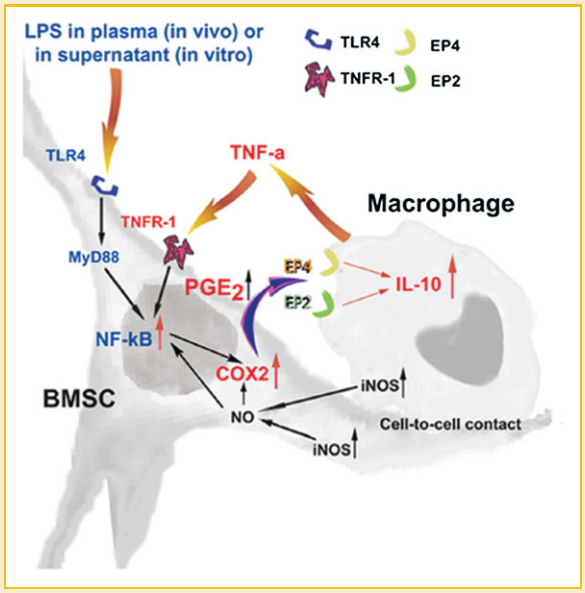

Fig. 5. Schematic for the anti-inflammatory effects of MSCs based on observations in a mouse model for sepsis. Bacterial toxins such as LPS and circulating TNF-α acted on the TLR4 and TNF receptor-1 (TNFR-1) of MSCs to activate the NF-κB signaling. Activation of NF-κB signaling up-regulated expression of cyclooxygenase 2 (COX2) and the COX2 increased synthesis of prostaglandin E2 (PGE2). PGE2 was secreted and bound to EP2 and EP4 receptors on macrophages. The PGE2 thereby increased IL-10 secretion by macrophages to reduce the inflammatory response. Reprinted with permission from Macmillan Publishers Ltd [Németh et al., 2009].

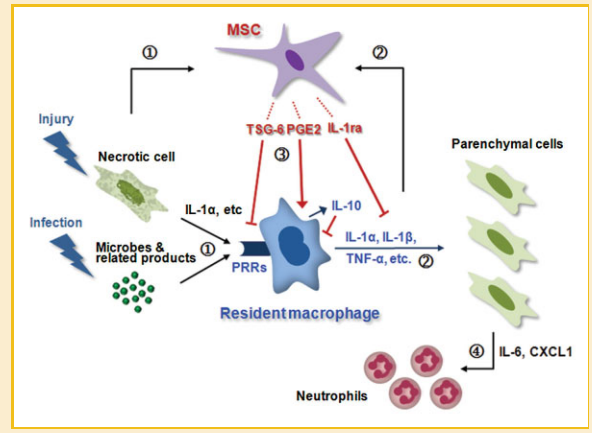

Fig. 6. Summary of some of the anti-inflammatory effects of MSCs. (1) Damage-associated molecular patterns (DAMPs) and IL-1α released by sterile injury or pathogen-associated molecular patterns (PAMPs) released by infectious injury to tissues activate resident macrophages through receptors involving pattern recognition receptors (PRRs). (2) The activated macrophages produce pro-inflammatory cytokines such as IL-1α, IL-1β, or TNF-α to initiate the inflammatory cascade. (3) Simultaneously, the pro-inflammatory cytokines and probably other signals from injured cells activate MSCs to secrete anti-inflammatory factors that include TSG-6, PGE2, and IL-1ra that either modulate the activation of the resident macrophages or decrease the downstream effects of the pro-inflammatory cytokines. (4) The net effect is to decrease the amplification of the pro-inflammatory signals from resident macrophages by parenchymal cells through the secretion of IL-6, CXCL1, and related factors and to decrease the recruitment of neutrophils. Reprinted with permission from Macmillan Publishers Ltd [Prockop and Oh, 2011].

stanniocalcin-1 (STC-1). STC1 is a secreted protein that exerts pleiotropic effects including alteration of mitochondrial function by up-regulation of uncoupling protein 2 (UCP2) [Ellard et al., 2007; Wang et al., 2009]. The uncoupling of oxidative phosphorylation increases the flow of electrons to reduce ROS, and thereby reduces apoptosis.

THERAPEUTIC IMPLICATIONS OF THE FACTORS SECRETED BY MSCs

The observations that many of the anti-inflammatory effects of MSCs can be reproduced by soluble factors produced by the cells raise an obvious question: Can therapies with the factors replace therapies with MSCs? Several of the factors are not attractive candidates. For example, therapy with recombinant IL-1ra has been introduced into clinical trials. It appears to have limited applications because improvements were seen in a small number of patients with gout [So et al., 2007], but there was no benefit in patients with osteoarthritis and rheumatoid arthritis [Gabay et al., 2010]. Other factors produced by MSCs, such as nitric oxide, IDO, or PGE2 are not promising candidates because they have short half-lives or they have adverse effects when administered systemically. However, there appear to be adequate reasons for testing the protein TSG-6 for therapeutic uses. The protein was previously shown to have multiple anti-inflammatory effects in addition to its modulation of NF-κB signaling in macrophages [Choi et al., 2011], without any apparent toxic effects, and even in transgenic mice over-expressing the gene [Wisniewski and Vilcek, 2004; Milner et al., 2007].

A similar question is raised by the observation that MSCs can be activated by signals from apoptotic cells to secrete STC-1. Since low levels of ROS are pro-inflammatory and high levels are pro-apoptotic, STC-1 may be both anti-inflammatory and anti-apoptotic.

Further examination of the beneficial effects of MSCs in disease models may identify additional factors that can be used therapeutically.

SUMMARY

The intensive interest in MSCs is in part driven by the unusual biological features of the cells that appear to place them somewhere on a continuum between ES cells and fully differentiated cells. Recent observations have emphasized that one of the most striking features of MSCs is their broad range of responses to different micro-environments, especially the micro-environments of injured tissues. The interest is also driven by the dramatic beneficial effects that the cells produce when administered to animal models of diseases. Because MSCs have demonstrated few if any adverse effects, they are likely to continue to be the most widely used cells for new clinical trials in patients.

Research on the therapeutic potentials was originally driven by the paradigm (Paradigm II) that the cells would home to and replace

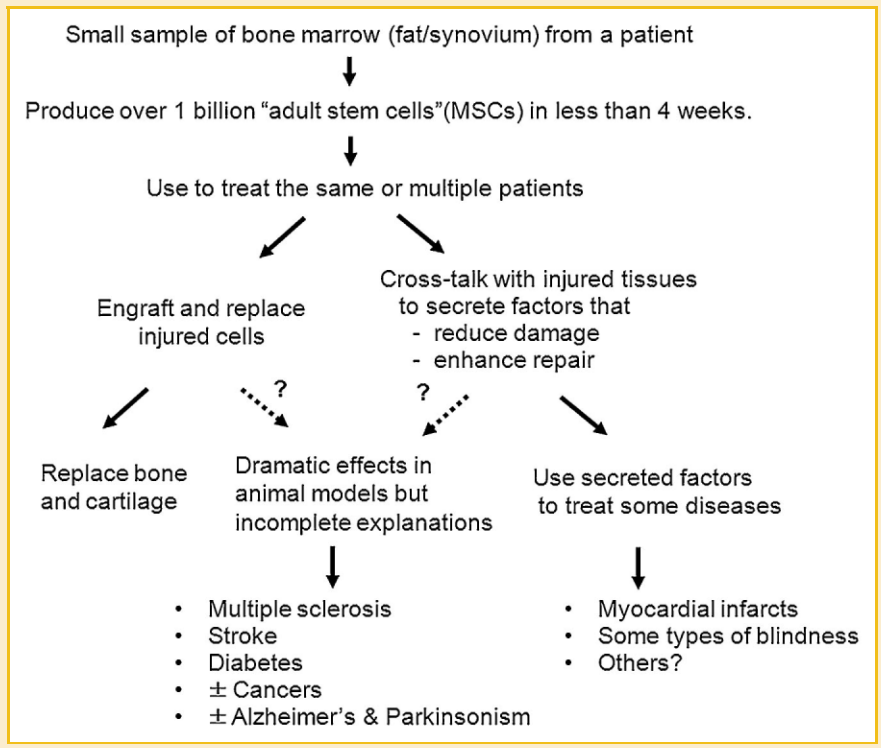

Fig. 7. Schematic representation of how paradigms for therapies with MSCs have evolved.

the cells in injured tissues (Fig. 7). The recent data demonstrate that many of the therapeutic benefits can be ascribed to cross-talk with injured tissues that activates the cells to produce a variety of soluble factors that modulate inflammation and immune responses. However, administration of MSCs has produced beneficial effects in a wide range of disease models, and there are as yet not adequate explanations for many of the beneficial effects. The disease models in which MSCs have produced beneficial effects include diabetes, stroke, spinal cord injury, Parkinsonism, Alzheimer's disease, liver disease, kidney disease, and some cancers. We are still at the beginning of the journey of discovering the secrets whereby MSCs produce their beneficial effects in all these conditions. And the secrets are almost certainly going to provide us with a deeper understanding of the biology and with new strategies for therapies for a long list of devastating diseases.

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REFERENCES

Ben-Ami E, Berrih-Aknin S, Miller A. 2011. Mesenchymal stem cells as an immunomodulatory therapeutic strategy for autoimmune diseases. Autoimmun Rev 10:410–415.

Bernardo ME, Locatelli F, Fibbe WE. 2009. Mesenchymal stromal cells. Ann NY Acad Sci 1176:101–117.

Bianco P, Sacchetti B, Riminucci M. 2011. Osteoprogenitors and the hematopoietic microenvironment. Best Pract Res Clin Haematol 24:37–47.

Block GJ, Ohkouchi S, Fung F, Frenkel J, Gregory C, Pochampally R, DiMattia G, Sullivan DE, Prockop DJ. 2009. Multipotent stromal cells are activated to reduce apoptosis in part by upregulation and secretion of stanniocalcin-1. Stem Cells 27:670–681.

Caplan AI. 1991. Mesenchymal stem cells. J Orthop Res 9:641–650.

Chen GY, Nuñez G. 2010. Sterile inflammation: Sensing and reacting to damage. Nat Rev Immunol 10:826–837.

Choi H, Lee RH, Bazhanov N, Oh JY, Prockop DJ. 2011. Anti-inflammatory protein TSG-6 secreted by activated MSCs attenuates zymosan-induced mouse peritonitis by decreasing TLR2/NF-κB signaling in resident macrophages. Blood 118:330–338.

Crobu F, Latini V, Marongiu MF, Sogos V, Scintu F, Porcu S, Casu C, Badiali M, Sanna A, Manchinu MF, Ristaldi MS. 2011. Differentiation of single cell derived human mesenchymal stem cells into cells with a neuronal phenotype: RNA and microRNA expression profile. Mol Biol Rep Epub ahead of print.

Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini F, Krause D, Deans R, Keating A, Prockop DJ, Horwitz E. 2006. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy 8:315–317.

Eigenbrod T, Park JH, Harder J, Iwakura Y, Nuñez G. 2008. Cutting edge: Critical role for mesothelial cells in necrosis-induced inflammation through the recognition of IL-1 alpha released from dying cells. J Immunol 181:8194–8198.

Ellard JP, McCudden CR, Tanega C, James KA, Ratkovic S, Staples JF, Wagner GF. 2007. The respiratory effects of stanniocalcin-1 (STC-1) on intact mitochondria and cells: STC-1 uncouples oxidative phosphorylation and

its actions are modulated by nucleotide triphosphates. Mol Cell Endocrinol 264:90–101.

Fang L, Gao XM, Moore XL, Kiriazis H, Su Y, Ming Z, Lim YL, Dart AM, Du XJ. 2007. Differences in inflammation, MMP activation and collagen damage account for gender difference in murine cardiac rupture following myocardial infarction. J Mol Cell Cardiol 43:535–544.

Friedenstein AJ, Gorskaja JF, Kulagina NN. 1976. Fibroblast precursors in normal and irradiated mouse hematopoietic organs. Exp Hematol 4: 267–274.

Gabay C, Lamacchia C, Palmer G. 2010. IL-1 pathways in inflammation and human diseases. Nat Rev Rheumatol 6:232–241.

Gordon S, Martinez FO. 2010. Alternative activation of macrophages: Mechanism and functions. Immunity 32:593–604.

Gregory CA, Ylostalo J, Prockop DJ. 2005. Adult bone marrow stem/progenitor cells (MSCs) are preconditioned by microenvironmental "niches" in culture: A two-stage hypothesis for regulation of MSC fate. Sci STKE 294:pe37.

Gregory LA, Ricart RA, Patel SA, Lim PK, Rameshwar P. 2011. MicroRNAs, Gap junctional intercellular communication and mesenchymal stem cells in breast cancer metastasis. Curr Cancer Ther Rev 7:176–183.

Guo L, Zhao RC, Wu Y. 2011. The role of microRNAs in self-renewal and differentiation of mesenchymal stem cells. Exp Hematol 39:608–616.

Izcue A, Coombes JL, Powrie F. 2009. Regulatory lymphocytes and intestinal inflammation. Annu Rev Immunol 27:313–338.

Karnoub AE, Dash AB, Vo AP, Sullivan A, Brooks MW, Bell GW, Richardson AL, Polyak K, Tubo R, Weinberg RA. 2007. Mesenchymal stem cells within tumour stroma promote breast cancer metastasis. Nature 449:557–563.

Kidd S, Spaeth E, Dembinski JL, Dietrich M, Watson K, Klopp A, Battula VL, Weil M, Andreeff M, Marini FC. 2009. Direct evidence of mesenchymal stem cell tropism for tumor and wounding microenvironments using in vivo bioluminescent imaging. Stem Cells 27:2614–2623.

Kim J, Hematti P. 2009. Mesenchymal stem cell-educated macrophages: A novel type of alternatively activated macrophages. Exp Hematol 37:1445–1453.

Koga H, Muneta T, Nagase T, Nimura A, Ju YJ, Mochizuki T, Sekiya I. 2008. Comparison of mesenchymal tissues-derived stem cells for in vivo chondrogenesis: Suitable conditions for cell therapy of cartilage defects in rabbit. Cell Tissue Res 33:207–215.

Laurent LC, Ulitsky I, Slavin I, Tran H, Schork A, Morey R, Lynch C, Harness JV, Lee S, Barrero MJ, Ku S, Martynova M, Semechkin R, Galat V, Gottesfeld J, Izpisua Belmonte JC, Murry C, Keirstead HS, Park HS, Schmidt U, Laslett AL, Muller FJ, Nievergelt CM, Shamir R, Loring JF. 2011. Dynamic changes in the copy number of pluripotency and cell proliferation genes in human ESCs and iPSCs during reprogramming and time in culture. Cell Stem Cell 8:106–118.

Le Blanc K, Rasmusson I, Sundberg B, Götherström C, Hassan M, Uzunel M, Ringdén O. 2004. Treatment of severe acute graft-versus-host disease with third party haploidentical mesenchymal stem cells. Lancet 363: 1439–1441.

Lee RH, Pulin AA, Seo MJ, Kota DJ, Ylostalo J, Larson BL, Semprun-Prieto L, Delafontaine P, Prockop DJ. 2009. Intravenous hMSCs improve myocardial infarction in mice because cells embolized in lung are activated to secrete the anti-inflammatory protein TSG-6. Cell Stem Cell 5:54–63.

Maggini J, Mirkin G, Bognanni I, Holmberg J, Piazzón IM, Nepomnaschy I, Costa H, Cañones C, Raiden S, Vermeulen M, Geffner JR. 2010. Mouse bone marrow-derived mesenchymal stromal cells turn activated macrophages into a regulatory-like profile. PLoS One 5:e9252.

Mahoney DJ, Mikecz K, Ali T, Mabilleau G, Benayahu D, Plaas A, Milner CM, Day AJ, Sabokbar A. 2008. TSG-6 regulates bone remodeling through inhibition of osteoblastogenesis and osteoclast activation. J Biol Chem 283:25952–25962.

Medzhitov R. 2010. Innate immunity: Quo vadis? Nat Immunol 11:551–553.

Mei SH, Haitsma JJ, Dos Santos CC, Deng Y, Lai PF, Slutsky AS, Liles WC, Stewart DJ. 2010. Mesenchymal stem cells reduce inflammation while enhancing bacterial clearance and improving survival in sepsis. Am J Respir Crit Care Med 182:1047–1057.

Méndez-Ferrer S, Michurina TV, Ferraro F, Mazloom AR, Macarthur BD, Lira SA, Scadden DT, Ma'ayan A, Enikolopov GN, Frenette PS. 2010. Mesenchymal and haematopoietic stem cells form a unique bone marrow niche. Nature 466:829–834.

Milner CM, Tongsoongnoen W, Rugg MS, Day AJ. 2007. The molecular basis of inter-alpha-inhibitor heavy chain transfer on to hyaluronan. Biochem Soc Trans 35:672–676.

Munoz JR, Stoutenger BR, Robinson AP, Spees JL, Prockop DJ. 2005. Human stem/progenitor cells from bone marrow promote neurogenesis of endogenous neural stem cells in the hippocampus of mice. Proc Natl Acad Sci USA 102:18171–18176.

Nathan C, Ding A. 2010. Nonresolving inflammation. Cell 140:871–882.

Németh K, Leelahavanichkul A, Yuen PS, Mayer B, Parmelee A, Doi K, Robey PG, Leelahavanichkul K, Koller BH, Brown JM, Hu X, Jelinek I, Star RA, Mezey E. 2009. Bone marrow stromal cells attenuate sepsis via prostaglandin E(2)-dependent reprogramming of host macrophages to increase their interleukin-10 production. Nat Med 15:42–49.

Oh JY, Roddy GW, Choi H, Lee RH, Ylöstalo JH, Rosa RH Jr, Prockop DJ. 2010. Anti-inflammatory protein TSG-6 reduces inflammatory damage to the cornea following chemical and mechanical injury. Proc Natl Acad Sci USA 107:16875–16880.

Ortiz LA, Gambelli F, McBride C, Gaupp D, Baddoo M, Kaminski N, Phinney DG. 2003. Mesenchymal stem cell engraftment in lung is enhanced in response to bleomycin exposure and ameliorates its fibrotic effects. Proc Natl Acad Sci USA 100:8407–8411.

Ortiz LA, Dutreil M, Fattman C, Pandey AC, Torres G, Go K, Phinney DG. 2007. Interleukin 1 receptor antagonist mediates the antiinflammatory and antifibrotic effect of mesenchymal stem cells during lung injury. Proc Natl Acad Sci USA 104:11002–11007.

Owen M, Friedenstein AJ. 1988. Stromal stem cells: Marrow-derived osteogenic precursors. Ciba Found Symp 136:42–60.

Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, Moorman MA, Simonetti DW, Craig S, Marshak DR. 1999. Multilineage potential of adult human mesenchymal stem cells. Science 284:143–147.

Pochampally RR, Smith JR, Ylostalo J, Prockop DJ. 2004. Serum deprivation of human marrow stromal cells (hMSCs) selects for a subpopulation of early progenitor cells with enhanced expression of OCT-4 and other embryonic genes. Blood 103:1647–1652.

Prockop DJ. 1997. Marrow stromal cells as stem cells for nonhematopoietic tissues. Science 276:71–74.

Prockop DJ, Brenner M, Fibbe WE, Horwitz E, Le Blanc K, Phinney DG, Simmons PJ, Sensebe L, Keating A. 2010a. Defining the risks of mesenchymal stromal cell therapy. Cytotherapy 12:576–578.

Prockop DJ, Kota DJ, Bazhanov N, Reger RL. 2010b. Evolving paradigms for repair of tissues by adult stem/progenitor cells (MSCs). J Cell Mol Med 14:2190–2199.

Prockop DJ, Oh JY. 2011. Mesenchymal Stem/Stromal Cells (MSCs): Role as Guardians of Inflammation. Mol Ther Epub ahead of print.

Rebbeck CA, Leroi AM, Burt A. 2011. Mitochondrial capture by a transmissible cancer. Science 331:303.

Ren G, Zhang L, Zhao X, Xu G, Zhang Y, Roberts AI, Zhao RC, Shi Y. 2008. Mesenchymal stem cell-mediated immunosuppression occurs via concerted action of chemokines and nitric oxide. Cell Stem Cell 2:141–150.

Rock KL, Latz E, Ontiveros F, Kono H. 2010. The sterile inflammatory response. Annu Rev Immunol 28:321–342.

Roddy GW, Oh JY, Lee RH, Bartosh TJ, Ylostalo J, Coble K, Rosa RH Jr, Prockop DJ. 2011. Action at a distance: Systemically administered adult

stem/progenitor cells (MSCs) reduce inflammatory damage to the cornea without engraftment and primarily by secretion of TNF-α stimulated gene/protein 6. Stem Cells 29:1572–1579.

Romieu-Mourez R, François M, Boivin MN, Bouchentouf M, Spaner DE, Galipeau J. 2009. Cytokine modulation of TLR expression and activation in mesenchymal stromal cells leads to a proinflammatory phenotype. J Immunol 182:7963–7973.

Rubin H. 2001. Multistage carcinogenesis in cell culture. Dev Biol (Basel) 106:61–66.

Serhan CN, Chiang N, Van Dyke TE. 2008. Resolving inflammation: Dual anti-inflammatory and pro-resolution lipid mediators. Nat Rev Immunol 8:349–361.

So A, De Smedt T, Revaz S, Tschopp J. 2007. A pilot study of IL-1 inhibition by anakinra in acute gout. Arthritis Res Ther 9:R28.

Spees JL, Olson SD, Whitney MJ, Prockop DJ. 2006. Mitochondrial transfer between cells can rescue aerobic respiration. Proc Natl Acad Sci USA 103: 1283–1288.

Tolar J, Nauta AJ, Osborn MJ, Panoskaltsis Mortari A, McElmurry RT, Bell S, Xia L, Zhou N, Riddle M, Schroeder TM, Westendorf JJ, McIvor RS, Hogendoorn PC, Szuhai K, Oseth L, Hirsch B, Yant SR, Kay MA, Peister A, Prockop DJ, Fibbe WE, Blazar BR. 2007. Sarcoma derived from cultured mesenchymal stem cells. Stem Cells 25:371–379.

Uccelli A, Moretta L, Pistoia V. 2008. Mesenchymal stem cells in health and disease. Nat Rev Immunol 8:726–736.

Wakao S, Kitada M, Kuroda Y, Shigemoto T, Matsuse D, Akashi H, Tanimura Y, Tsuchiyama K, Kikuchi T, Goda M, Nakahata T, Fujiyoshi Y, Dezawa M. 2011. Multilineage-differentiating stress-enduring (Muse) cells are a primary source of induced pluripotent stem cells in human fibroblasts. Proc Natl Acad Sci USA 108:9875–9880.

Wan YY. 2010. Regulatory T cells: Immune suppression and beyond. Cell Mol Immunol 7:204–210.

Wang Y, Huang L, Abdelrahim M, Cai Q, Truong A, Bick R, Poindexter B, Sheikh-Hamad D. 2009. Stanniocalcin-1 suppresses superoxide generation in macrophages through induction of mitochondrial UCP2. J Leukoc Biol 86:981–988.

Wisniewski HG, Vilcek J. 2004. Cytokine-induced gene expression at the crossroads of innate immunity, inflammation and fertility: TSG-6 and PTX3/TSG-14. Cytokine Growth Factor Rev 15:129–146.

Yagi H, Soto-Gutierrez A, Navarro-Alvarez N, Nahmias Y, Goldwasser Y, Kitagawa Y, Tilles AW, Tompkins RG, Parekkadan B, Yarmush ML. 2010. Reactive bone marrow stromal cells attenuate systemic inflammation via sTNFR1. Mol Ther 18:1857–1864.

Ylöstalo J, Bazhanov N, Prockop DJ. 2008. Reversible commitment to differentiation by human multipotent stromal cells in single-cell-derived colonies. Exp Hematol 36:1390–1402.

Zhang JF, Fu WM, He ML, Xie WD, Lv Q, Wan G, Li G, Wang H, Lu G, Hu X, Jiang S, Li JN, Lin MC, Zhang YO, Kung H. 2011. MiRNA-20a promotes osteogenic differentiation of human mesenchymal stem cells by co-regulating BMP signaling. RNA Biol Epub ahead of print.

Zhang QZ, Su WR, Shi SH, Wilder-Smith P, Xiang AP, Wong A, Nguyen AL, Kwon CW, Le AD. 2010. Human gingiva-derived mesenchymal stem cells elicit polarization of m2 macrophages and enhance cutaneous wound healing. Stem Cells 28:1856–1868.