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REVIEW

Therapeutic Potential of the Immunomodulatory Activities of Adult Mesenchymal Stem Cells

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Adult mesenchymal stem cells (MSCs) include a select population of resident cells within adult tissues, which retain the ability to differentiate along several tissue-specific lineages under defined media conditions and have finite expansion potential in vitro. These adult progenitor populations have been identified in various tissues, but it remains unclear exactly what role both transplanted and native MSCs play in processes of disease and regeneration. Interestingly, increasing evidence reveals a unique antiinflammatory immunomodulatory phenotype shared among this population, lending support to the idea that MSCs play a central role in early tissue remodeling responses where a controlled inflammatory response is required. However, additional evidence suggests that MSCs may not retain infinite immune privilege and that the context with which these cells are introduced in vivo may influence their immune phenotype. Therefore, understanding this dynamic microenvironment in which MSCs participate in complex feedback loops acting upon and being influenced by a plethora of secreted cytokines, extracellular matrix molecules, and fragments will be critical to elucidating the role of MSCs in the intertwined processes of immunomodulation and tissue repair. **Birth Defects Research (Part C) 90:67–74, 2010.**

Key words: mesenchymal stem cells; immunomodulation; regenerative medicine

INTRODUCTION

Adult Mesenchymal Stem Cells

First identified in 1966 within the stromal compartment of bone marrow (Friedenstein et al., 1968), adult mesenchymal stem cells (MSCs) include a select population of cells that exhibit extensive proliferative potential and retain the ability to differentiate along multiple tissue-specific lineages, including osteoblasts, chondrocytes, and adipocytes (Pittenger et al., 1999). Tissue sources from which these cells can be isolated have expanded to include bone (Noth et al., 2002), tonsil (Janjanin et al., 2008), dental pulp (Perry et al., 2008), dura mater (Petrie et al., 2008), adipose (Zuk et al., 2001), cartilage (Hiraoka et al., 2006), synovial fluid (de Bari et al., 2001), skin (Shih et al., 2005), and hair (Sieber-Blum and Grim, 2004). The presence of adult progenitor populations resident within various tissue spaces and their ability to adopt tissue-specific phenotypes given appropriate differentiation conditions, has led many investigators to suggest that the primary role of resident MSCs is to serve as a replacement cell type during the natural course of tissue turnover and homeostasis (Caplan, 2005). Consequently, there has been an explosion of work set out to evaluate the potential of MSCs as replacement and repair parts for damaged tissues. Isolation, ex vivo expansion, differentiation, and re-delivery of MSCs in vivo have yielded promising results.

MSCs as Replacement Cells

MSCs, delivered alone or in concert with a biomaterial, either native or synthetic, have been used in a variety of regenerative medicine strategies. Various tissue-engineering approaches that utilize adult MSCs as the replacement cell of choice include: (a) undifferentiated MSCs delivered to a tissue-specific site of repair where endogenous factors will provide appropriate differentiation cues (Mirza et al., 2008), (b) predifferentiated MSCs seeded onto scaffolding materials and subsequently implanted at the site of repair (Sheng et al., 2009), (c) MSCs seeded onto a biomaterial with controlled release systems to deliver appropriate differentiation factors to transplanted MSCs in situ (Hsiong et al., 2009), or (d)

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biomaterials with controlled release systems to enhance horning and differentiation of endogenous MSCs (Zhao et al., 2008). Many of these tissue-engineered strategies utilizing MSCs have shown promise in replacing various tissues, including cartilage (Tuan, 2006), bone (El-Amin et al., 2006), tendon (Kuo and Tuan, 2008), vasculature (Gong and Niklason, 2008), liver (Aurich et al., 2009), kidney (Li et al., in press), nerve (Prabhakaran et al., 2009), and others. However, in many of these studies it remains unclear how many of the originally delivered MSCs retain residency in the wounded tissue and maintain the appropriate, terminally differentiated phenotype. In many cases, large amounts of the original transplant population become apoptotic within the initial wound healing phase, collect in both the lungs and liver, leave the initial site of implantation, or never extravagate from the vascular system. Interestingly, in cardiac and stroke studies where the primary concern is improving local vascularization, the transient MSC presence appears to be sufficient to elicit a therapeutic effect (Li et al., 2002; Askari et al., 2003). In other words, the primary role of the MSCs may be that of a trophic support cell and not that of a permanent replacement cell. In myocardial infarctions and rodent stroke models, the exogenously introduced MSCs improve functional recovery, enhance vascularization, and secrete trophic factors without terminally differentiating to myocytes and neurons. These more recent studies suggest that the intended role of endogenous MSCs in processes of disease, damage, and repair remains unclear, and that the full therapeutic potential has not yet been realized.

MSCs AND IMMUNOMODULATION

MSCs as Immunomodulators

In 2002, emerging in vitro evidence began to suggest a new immunomodulatory role for adult MSCs regulating transplantation tolerance, autoimmunity, and tumor evasion. Specifically, in vitro T-lymphocyte activation and proliferation assays were performed in the presence of both autologous and allogeneic human (Di Nicola et al., 2002), baboon (Bartholomew et al., 2002), and murine (Djouad et al., 2003) MSCs. These studies demonstrated that MSC were capable of suppressing both lymphocyte proliferation and activation in response to allogeneic antigens. These immunomodulatory properties were not limited to bone marrow–derived MSCs, but have been shown in other MSC-derived tissues as well (Weiss et al., 2008).

Interestingly, T lymphocytes are not the only cell type within the immune system on which MSCs have been shown to exert their modulating effects. In culture, MSCs can induce development of CD8+ regulatory T cells that can in turn successfully suppress allogeneic lymphocyte responses (Djouad et al., 2003). MSCs also play an influential role in dendritic cell (DC) differentiation, maturation, and phenotype maintenance. Specifically, coculture with both human and murine MSCs in vitro prohibits differentiation of monocytes and CD34+ progenitors into antigen presenting DCs (Djouad et al. 2007). Furthermore, MSCs reduce expression of major histocompatibility complex class II (MHCII), CD40, and CD86 on DCs following maturation induction (Jiang et al., 2005; Nauta et al., 2006a,b). MSCs have also been shown to alter B-cell proliferation (Augello et al., 2005; Glennie et al., 2005), activation, IgG secretion (Deng et al., 2005), differentiation, antibody production, and chemotactic behaviors (Corcione et al., 2006).

Perhaps the most exciting data to date supporting the immunomodulatory properties of MSCs are from in vivo studies conducted in humans demonstrating successful treatment of graft-versus-host disease (GVHD). Specifically, treatment with ex vivo expanded allogeneic MSCs successfully resolved severe grade IV acute GVHD (Le Blanc et al., 2006). Furthermore, characterization of MSC immunophenotype reveals expression of major histocompatibility complex class I (MHCI), but no expression of MHCII or several costimulatory molecules (Le Blanc et al., 2003), suggesting that MSCs may not be capable of acting as antigen presenting cells and thus evade local immune surveillance (Krampera et al., 2003). However, new in vivo studies demonstrate MSCs can cross-present soluble ovalbumin (OVA) to naïve CD8(+) T cells from OT-1 mice (Francois et al., 2009), a feature that could be exploited for treatment in infectious disease or regenerative strategies. Collectively, these lines of evidence challenge the idea that cell replacement is the primary and sole role of resident adult MSCs. Alternatively, new ideas suggest that resident MSCs also work to suppress both transient and perpetual immune surveillance systems and create an ideal healing environment by secreting factors and altering the local microenvironment.

Universal and Unconditional Immune Privilege?

A growing body of in vivo evidence now supports the hypothesis that MSCs have immunosuppressive properties, including: (murine) prevention of GVHD (Yanez et al., 2006), decreased graft rejection (Nauta et al., 2006a,b), prevention of experimental acute encephalomyelitis (Zappia et al., 2005), (baboon) prolonged skin graft survival (Bartholomew et al., 2002), (rat) protection against renal ischemia/reperfusion injury (Togel et al., 2005), (human) hematopoietic stem cell (HSC) engraftment with no GVHD (Lee et al., 2002), platelet engraftment with low incidence of GVHD (Le Blanc et al., 2006), and resolution of severe acute GVHD (Lazarus et al., 2005). Furthermore, new studies suggest that MSCs can retain these immunosuppressive properties even after manipulation, such as phenotypic differentiation and genetic

engineering. Specifically, allogeneic MSCs that were differentiated toward a chondrogenic phenotype continued to suppress antigen-specific T-cell proliferation in rheumatoid arthritis (Zheng et al., 2008), and locally transplanted MSCs genetically engineered to produce bone morphogenetic protein 2 (BMP-2) escaped immune rejection and induced ectopic bone formation in vivo (Djouad et al., 2003).

However, Djouad et al. (2005) reported the first indication that immunomodulatory properties were not universal and unconditional. Specifically, allogeneic MSCs delivered to a murine collagen-induced arthritis model did not confer any benefit, and paw swelling worsened compared with control groups. Furthermore, coincubation with tumor necrosis factor-α (TNF-α) was sufficient to reverse the immunosuppressive effects of MSCs on T-cell proliferation. Others reported similar results, whereby MSC transplantation failed to alleviate GVHD in a murine model, even though in vitro assays confirmed suppression of T-lymphocyte proliferation (Sudres et al., 2006). More recently, repeated intravenous injections of MSCs failed to reduce GVHD-related recipient mortality. Most interestingly, local implantation of MSCs derived from bone marrow, placenta, or umbilical cord prompted ectopic bone formation in syngeneic recipients, and ultimately led to transplant rejection by allogeneic mice (Prigozhina et al., 2008). Taken together, these results suggest that MSC phenotype is transient and context dependent.

Thus, MSC phenotype and function may be particularly susceptible to the microenvironmental context. In other words, in terms of their trophic and phenotypic characteristics, MSCs may not have an entirely autonomous program, but instead may be influenced by the surrounding microenvironment. In this way, isolation from resident niches, subsequent expansion ex vivo, and redelivery to a remote inflamed or injured site where inflammatory chemokines and bioactive extracellular matrix (ECM) fragments are abundantly present may heavily influence MSC behavior and function. Therefore, issues related to appropriate delivery mechanisms, timing of delivery, and manipulation of the injury site microenvironment are of utmost importance in the study and application of MSCs.

Immune Privilege Driven by Secreted Factors?

Even the first characterization studies defining MSCs discussed the trophic role for this population by secreting various cytokine profiles. More recently, several key components from these profiles have been suggested as the mechanisms by which MSCs exert their immunosuppressive effects. These include but are not limited to: transforming growth factor-β (TGF-β), interleukin (IL)-10, IL-6, cyclooxygenase-1 (COX-1), and COX-2, which synthesizes prostaglandin E2 (PGE2) (Noel et al., 2007). For example, inhibition of DC differentiation is mediated partly through IL-6 in MSC conditioned media (Djouad et al., 2007). In chemically burned corneas, observed reductions in tissue inflammation by MSCs were attributed to IL-10, TGF-β1, and IL-6 in MSC-conditioned media (Oh et al., 2008). At low ratios, MSCs suppress natural killer (NK) cell proliferation, cytokine production, and cytotoxicity against HLA-class 1 expressing targets via TGF-β1 and PGE2 secretion. Interestingly, although MSCs can suppress NK activity via soluble factors, they remain a target for activated NK cells (Sotiropoulou et al., 2006). TGF-β1 secretion by MSCs suppressed alloreactive T-lymphocyte proliferation and activation, initiated by IL-1β secretion from CD14+ monocytes (Groh et al., 2005). Furthermore, key observations that MSC-conditioned media do not suppress lymphocyte proliferation (Augello et al., 2005) unless previously exposed to T lymphocytes (Djouad et al., 2003), suggest that key mediators are not constitutively expressed by MSCs (Nauta and Fibbe, 2007) and may need to be activated or primed.

Just as MSCs mediate immune suppression via soluble factors, exogenous signals equally influence MSCs behavior and function. Specifically, MSCs pretreated with TNF-α have heightened sensitivity and enhanced migratory capacity in response to various chemokines, compared with unstimulated MSCs (Ponte et al., 2007). In similar studies using TNF-α pretreatment, observed increases in MSC adhesiveness and migration in vitro were followed by increased engraftment and improved cardiac function in ischemic cardiac tissue in vivo (Kim et al., 2009a,b). Finally, novel soluble peptides have been identified in conditioned medium collected from breast carcinoma cell line that are chemotactic to MSCs, and include cyclophilin B [also known to be chemotactic to T cells and neutrophils (Allain et al., 2002; Pakula et al., 2007)] and hepatoma-derived growth factor (Lin et al., 2008), highlighting the role of tumor secretion in altered MSC behavior and function (Kuhn and Tuan, 2010). Collectively, these studies suggest that specific inflammatory environments may prime MSCs via soluble factor release to become mobile and migrate toward inflamed tissue spaces.

Recalling earlier discussions of transient MSC immune privilege, exposure to soluble factors, TGF-β and hepatocyte growth factor (HGF), is thought to drive the reversal of MSC immunosuppressive effects on T-cell proliferation (Di Nicola et al., 2002). Furthermore, overnight pretreatment with proinflammatory cytokines, interferon-γ (INF-γ) and TNF-α, dramatically alters MSC cytokine secretion profiles, including those that have been implicated in immunomodulation (English et al., 2007). In contrast, MSC populations that typically support osteoclast development were shown to inhibit osteoclast formation following TNF-α pretreatment, but retain immunosuppressive phenotype via lymphocyte proliferation assay. Pretreating MSCs with arthritic synovial fluid yielded similar results (Zhu et al., 2009). Similarly, pretreatment with INF-γ enhanced MSC cross-presentation of OVA to naïve CD8(+) T in OT-1 mice. Therefore, various proinflammatory

microenvironments with high levels of soluble cytokines may influence not only migratory behavior but also the immunomodulatory phenotype of both transplanted and endogenous MSCs.

Role of the ECM in Regulating MSC Immunomodulatory Properties

Similar to soluble factor profiles, there is now a large body of evidence regarding the cocktail of ECM molecules that MSCs secrete and, conversely, an expanding list of ECM molecules that actively regulate MSC phenotype. As evident from numerous multipotential and differentiation studies, MSCs are capable of secreting a variety of ECM molecules, including collagen types I and II and osteopontin. Furthermore, various ECM-ligand interactions enhance MSC differentiation along tissue-specific phenotypes, including vitronectin binding to enhance osteogenesis and collagen type II binding to enhance chondrogenesis (Lozito et al., 2008). Furthermore, ECM produced by adult MSCs facilitates ex vivo expansion of identical populations, suggesting that MSCs may self regulate stemness and prevent lineage differentiation by secreting a specific cocktail of ECM proteins, including decorin, biglycan, and others (Chen et al., 2007; Lai et al., in press). More recently, studies from our laboratory showed that endothelial cell matrix components that drive MSC differentiation are the same elements modified by MSC-secreted agents. Thus, we begin to appreciate the dynamic and intertwined feedback system in which MSCs are able to alter the very matrix signals acting upon them (Lozito et al., 2009).

Much attention has been given to understanding the relationship between soluble factors and MSC immunomodulation, that is, addressing the issues of what factors MSCs secrete to regulate immune privilege and what factors act on MSCs to alter their immunomodulatory properties. However, the connection between MSCs and their ECM niche has been underappreciated in relation to their immunomodulatory phenotype. There is high probability that ECM composition may be a critical factor in maintaining immune tolerance and regulating both inflammation and autoimmunity.

Conceptually, intact ECM molecules have been viewed as relatively inert in terms of tissue inflammatory responses. At least one study suggests that intact ECM, high-molecular-weight hyaluronic acid (HMW-HA), communicates an "all clear" signal to the local immune surveillance system by providing a costimulatory signal via CD44 (Bollyky et al., 2009), whereas fragmented ECM molecules are often described as proinflammatory chemoattractants. Thus matrix fragments are thought to act as "danger signals" to the immune system much in the same way that exogenous pathogen-associated molecular pattern (PAMP) molecules derived from viruses and bacteria act. Fragments of many ECM molecules, including those of versican (Kim et al., 2009a, b), fibronectin (Okamura et al., 2001), fibrinogen (Kuhns et al., 2007), biglycan (Schaefer et al., 2005), soluble heparate sulfate (Johnson et al., 2002), and low-molecular weight hyaluronic acid (Termeer et al., 2002), act as signaling moieties through Toll-like receptors (TLRs). Furthermore, knockout mice for mindin show normal leukocyte development but decreased recruitment of activated neutrophils and macrophages to areas of peripheral tissue insult (Jia et al., 2005). Osteonectin knockout mice exhibit reduced collagen type IV matrix deposition in dermal tissues. This defect results in increased pore size and interstitial volume space, thus allowing faster infiltration of DCs and accelerating T-cell priming (Sangaletti et al., 2005). Decorin has been shown to enhance macrophage sensitivity to lipopolysaccharides (LPS), promote macrophage survival, and upregulate MHCII expression (Comalada et al., 2003). In addition, both decorin and biglycan have been implicated in the complement activation cascade (Groeneveld et al., 2005).

More recently, connections between matrix molecules regulating immune response and MSCs modulating matrix composition are beginning to surface. Cultured MSCs are found to exhibit robust expression of galectin-1 (Gal1) (Kadri et al., 2005), a matrix protein implicated in T-cell homeostasis, which localizes at immune privileged sites, and ameliorates graft-versus-host disease following allogenic stem cell transplant (Baum et al., 2003). Moreover, Gal1 is secreted and found at the cell surface of MSCs, and is thus capable of participating in ECM-cell interactions. In vitro transmembrane assays report that MSCs respond chemotactically to both soluble and insoluble forms of fibronectin, vitronectin, and collagen type I, suggesting that matrix composition may be important for in situ MSC recruitment to a wound site (Thibault et al., 2007). Perhaps the most compelling recent study to argue a link between MSC immune privilege and matrix alterations involves the matrix metalloproteinases (MMPs), MMP-2 and -9. Blocking MMP-2 and -9 in vitro wipes out MSC immunosuppression by reducing surface expression of CD25 on responding allogeneic T cells. Furthermore, cotransplantation of MSCs with allogeneic islets prolonged graft survival, whereas in vivo results were completely reversed by inhibition of MMP-2 and -9 (Ding et al., 2009). Thus, MSCs participate in complex feedback loops by acting upon ECM networks and cell surface receptors via MMP activity and being influenced by matrix fragments via receptor binding (Sangaletti et al., 2005). In fact, our recent findings (Lozito et al., submitted for publication) demonstrate that MSCs fine-tune their proteolytic microenvironment with highly regulated expression of both MMPs and their native inhibitors, tissue inhibitors of metalloproteinases (TIMPs).

MSC-BASED THERAPY

Role of Matrix in MSC Therapy

Studies done in microvascular transplantation suggest that the immunobiology of transplanted cells may be anchorage depend-

ent. Here, matrix-embedded endothelial cells had reduced expression of costimulatory molecules and MHCII, suppressed allogeneic T-cell proliferation, and decreased NK cell adhesion, compared with free cell suspensions (Methe et al., 2007). Furthermore, no humoral or cellular rejection was detected after implantation of matrix-embedded allogeneic and xenogeneic endothelial cells in immunocompetent mice (Methe et al., 2005). Collectively, these studies support the hypothesis that immunomodulation is context dependent, and delivery methods for cell transplantation should therefore capitalize on these characteristics. Although immunogenicity was not directly addressed in a similar MSC transplantation study, the authors did report significantly greater numbers of MSCs retained at the transplant site, significant increases in vascular density, and superior heart function of matrix-embedded MSCs, compared with free cell suspension (Wang et al., 2008). Another study involving transplantation of MSC-derived endothelial progenitors suggested loss of survival signal derived from cell–cell contact, as one of several factors inducing acute donor cell death within 4 days delivery to ischemic cardiac tissue (Wu et al., 2006). Other factors that have been suggested to contribute to significant apoptosis of transplanted MSCs include local tissue ischemia, host inflammatory responses, and high concentration of cytotoxic factors (Ohnishi et al., 2007). Thus, designing delivery strategies to preserve cell–cell contacts, preserve immunomodulatory properties, and provide an appropriate microenvironment to cushion the hostile-inflamed site will be a key to improving effective MSC strategies. For example, covalently tethering epidermal growth factor (EGF) to a biomaterial surface that enhances MSC spreading and survival is a technique that offers a ''protective advantage'' to transplanted MSCs during the early acute phases of inflammation at the implantation site (Fan et al., 2007). Alternatively, linking biomaterials with HMW-HA, compared with short HA fragments, may help preserve MSC evasion of immune surveillance following transplantation.

Timing of MSC Therapy

Microenvironments are dynamic, with a myriad of feedback systems in place to regulate both homeostasis and repair. For example, dermal skin lacerations elicit massive neutrophil swarming to damaged tissue 30 min postinjury. These neutrophils arrive to spill toxic chemokines and eventually undergo self-induced apoptosis. Subsequently, monocytes infiltrate, differentiate to macrophages, phagocytose cellular debris, and secrete new chemokines. Finally, vascular, progenitor, and fibroblastic cells invade to repair and fill in the damaged tissue site. Should MSC phenotype and immunomodulatory function be particularly susceptible to chemokines, such as TNF-α and IFN-γ, and build-up of ECM fragments such as decorin and biglycan, the timing of MSC delivery within the wound healing cascade will be important. To date, timing of MSC treatment and its influence on both functional recovery and donor immunogenicity have not been systemically analyzed. For example, in studies evaluating MSC therapy to improve functional recovery of stroke patients, immediate transplantation following ischemia improved cerebral blood flow (Borlongan et al., 2004). Treatment within 2 hr but not after 24 hr significantly reduced infarcted tissue volume (Zhao et al., 2006). In comparison, neurological function but not infarct area was significantly improved when MSCs were delivered 24 hr following the initial stroke (Li et al., 2000). More recent studies suggest that MSC transplantation 1 month following injury can still yield functional recovery (Shen et al., 2007). Most interestingly, treatment with fibroblastic growth factor-2 (FGF-2) modified MSCs at 24 hr postinjury yielded both functional recovery and reduced infarcted area (Ikeda et al., 2005). Thus, for each disease and each injury, timing of MSC delivery must be optimized.

Tissue Site Specificity of MSC Therapy

Finally, site-specific delivery is also important. The nature and identity of the endogenous cell populations, frequency of immune cell patrolling, and matrix composition and density are all properties unique to each tissue type. Furthermore, treatment of diseases with chronic inflammation, such as rheumatoid arthritis, diabetic ulcers, and atherosclerosis, may be very different from that of transient skin lacerations. Likewise, delivering MSCs to an inflamed tissue site might elicit very different therapeutic response than MSCs delivered to a hypoxic tissue space. These site-specific differences may in part explain differences observed between resolution of GVHD following MSC treatment and exacerbated swelling in arthritic joints following MSC delivery. As with the design of any therapy, context and description of the host site will be important for appropriate modification, and thus maximal productivity of each MSC strategy.

CONCLUSION

In summary, recent investigations suggest that both soluble factors and matrix components influence MSC phenotype and function, which can be transient and context dependent (Kolf et al., 2007; Lozito et al., 2008; Kuhn and Tuan, 2010). Emerging findings continue to shed light on the diverse functional roles of MSCs, including those of replacement cell, support cell to endogenous tissues and tumors, and ''invisible cloak'' to immune surveillance. Hence, given these unique characteristics, MSCs represent a potentially highly rewarding target cell type to pursue as a tool to replace whole tissues, augment immune responses, and support native tissue repair in clinical applications. Elucidating the dynamic feedback networks and isolating the main signaling points will be essential in

harnessing and translating the therapeutic response of MSC-based strategies. Additionally, incorporation of biomaterial design strategies to work seamlessly with the therapeutic goals of transplanted MSCs will be a key to supporting, preserving, and controlling MSC response in vivo.

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