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Concise Review: Mesenchymal Stem Cells: Their Phenotype, Differentiation Capacity, Immunological Features, and Potential for Homing

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ABSTRACT

MSCs are nonhematopoietic stromal cells that are capable of differentiating into, and contribute to the regeneration of, mesenchymal tissues such as bone, cartilage, muscle, ligament, tendon, and adipose. MSCs are rare in bone marrow, representing ~1 in 10,000 nucleated cells. Although not immortal, they have the ability to expand manyfold in culture while retaining their growth and multilineage potential. MSCs are identified by the expression of many molecules including CD105 (SH2) and CD73 (SH3/4) and are negative for the hematopoietic markers CD34, CD45, and CD14. The properties of MSCs make these cells potentially ideal candidates for tissue engineering. It has been shown that MSCs, when transplanted systemically, are able to migrate to sites of injury in animals, suggesting that MSCs possess migratory capacity. However, the mechanisms underlying the migration of these cells remain unclear. Chemokine receptors and their ligands and adhesion molecules play an important role in tissue-specific homing of leukocytes and have also been implicated in trafficking of hematopoietic precursors into and through tissue. Several studies have reported the functional expression of various chemokine receptors and adhesion molecules on human MSCs. Harnessing the migratory potential of MSCs by modulating their chemokine-chemokine receptor interactions may be a powerful way to increase their ability to correct inherited disorders of mesenchymal tissues or facilitate tissue repair in vivo. The current review describes what is known about MSCs and their capacity to home to tissues together with the associated molecular mechanisms involving chemokine receptors and adhesion molecules. STEM CELLS *2007;25:2739–2749*

Disclosure of potential conflicts of interest is found at the end of this article.

THE DISCOVERY OF MESENCHYMAL STEM CELLS

The presence of nonhematopoietic stem cells in bone marrow was first suggested by the observations of the German pathologist Cohnheim 130 years ago. His work raised the possibility that bone marrow may be the source of fibroblasts that deposit collagen fibers as part of the normal process of wound repair [1].

Evidence that bone marrow contains cells that can differentiate into other mesenchymal cells, as well as fibroblasts, is now available, starting with the work of Friedenstein and colleagues [2]. They placed whole bone marrow in plastic culture dishes and removed the nonadherent cells after 4 hours, thus discarding most of the hematopoietic cells. They reported that the adherent cells were heterogeneous in appearance, but the most tightly adherent cells were spindle-shaped and formed foci of two to four cells, which remained inactive for 2–4 days and then began to multiply rapidly. After passaging several times in culture, the adherent cells became more homogeneously fibroblastic in appearance. They also found that the cells could differentiate into colonies that resembled small deposits of bone or cartilage. Friedenstein's observations were extended by other groups throughout the 1980s [3–5], and it was established that the cells isolated by Friedenstein's method were multipotential and could differentiate into osteoblasts, chondrocytes, adipocytes, and even myoblasts. They are currently referred to as either mesenchymal stem cells (MSCs), because of their ability to differentiate into mesenchymal-type cells, or as marrow stromal cells, because they appear to arise from the complex array of supporting structures found in the marrow [1].

IN VITRO CHARACTERISTICS OF MSCs

Human MSCs (hMSCs) are typically isolated from the mononuclear layer of the bone marrow after separation by density gradient centrifugation [6]. The mononuclear cells are cultured in medium with 10% fetal calf serum, and the MSCs adhere to the tissue culture plastic. Some hematopoietic cells also adhere, but over time in culture these are washed away, leaving adherent, fibroblast-like cells. After an initial lag

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phase, the cells divide rapidly, with population doubling time depending on the donor and the initial plating density. MSCs and MSC-like cells have now been isolated from various sites other than the bone marrow, including adipose tissue, amniotic fluid, periosteum, and fetal tissues, and show phenotypic heterogeneity [7–10]. MSC-like cells have been isolated from pathological tissues such as the rheumatoid arthritic joint, and these cells express bone morphogenetic protein receptors [11]. Indeed, it has been suggested that cells with mesenchymal stem characteristics reside in virtually all postnatal organs and tissues. MSCs have been isolated and cultured from many other species including mice, rats, cats, dogs, rabbits, pigs, and baboons, albeit with varying success, as it can be difficult to remove contaminating hematopoietic cells from species such as mice [12]. Nevertheless, enrichment for some species' MSCs can be achieved by expansion and passaging in deprivational medium to eliminate contamination. The resulting cultures are still morphologically heterogeneous, containing cells ranging from narrow spindle-shaped cells to large polygonal cells and, in confluent cultures, some slightly cuboidal cells [12].

Phenotypically, MSCs express a number of markers, none of which, unfortunately, are specific to MSCs. It is generally agreed that adult human MSCs do not express the hematopoietic markers CD45, CD34, CD14, or CD11. They also do not express the costimulatory molecules CD80, CD86, or CD40 or the adhesion molecules CD31 (platelet/endothelial cell adhesion molecule [PECAM]-1), CD18 (leukocyte function-associated antigen-1 [LFA-1]), or CD56 (neuronal cell adhesion molecule-1), but they can express CD105 (SH2), CD73 (SH3/4), CD44, CD90 (Thy-1), CD71, and Stro-1 as well as the adhesion molecules CD106 (vascular cell adhesion molecule [VCAM]-1), CD166 (activated leukocyte cell adhesion molecule [ALCAM]), intercellular adhesion molecule (ICAM)-1, and CD29 [13–18].

There are several reports that describe the isolation of both human and rodent MSCs using antibody selection based on the phenotype of MSCs. Some have used a method of negative selection to enrich for MSCs, whereby cells from the hematopoietic lineage are removed [19]; others have used antibodies to positively select for MSCs [20, 21].

MSCs from other species do not express all the same molecules as those on human cells; for example, although human and rat MSCs have been shown to be CD34−, some papers report variable expression of CD34 on murine MSCs [22]. It is generally accepted that all MSCs are devoid of the hematopoietic marker CD45 and the endothelial cell marker CD31. However, it is important to note that differences in cell surface expression of many markers may be influenced by factors secreted by accessory cells in the initial passages, and the in vitro expression of some markers by MSCs does not always correlate with their expression patterns in vivo [23].

There is also variable expression of many of the markers mentioned due to variation in tissue source, the method of isolation and culture, and species differences [12, 19]. For example, human adipose tissue is a source of multipotent stem cells called processed lipoaspirate (PLA) cells which, like bone marrow MSCs, can differentiate down several mesenchymal lineages in vitro. However, there are some differences in the expressions of particular markers: CD49d is expressed on PLA cells but not MSCs, and CD106 is expressed on MSCs but not PLA cells. CD106 on MSCs in bone marrow has been functionally associated with hematopoiesis, so the lack of CD106 expression on PLA cells is consistent with localization of these cells to a nonhematopoietic tissue [10].

Blood-derived mesenchymal precursor cells (BMPCs) have also been described in the blood of normal individuals, and these express many of the same markers as bone marrow MSCs, as well as differentiating down the osteoblastic and adipogenic lineages [24]. However, these appear to be a separate population from fibrocytes, which are mesenchymal precursor cells that circulate in the blood and can migrate into tissues [25]. Fibrocytes express CD34 and CD45 and appear to differentiate into myofibroblasts, whereas BMPCs are reported to be CD34 negative.

Mesenchymal stem cells have also been isolated from human first- and second-trimester fetal blood, liver, spleen, and bone marrow [7, 26]. Although phenotypically similar, these culture-expanded MSCs exhibited heterogeneity in differentiation potential, which related to the tissue source. Taken together, these examples illustrate that mesenchymal precursor cells are phenotypically heterogeneous, and the relationship between traditional bone marrow-derived MSCs and these other MSC-like populations remains to be fully clarified.

Adult human MSCs are reported to express intermediate levels of major histocompatibility complex (MHC) class I but do not express human leukocyte antigen (HLA) class II antigens on the cell surface [27]. The expression of HLA class I on fetal hMSCs is lower [28]. Le Blanc and colleagues did detect HLA class II by Western blot on lysates of unstimulated adult hMSCs, suggesting intracellular deposits of the antigen [18], and found that cell-surface expression can be induced by treatment of the cells with interferon-γ for 1 or 2 days [27]. Unlike adult hMSCs, human fetal liver-derived hMSCs have no MHC class II intracellularly or on the cell surface [28], suggesting that MHC antigen expression by hMSCs changes from fetal to adult life.

In Vitro Differentiation of MSCs

In addition to the identification of MSCs based on their morphologic or phenotypic characteristics, a further way to identify supposed MSC populations is by their capacity to be induced to differentiate into bone, fat, and cartilage in vitro. The classic method for differentiation of MSCs to osteoblasts in vitro involves incubating a confluent monolayer of MSCs with ascorbic acid, β-glycerophosphate, and dexamethasone for 2–3 weeks. The MSCs form aggregates or nodules and increase their expression of alkaline phosphatase; calcium accumulation can be seen over time [15]. These bone nodules stain positively by alizarin red and von Kossa techniques. These conditions, however, are unlikely to reflect the physiological signals MSCs receive that induce osteogenesis in vivo. There have been some recent reports investigating the role of bone morphogenic proteins (BMPs) on osteogenesis [29–31], but there appears to be species-specific differences in the effect of BMPs in vitro.

To promote adipogenic differentiation, MSC cultures are incubated with dexamethasone, insulin, isobutyl methyl xanthine, and indomethacin. There is an accumulation of lipid-rich vacuoles within cells, and they express peroxisome proliferation-activated receptor γ2, lipoprotein lipase, and the fatty acid-binding protein aP2 [15]. Eventually, the lipid vacuoles combine and fill the cells. Accumulation of lipid in these vacuoles is assayed histologically by oil red O staining. Having first been identified for their ability to differentiate into bone and adipocytes, further studies have demonstrated that MSCs can also differentiate, under appropriate in vitro conditions, to form chondrocytes, tenocytes, skeletal myocytes, neurons, and cells of visceral mesoderm (endothelial cells) [15, 32–34].

To promote chondrogenic differentiation, MSCs are centrifuged to form a pelleted micromass and cultured in the presence of transforming growth factor-β [35]. The cell pellets develop a multilayered, matrix-rich morphology, and histological analysis shows strong staining with toluidine blue, indicating an abundance of glycosaminoglycans within the extracellular matrix [36]. The cells also produce type II collagen, which is typical of articular cartilage [15].

It has also been demonstrated that, when treated with 5-azacytidine and amphotericin B, MSCs differentiate into myoblasts that fuse into rhythmically beating myotubes [32]. In addition, differentiation into neuron-like cells expressing markers typical for mature neurons has been reported [33, 37]. However, Hofstetter and colleagues reported that these neuron-like cells lack voltage-gated ion channels necessary for generation of action potentials; therefore, these cells may not actually be classified as true neurons [38].

Immunological Characteristics of MSCs

The immune phenotype of MSCs (widely described as MHC I^+, MHC II^-, $CD40^-$, $CD80^-$, $CD86^-$) is regarded as nonimmunogenic and, therefore, transplantation into an allogeneic host may not require immunosuppression. MHC class I may activate T cells, but, with the absence of costimulatory molecules, a secondary signal would not engage, leaving the T cells anergic [12]. Many reports have also described MSCs as having immunosuppressive properties, specifically that MSCs can modulate many T-cell functions including cell activation [39, 40]. This suppression appears to be independent of MHC matching between the MSCs and the T cells. Some reports have demonstrated that direct cell-cell contact is required for suppression [41], whereas others have shown that the suppressor activity depends on a soluble factor [39, 42]. It has also been shown that MSCs have immunomodulatory properties impairing maturation and function of dendritic cells and that hMSCs inhibit in vitro human B-cell proliferation, differentiation, and chemotaxis [43–46].

Despite some disagreement on the mechanisms by which MSCs exert their immunosuppressive effects, there is some evidence that these in vitro observations may translate to the in vivo setting. It has been reported that in vivo administration of baboon MSCs in immunocompetent outbred baboons significantly prolongs the survival of MHC-mismatched skin grafts [40]. Also, hMSCs have been administered in vivo to improve the outcome of allogenic transplantation by promoting hematopoietic engraftment [47] and to hamper graft-versus-host disease [48]. More recently, systemic administration of murine MSCs to mice affected by experimental autoimmune encephalomyelitis (a model of multiple sclerosis), a disease mediated by self-reactive T cells, resulted in striking improvement in disease symptoms, mediated by the induction of peripheral tolerance [49]. Therefore, targeting MSCs to inflamed tissues may have therapeutic benefit due to their immunosuppressive properties.

However, another study investigated whether the immunosuppressive properties of murine MSCs could be of therapeutic value in the collagen-induced arthritis (CIA) mouse model (an established model of rheumatoid arthritis) to explore the effect of MSCs on disease progression [50]. Interestingly, they found that MSCs offered no benefit in the CIA model of arthritis; indeed, they found that MSCs were associated with accentuation of the Th1 response. Experiments in vitro showed that the addition of tumor necrosis factor α (TNFα) was sufficient to reverse the immunosuppressive effect of MSCs on T-cell proliferation, possibly accounting for the lack of improvement of CIA. Hence, nonengineered MSCs may be unsuitable for the treatment of certain inflammatory diseases.

MSCs for Tissue Repair and Gene Therapy: Site-Directed and Systemic Delivery

The fact that MSCs can be differentiated into several different cell types in vitro, their relative ease of expansion in culture, and their immunologic characteristics clearly make MSCs and MSC-like cells a promising source of stem cells for tissue repair and gene therapy. However, compared with in vitro characterization, there is less information on the in vivo behavior of MSCs. The studies that have been performed can be split into observations following site-directed or systemic administration of cells.

Site-directed delivery of MSCs has shown their engraftment in several tissues, particularly after injury. Several groups have used bone marrow cells to repair infarcted myocardium [51–53]. Another group injected isolated murine MSCs directly into healthy adult myocardium and noted neoangiogenesis near the injection site within 1 week after transplantation [54]. Donor cells could be identified within these vessels, and it was shown that transplanted cells had differentiated into cardiomyocytes, endothelial cells, and pericytes or smooth muscle cells, demonstrating that cultured MSCs have the ability to engraft into healthy as well as injured tissue and can differentiate into several cell types in vivo.

Hofstetter and colleagues injected rat MSCs into the spinal cords of rats rendered paraplegic 1 week after injury. They found that MSCs formed bundles bridging the epicenter of the injury and guided regeneration through the spinal cord lesion, thus promoting recovery [38]. This implies that the beneficial effect of MSCs in sites of injury may not necessarily involve their differentiation into the regenerating tissue type but rather the local production of growth or other factors or physical attributes such as forming guiding strands in the injured spinal cord.

Some reports showed that when MSCs are transplanted into fetal or neonatal animals, they engraft and contribute to many different tissues. Liechty and colleagues transplanted hMSCs into fetal sheep early in gestation before and after the expected development of immune competence [55]. In this xenogenic system, hMSCs engrafted and persisted in multiple tissues for as long as 13 months after transplantation. Transplanted cells underwent site-specific differentiation into chondrocytes, adipocytes, myocytes and cardiomyocytes, bone marrow stromal cells, and thymic stroma. Even after development of immunocompetence, cells were present in liver, bone marrow, spleen, thymus, adipose tissue, lung, articular cartilage, perivascular areas of the central nervous system, and cardiac and skeletal muscle, indicative of migration and engraftment in multiple tissues throughout the body without provoking an immune response. Another group injected murine MSCs into the lateral ventricle in the brains of 3-day-old mice and examined the brains 12 days later [36]. They found that MSCs migrated throughout the forebrain and cerebellum, suggesting that MSCs mimic the behavior of neural progenitor cells in this setting. Some MSCs differentiated into astrocytes, and others may have differentiated into neurons, as indicated by the expression of neurofilaments. It is likely that a major contributing factor to the behavior of the MSCs in these two studies is their exposure to tissues and

organs still undergoing extensive development. The signals they respond to in the fetus or neonate will be very different from those in the adult animal, and hence MSCs may be capable of differentiating into more cell types in the embryo than in the adult.

Systemic delivery of MSCs has been reported by several groups. Barbash and colleagues investigated whether cultured MSCs could be successfully delivered to the infarcted myocardium with a view to repair [56]. They delivered cultured rat MSCs into the left ventricular cavity of rats 2, 10, and 14 days after induced myocardial infarction (MI) and compared with sham-MI rats. MSC infusion into MI rats resulted in significantly higher uptake in the heart than in sham-MI rats; however, less than 1% of the infused cells resided in the infarcted heart 4 hours after infusion. Early infusion (2 days compared with 14 after MI) also resulted in significantly higher uptake in the heart. MSCs were preferentially attracted to, and retained in, the ischemic tissue but not in the remote or intact myocardium. This suggests that injured tissue might express specific receptors or ligands to facilitate trafficking, adhesion, and infiltration of MSCs to the site of injury, but these may be downregulated a fairly short time after injury occurs. Barbash and colleagues also infused rat MSCs to their MI rats by the intravenous (IV) route but found the majority of cells in the lungs, with a small amount engrafting in the heart, liver, and spleen. Some MSCs had still homed to the site of injury in the heart, but much fewer than after delivery into the ventricle [56]. Entrapment of donor cells in the lung occurs in other studies where cultured MSCs are delivered intravenously. This is most likely explained because expanded MSCs are relatively large and activated and express adhesion molecules. However, Gao and colleagues found that treatment with the vasodilator sodium nitroprusside decreased the number of cells entrapped [57].

Despite the fact that MSCs can get trapped in the lungs, evidence has accumulated to show that MSCs are capable of homing to injured tissues after IV delivery (Table 1). Cultured rat and human MSCs have been shown to migrate into sites of brain injury after cerebral ischemia when transplanted intravenously in rats [58, 59]. Wu and colleagues delivered rat MSCs by the IV route to treat heart allograft rejection in rats and found that they "vigorously migrated to sites of allograft rejection," mainly differentiating into fibroblasts and a small number of myocytes [60]. MSCs have also been used to treat lung injury in mice when administered by the IV route. Ortiz and colleagues found that murine MSCs home to lung in response to injury, adopt an epithelium-like phenotype, and reduce inflammation and collagen deposition in the lung tissue of mice challenged with bleomycin (a model of pulmonary fibrosis) [61]. They found a 23-fold increase in engraftment levels of donor-derived cells when compared with mice not exposed to bleomycin.

Cultured MSCs have also been administered systemically to humans to treat several conditions, including osteogenesis imperfecta (OI), a disease in which osteoblasts produce defective type I collagen, which leads to osteopenia, multiple fractures, bone deformities, and shortened stature. Horwitz and colleagues used bone marrow transplant (BMT) after ablative chemotherapy to treat children with severe deforming OI. After 3 months, there was new dense bone formation, an increase in total body bone mineral content, an increase in growth velocity, and reduced frequency of bone fracture in all patients [62]. This study demonstrates that mesenchymal progenitors in transplanted marrow can migrate to bone in children with OI and then give rise to osteoblasts whose presence correlates with an improvement in bone structure and function. However, with increasing time post-transplantation, growth rate slowed and eventually reached a plateau, so it was hypothesized that additional therapy using isolated hMSCs without marrow ablative therapy would safely boost responses. They infused culture-expanded hMSCs into children who had previously undergone conventional BMT and found that some cells engrafted in defective bone and differentiated to osteoblasts capable of extending the clinical benefits of BMT [63]. Thus, allogeneic MSCs can be safely transplanted to children with OI without provoking an immune response, and some cells home to the bone marrow.

Many studies have also investigated the use of MSCs for gene therapy, including transplantation of MSCs transfected with vascular endothelial growth factor for the improvement of heart function after MI in rats [64, 65], MSCs as vehicles for interferon-β delivery into tumors in mice [66], and gene therapy with MSCs expressing BMPs to promote bone formation [67–69]. There is much evidence to support the theory that MSCs can home to tissues, particularly when injured or inflamed, involving migration across endothelial cell layers. The mechanism by which MSCs home to tissues and migrate across endothelium is not yet fully understood, but it is likely that injured tissue expresses specific receptors or ligands to facilitate trafficking, adhesion, and infiltration of MSCs to the site of injury, as is the case with recruitment of leukocytes to sites of inflammation. Chemokine receptors and their chemokine ligands are essential components involved in the migration of leukocytes into sites of inflammation, and it has recently been shown that MSCs also express some of these molecules. In addition, some of the adhesion molecules known to be involved in migration of leukocytes across the endothelium are also reported to be expressed on MSCs.

Chemokines and Their Receptors

Chemokines (chemotactic cytokines) are a large superfamily of small (8–10 kDa) glycoproteins that are involved in a diverse range of biological processes, including leukocyte trafficking, hematopoiesis, angiogenesis, and organogenesis. They are distinguished from other cytokines by being the only members of the cytokine family that bind to the superfamily of G protein-coupled 7-transmembrane domain receptors (also called serpentine receptors). They are small proteins with four conserved cysteines in their primary structure that form two essential disulfide bonds (Cys1-Cys3 and Cys2-Cys4) [70]. Chemokines have a short amino-terminal domain preceding the first cysteine, a backbone made of β-strands and the connecting loops found between the second and fourth cysteines, and a carboxy-terminal α-helix of 20–30 amino acids [71]. There are approximately 50 human chemokines that segregate into four categories based on the positioning of the cysteine residues within the primary amino acid sequence. The largest family is the CC chemokines, so called because the first two of four cysteine residues are adjacent to each other (Table 2). A second family is CXC chemokines, which have a single amino acid residue between the first two cysteines (Table 3). The third family is the CX_3C family, of which fractalkine (CX_3CL1) is a member [72]. Lymphotactin, the sole member of the fourth family, has a single disulfide-linked pair of cysteine residues [73] (Table 3).

There are two chemokine nomenclature systems used in the literature: the traditional abbreviations dating back to when the first chemokines were discovered and were commonly named according to their function, such as monocyte chemoattractant

Table 1. Publications showing homing of systemically administered MSCs

MSC administration	Recipient species	Tissue or condition and outcome	Reference
5×10^7 Allogeneic MSCs, iv, ic, or ec	Swine	Myocardial infarction model Increased engraftment, especially ic; ec safer and less engraftment in nonheart organs	[113]
$3.2 \pm 0.4 \times 10^8$ Allogeneic MSCs, iv infusion	Swine	Myocardial infarction model. Improved left ventricular function and remodelling	[114]
5×10^6 Isogenic MSCs, iv	Rat	Acute myocardial infarction MSCs migrated into infarcted myocardium leading to enhanced cardiac function, angiogenesis, and myogenesis	[115]
3×10^5 Allogeneic MSCs, iv	Mouse	Myocardial infarction model MSC homing to heart involving SDF-1α (CXCL12)	[116]
Allogeneic MSCs, iv	Mouse	Myocardial infarction MSCs migrate to heart and differentiate into cardiomyocytes	[117]
4×10^6 Allogeneic MSCs, iv or lvc	Rat	Myocardial infarction model MSC trapping mainly in lungs when iv; enhanced homing to ischemic myocardium when lvc	[56]
1×10^7 Allogeneic MSCs, iv	Rat	Myocardial ischemia Increased migration of MSC into injured heart	[111]
1×10^7 Immortalised rat MSCs, iv	Rat	Heart allograft during chronic rejection MSC migration into lesions of chronic rejection	[60]
2×10^6 Xenogeneic hMSCs, iv	Rat	Traumatic brain injury hMSCs migrated into injured brain and neurological function improved	[58]
1×10^7 Xenogeneic hMSCs transfected with glial cell-line derived neurotrophic factor, iv	Rat	Cerebral ischemic stroke model MSCs recruited to brain and increased functional recovery (especially using transfected MSCs)	[118]
3×10^6 Allogeneic MSCs, iv	Rat	Cerebral ischemic stroke model MSC recruitment to brain leading to therapeutic benefit	[59]
5×10^5 Allogeneic MSCs, iv	Mouse	Pulmonary fibrosis model (using bleomycin) Homing of MSCs to lung, reducing lung inflammation and collagen deposition	[61]
$8–10 \times 10^6$ Allogeneic MSCs, iv	Rat	Nephropathy model MSCs specifically home to focal areas of kidney damage and are detected by MR imaging	[119]
$1–5 \times 10^6$ Per kg allogeneic hMSCs, iv	Human	Graft-versus-host disease. Improved disease or progression-free survival	[47]
5×10^6 hMSCs, iv	Mouse	Total body and local irradiation Increased engraftment in response to tissue injury produced by irradiation	[120]
Allogeneic hMSCs, iv, $>1 \times 10^6$ MSCs per kg body weight	Human	Ontogenesis imperfecta MSC engraftment (e.g., bone and skin) and differentiation into osteoblasts. Acceleration of patient growth	[63]
1×10^6 Allogeneic MSCs, iv	Mouse	Experimental autoimmune encephalomyelitis (model of multiple sclerosis) MSC homing to lymphoid organs and striking amelioration of disease	[49]
5×10^6 Xenogeneic hMSCs transfected with interferon-β, iv	Mouse	Preferential engraftment of MSCs to tumors. Inhibition of growth of malignant cells	[66]
$2 \times 10^{5–6}$ Allogeneic MSCs, iv	Mouse	MSCs containing osteocalcin promoter transgene home to bone	[121]
$8–10 \times 10^5$ Xenogeneic iron-labeled hMSCs, iv	Rat	MSC homing to liver enhanced by magnet	[122]
$1–2 \times 10^6$ Xenogeneic hMSCs per kg fetal weight, ip	Fetal sheep	Normal fetus hMSCs engrafted, persisted, and differentiated into tissue-specific cells	[55]
1×10^6 Allogeneic MSCs, iv	Mouse	Normal mouse MSCs homed to several tissues (e.g., lymph node, thymus, salivary gland, and intestine)	[95]

Abbreviations: ec, endocardial; hMSC, human MSC; ic, intracoronary; ip, intraperitoneal; iv, intravenous; lvc, left ventricular cavity; MR, magnetic resonance; SDF-1, stromal cell-derived factor-1.

proteins, and newer systematic nomenclature that combines structural motifs (CXC, CC, XC, or CX_3C) with "L" for ligand and the number of the respective gene. Chemokine receptors are named according to the type(s) of chemokine(s) they bind (CXC, CC, XC, or CX_3C) followed by "R" for receptor and a number indicating the order of discovery [74].

Table 2. CC family of chemokines and chemokine receptors

Receptor	Chemokine ligands	Cell types expressing receptor	Associated disease or normal physiology
CCR1	CCL3 (MIP-1α), CCL5 (RANTES), CCL7 (MCP-3), CCL14 (HCC1)	Monocytes, memory T cells, basophils, eosinophils	Rheumatoid arthritis, multiple sclerosis
CCR2	CCL2 (MCP-1), CCL8 (MCP-2), CCL7 (MCP-3), CCL13 (MCP-4), CCL16 (HCC4)	Monocytes, dendritic cells (immature), memory T cells	Resistance to intracellular pathogens, atherosclerosis, rheumatoid arthritis, multiple sclerosis, type 2 diabetes mellitus
CCR3	CCL11 (eotaxin), CCL13 (eotaxin 2), CCL7 (MCP-3), CCL5 (RANTES), CCL8 (MCP-2), CCL13 (MCP-4)	Eosinophils, basophils, mast cells, T cells (Th2), platelets	Allergic asthma, rhinitis
CCR4	CCL17 (TARC), CCL22 (MDC)	T cells (Th2), dendritic cells (mature), basophils, macrophages, platelets	T-cell homing to skin, parasitic infection, graft rejection
CCR5	CCL3 (MIP-1α), CCL4 (MIP-1β), CCL5 (RANTES), CCL11 (eotaxin), CCL14 (HCC1), CCL16 (HCC4)	T cells, monocytes	HIV-1 coreceptor, transplant rejection
CCR6	CCL20 (MIP-3β, LARC)	T cells (regulatory and memory), B cells, dendritic cells	Mucosal humoral immunity, intestinal T cell homing, allergic asthma
CCR7	CCL19 (ELC), CCL21 (SLC)	T cells, dendritic cells (mature)	Transport of T cells and dendritic cells to lymph node, antigen presentation, cellular immunity
CCR8	CCL1 (I-309)	T cells (Th2), monocytes, dendritic cells	Dendritic cell migration to lymph node, type 2 cellular immunity, granuloma formation
CCR9	CCL25 (TECK)	T cells, IgA+ plasma cells	Homing of T cells and IgA+ plasma cells to intestine, inflammatory bowel disease
CCR10	CCL27 (CTACK), CCL28 (MEC)[a]	T cells	T-cell homing to intestine and skin

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Abbreviations: CTACK, cutaneous T cell-attracting chemokine; ELC, Epstein-Barr I1-ligand chemokine; HCC, hemofiltrate chemokine; HIV, human immunodeficiency virus; LARC, liver and activation-regulated chemokine; MCP, monocyte chemoattractant protein; MDC, macrophage-derived chemokine; MEC, mammary-enriched chemokine; MIP, macrophage inflammatory protein; RANTES, regulated upon activation normal T cell expressed and secreted; SLC, secondary lymphoid-tissue chemokine; TARC, thymus and activation-regulated chemokine; TECK, thymus expressed chemokine.

Table 3. CXC, CX_3C, and XC families of chemokines and chemokine receptors

Receptor	Chemokine ligands	Cell types expressing receptor	Associated disease or normal physiology
CXCR1	CXCL8 (interleukin-8), CXCL6 (GCP2)	Neutrophils, monocytes	Inflammatory disease, COPD
CXCR2	CXCL8, CXCL1 (GROα), CXCL2 (GROβ), CXCL3 (GROγ), CXCL5 (ENA-78), CXCL6	Neutrophils, monocytes, microvascular endothelial cells	Inflammatory lung disease, COPD, angiogenic for tumor growth
CXCR3-A	CXCL9 (MIG), CXCL10 (IP-10), CXCL11 (I-TAC)	Type 1 helper cells, mast cells, mesangial cells	Inflammatory skin disease, multiple sclerosis, transplant rejection
CXCR3-B	CXCL4 (PF4), CXCL9 (MIG), CXCL10 (IP-10), CXCL11 (I-TAC)	Microvascular endothelial cells, neoplastic cells	Angiostatic for tumor growth
CXCR4	CXCL12 (SDF-1)	Widely expressed	HIV-1 coreceptor, tumor metastases, hematopoiesis
CXCR5	CXCL13 (BCA-1)	B cells, follicular helper T cells	Formation of B cell follicles
CXCR6	CXCL16 (SR-PSOX)	CD8+ T cells, natural killer cells, and memory CD4+ T cells	Inflammatory liver disease, atherosclerosis
CX_3CR1	CX_3CL1 (fractalkine)	Macrophages, endothelial cells, smooth-muscle cells	Atherosclerosis
XCR1	XCL1 (lymphotactin), XCL2	T cells, natural killer cells	Rheumatoid arthritis, IgA nephropathy, tumor response

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Abbreviations: BCA-1, B cell chemoattractant 1; COPD, chronic obstructive pulmonary disease; ENA, epithelial cell-derived neutrophil-activating peptide; GCP, granulocyte chemotactic protein; GRO, growth-related oncogene; HIV, human immunodeficiency virus; IP-10, interferon-inducible protein 10; I-TAC, interferon-inducible T cell alpha chemoattractant; MIG, monokine induced by interferon-γ; PF, platelet factor; SDF, stromal cell-derived factor; SR-PSOX, scavenger receptor for phosphatidylserine-serine containing oxidized lipids.

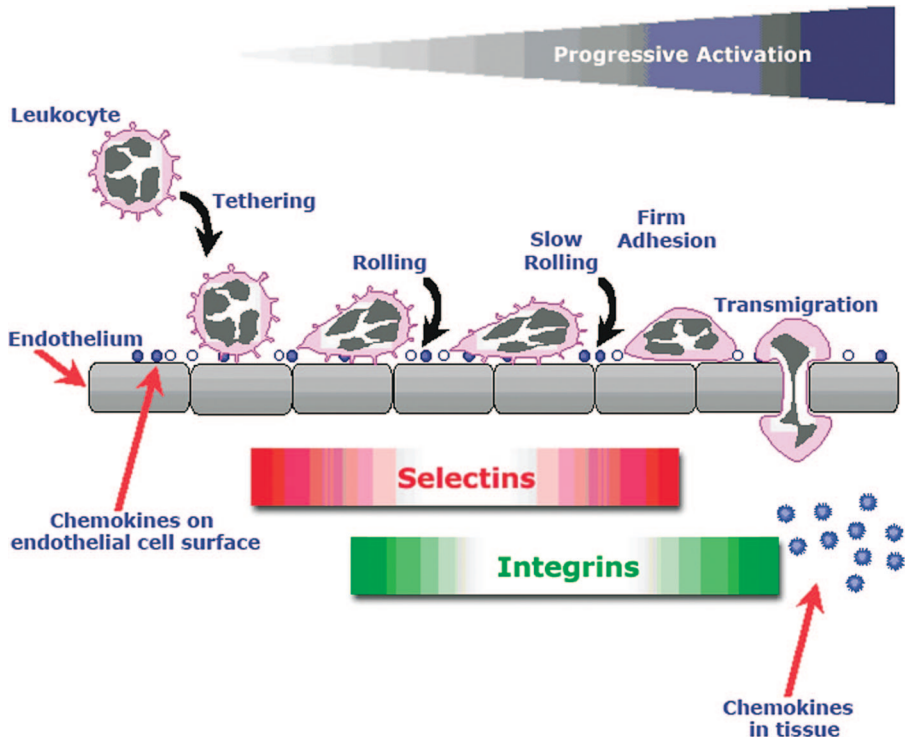

Figure 1. Schematic of the transmigration of leukocytes across the endothelium.

Chemokines and Leukocyte Traffic

The role of chemokines and their receptors in leukocyte trafficking has been extensively investigated (Fig. 1). Initially, contact between microvascular endothelial cells and blood leukocytes is mediated by interactions among adhesion molecules, such as selectins, β1-integrins, and their respective counterligands, resulting in a rolling motion of weakly adherent leukocytes [74]. This does not involve chemokines; however, some chemokines have recently been shown to destabilize the rolling of lymphocytes on L-selectin ligands, suggesting that chemokines can perhaps regulate the rolling process [75].

Chemokines presented on endothelial cells then trigger integrin activation and arrest of those leukocytes that carry the corresponding receptors [76]. Therefore, even though a leukocyte may express the appropriate molecules for capture and loose adhesion, this alone will not lead to transendothelial migration. A leukocyte is only able to cross a particular endothelial barrier if it is also capable of responding to the chemokine(s) present at this location.

Subsequently, adherent leukocytes move across the endothelial cell layer and the underlying basement membrane and into the tissue. Recent evidence suggests that this transendothelial migration is mediated by PECAM-1, junction adhesion molecules, CD99, and ICAM-1 [77, 78]. Once in the tissue, cells migrate along a chemokine gradient, which involves the sensing of subtle differences in chemokine concentrations and the establishment of cell polarity. This is followed by directional cell locomotion via cytoskeletal rearrangements and adhesive interactions with the extracellular matrix [79].

The blood vessel at which a leukocyte undergoes extravasation is not random but is tightly controlled by the range of chemokine receptors and adhesion molecules expressed on the cell surface, often referred to as the cell's address code. For example, CCR10 and its ligands CCL27 (cutaneous T cell-attracting chemokine) and CCL28 (mammary-enriched chemokine) are associated with migration of T cells to skin [80, 81], as is CCR4 and one of its ligands, CCL17 (thymus and activation-regulated chemokine) [82]. In contrast, CCR9 and its ligand CCL25 (thymus expressed chemokine) are involved in the migration of memory T cells to intestinal tissue [83, 84]. CCR3 and its ligands are involved in the recruitment of T_H2 cells during allergic inflammation [85, 86] and, in addition, activated T_H2 cells selectively express CCR4 and CCR8 [87]. CCR5 and CXCR3 are preferentially expressed on T_H1 cells, and their chemokine ligands are produced in many T_H1-driven inflammatory lesions, including rheumatoid arthritis and multiple sclerosis [88, 89].

Chemokine Receptor Expression on MSCs

As has been previously discussed, MSCs do have the ability to migrate into tissues from the circulation, possibly in response to signals that are upregulated under injury conditions. Although the mechanisms by which MSCs are recruited to tissues and cross the endothelial cell layer are not yet fully understood, it is probable that chemokines and their receptors are involved, as they are important factors known to control cell migration. Chemokines are reported to be involved in migration of other types of progenitor cells; CXCL12 (stromal cell-derived factor-1) and its receptor CXCR4 are crucial for bone marrow retention, mobilization, and homing of hematopoietic stem cells [90, 91] and are involved in migration of primordial germ cells [92] and recruitment of endothelial-cell progenitor cells to sites of ischemic tissue [93].

Consequently, several groups have recently been studying the expression of chemokine receptors on hMSCs, although results have been variable. Wynn and colleagues examined expression of CXCR4 on hMSCs and showed the receptor was present on the cell membrane of less than 1% of cells, although high levels (83%–98%) of intracellular CXCR4 expression were noted [94]. In contrast, Von Lüttichau and colleagues reported expression of CCR1, CCR4, CCR7, CXCR5, and CCR10 but not CXCR4, and these chemokine receptors were functional in driving MSC migration [95]. Other reports have shown functional expression of CXCR4 [96]; CCR1, CCR7, CXCR4, CXCR6, and CX_3CR1 on a minority of cells (2%–25%) [17]; and CXCR4 and CX_3CR1 [97]. However, these reports have not studied the expression of the whole repertoire of functional chemokine receptors at the protein level, that is, by flow cytometry analysis coupled with, for example, chemotaxis assay. In a recent report, expressions of all the CC chemokine receptors (except CCR10), the CXC receptors, and CX_3CR1 were studied by flow cytometry [98]. It was found that hMSCs expressed functional (as determined by chemotaxis) CCR1, CCR7, CCR9, CXCR4, CXCR5, and CXCR6 on 43%–70% of cells. Another group reported expression of CCR2, CCR8, CXCR1, CXCR2, and CXCR3, as detected by real-time polymerase chain reaction and immunohistochemistry [99]. Ponte and colleagues demonstrated expression of CCR2, CCR3, CCR4, and CXCR4 on hMSCs and found that TNFα increased CCR2, CCR3, and CCR4 expression but not CXCR4 [100]. Thus, MSCs express a variety of chemokine receptors, although there is much variability among different reports. These differences reflect the heterogeneity of cultured MSCs, which appears to be a feature of these cells. The fact that they express a variety of chemokine receptors suggests that they show the potential to home to different tissues where they could be used to enhance tissue repair or dampen inflammation. For example, assuming an analogous situation to leukocytes, MSCs could use CCR9 to enter intestine or CCR1 to enter inflamed joint tissues or brain in rheumatoid arthritis or multiple sclerosis.

ADHESION MOLECULES

A recent study by Ruster and colleagues [101] suggested that P-selectin and a counterligand are involved in the extravasation of hMSCs. Using intravital microscopy, it was observed that intravenously administered hMSCs can roll along the walls of the blood vessels in the ear veins of mice, and this phenomenon was significantly decreased in mice genetically deficient of P-selectin. Moreover, in an in vitro assay, hMSCs rolling upon human umbilical vein endothelial cells under shear flow conditions were significantly reduced in the presence of a neutralizing P-selectin antibody. As neither P-selectin glycoprotein ligand-1 nor the alternative ligand CD24 were present on hMSCs, it was proposed that a novel MSC-expressed carbohydrate ligand was the counterligand for endothelially expressed P-selectin. These data suggest hMSCs, like leukocytes, roll upon endothelial cells as the first stage in their recruitment. E- and L-selectins have been reported to be absent or present only in low amounts on hMSCs, and their significance in MSC trafficking, compared with P-selectin, may thus be unimportant [15, 101–103].

Various integrin molecules, such as $\alpha1$, $\alpha2$, $\alpha3$, $\alpha4$, $\alpha5$, αv, $\beta1$, $\beta3$, and $\beta4$, are known to be expressed on hMSCs. Also, other adhesion molecules, which include VCAM-1, ICAM-1, ICAM-3, ALCAM, and endoglin/CD105, are expressed [104, 105]. Approximately 50% of hMSCs are thought to express the integrin very late antigen (VLA)-4 ($\alpha4\beta1$, CD49d), and when neutralizing antibodies to this integrin were present it was shown that firm adherence of hMSCs to endothelial cells, under conditions of shear flow, occurred in a VLA-4 dependent manner [101]. Additionally, it was observed that treating endothelial cells with a blocking antibody to its counterpart adhesion molecule, namely VCAM-1, induced a similar decrease in hMSC adherence. This demonstrates a dependence upon the VLA-4/VCAM-1 axis for firm hMSC adherence to endothelial cells.

Segers and colleagues [106] demonstrated that adhesion of rat MSCs to TNFα or interleukin-1β stimulated cardiac microvascular endothelial cells (CMVE) under static and flow conditions. Adhesion was confirmed in vivo by observing rat MSC transmigration in the capillaries of TNFα stimulated rat hearts 24 hours after MSC injection into the left ventricular cavity. Analysis of the levels of VCAM-1 expressed upon CMVE showed that levels were increased at least 50-fold by cytokine stimulation and, accordingly, the adhesion of MSCs to the endothelial cells was significantly inhibited after their treatment with a blocking antibody against VCAM-1. TNFα-stimulated rat MSCs also increased their VCAM-1 expression, and their adhesion in static culture to CMVE was abolished after anti-VCAM-1 antibody treatment, whereas blocking antibodies against ICAM-1 were ineffective. Therefore, in summary, adhesion molecules so far reported to be functionally important in the adhesion of MSCs to the endothelium are P-selectin and a counterligand and VCAM-1 and its counterligand VLA-4.

MSCs have been shown to mobilize into peripheral blood in response to injury such as acute burns [107] and skeletal muscle injury [108] and in response to chronic hypoxia [109]. A comparison between the cell adhesion molecule expression profile of these mobilized, circulating MSCs and tissue-derived MSCs may provide further insight into the potential mechanisms of MSC homing.

Numerous in vivo studies have shown that MSCs have the capability to migrate from the blood, across endothelial cells, and into tissues. For example, this has been demonstrated after injury to the brain and heart in animal models [59, 110, 111]. However, little is known about the mechanism of MSC transendothelial migration and what adhesion molecules are involved. One study has examined this mechanism in vitro using a coculture of endothelial cells (derived from differentiated embryonic stem cells) and hMSCs from human bone marrow aspirates [112]. MSCs showed morphological changes after 30 minutes that resulted in contact with the endothelium and, after 2 hours, subsequent flattening and integration within the endothelial monolayer. These results showed that, albeit in vitro and in the absence of shear flow, hMSCs could make efficient cell-cell contact and commence migration across endothelial cells. Supporting in vivo experiments, consisting of cannulation of the aorta of mice and perfusion of MSCs, demonstrated that, after 2 hours, endothelial tight junctions in the heart had been abolished, and the MSCs had become associated with the endothelial cells. Electron microscopy revealed that 30 minutes of MSC perfusion was sufficient to observe transmigration across the endothelium of approximately 30% of the cells—this percentage rose to 50% after 60 minutes but remained continuous thereafter.

Many of the molecules known to be involved in the tethering, rolling, adhesion, and transmigration of leukocytes from the bloodstream into tissues are known to be expressed on MSCs. These include integrins, selectins, and chemokine receptors. Furthermore, P-selectin and VCAM-1, which function in leukocyte adhesion, have been shown to be functionally important

in the adhesion of MSCs to the endothelium. However, L- and E-selectin are involved in the initial rolling stage on leukocytes, whereas L-selectin expression is low or absent on the surface of MSCs, and the role of E-selectin has not yet been determined [101]. Another difference is that PECAM-1/CD31, which is involved in leukocyte transmigration across the endothelium, is not expressed on MSCs. So although it would seem likely that MSCs transmigrate into tissues by a similar mechanism to that of leukocytes employing some of the same molecules, specific differences in the use of adhesion molecules may also exist between these two cell types.

CONCLUDING REMARKS

MSCs are under investigation for a number of therapeutic applications. These cells are known to home to some tissues, particularly when injured or under pathological conditions. The mechanisms underlying migration of MSCs remain to be clarified, although evidence suggests that both chemokines and their receptors and adhesion molecules are involved. Studying the role of chemokine receptors and adhesion molecules on MSCs may allow the development of therapeutic strategies to enhance the recruitment of ex vivo-cultured MSCs to damaged or diseased tissues. This could lead to various therapeutic possibilities such as supporting tissue regeneration, correcting inherited disorders (e.g., of bone), dampening chronic inflammation, and using these cells as vehicles for the delivery of biological agents.

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DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST

The authors indicate no potential conflicts of interest.

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