Homing of mesenchymal stem cells: mechanistic or stochastic? Implications for targeted delivery in arthritis

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Abstract

Mesenchymal stem cells (MSCs) are multipotent cells with the capacity to undergo chondrogenic differentiation. Systemically administered MSCs have been shown to preferentially accumulate at sites of tissue damage and inflammation, thus MSC-based therapy holds great promise for the treatment of inflammatory diseases such as RA. Modulation of MSC homing may allow targeted delivery of systemically administered MSCs to damaged articular cartilage, where they can suppress immune-mediated cartilage destruction and contribute to cartilage repair via a combination of chondrogenic differentiation and paracrine stimulation of intrinsic residual repair. To harness the potential of MSC homing, a thorough understanding of the mechanism is key. This review discusses current knowledge of the mechanism of MSC homing to injured/inflamed tissue and its implications for targeted MSC-based therapy in arthritis.

Key words: mesenchymal stem cells, rheumatoid arthritis, osteoarthritis, chemotaxis, homing, chemokines.

Introduction

RA and OA are diseases of the synovial joints. They are the main forms of joint disease in industrialized countries. OA is a degenerative joint disease in which there is progressive destruction of articular cartilage associated with subchondral bone remodelling and sclerosis, causing pain and debilitating loss of joint function. It is a major cause of morbidity in the UK; ~2.4% of all general practice consultations are due to problems related to OA [1] and as such it is a major public health concern.

RA is an autoimmune disorder characterized by chronic synovitis, i.e. the synovium undergoes a pathological outgrowth (forming the so-called pannus), sustained by proliferation of synovial fibroblasts and infiltration of blood-borne inflammatory/immune cells, that invades and erodes articular cartilage and bone, leading to deformity, pain and disability. It is also a major cause of morbidity in the UK, affecting 0.5–1.0% of the population [2].

The main therapeutic options at present are analgesia and anti-inflammatory agents for OA and DMARDs and biologic agents (such as anti-TNF-α therapy) for RA. For both conditions, replacement of damaged articular surfaces with prostheses is required when joint disease becomes severe. None of these treatment options are curative or ideal. Analgesia does not deal with the underlying disease process. DMARDs, used to arrest disease progression, can cause toxic side effects; biologic agents are expensive and weaken the immune system, potentially increasing the risk of infections [3]; and prostheses have a limited lifespan, which increases the risk of revision in young patients [4–7]. When the joint tissue damage is established, no pharmacological intervention can trigger a reparative response. In addition, the biomechanics and homeostatic mechanisms of damaged joints are inevitably compromised, leading to a progressive and irreversible loss of tissue. Thus there is a need for novel treatment options that activate repair mechanisms.

Mesenchymal stem cells (MSCs) are multipotent cells derived from bone marrow [8] and other connective tissues such as synovium [9], periosteum [10], articular cartilage [11] and adipose tissue [12] that have the capacity for self-renewal and differentiation into mesenchymal tissues [8, 13, 14]. MSCs are able to undergo chondrogenic differentiation, generating new cartilaginous tissue, and therefore MSC-based therapy holds great promise for the treatment of OA and RA with established damage.

One major constraint on the clinical use of MSC-based approaches in OA and RA is the lack of a reliable delivery system that would allow cells, either in suspension or in...
biomaterials, to engraft into damaged cartilage and then integrate and differentiate into hyaline-like cartilage with the biomechanics of normal cartilage. Several methods for local cell delivery currently in use are cell-carrying membranes or cell-seeded scaffolds [15–18] for localized cartilage defects caused by trauma. However, these methods have yet to be robustly applied to the more widespread cartilage damage seen in OA and RA.

Systemically administered MSCs have been shown to preferentially accumulate at sites of tissue damage and inflammation [19–22]. This phenomenon of MSC homing to injured sites presents an opportunity for MSC delivery to the damaged articular surface in arthritis, which may be both cost effective and minimally invasive. Modulation of MSC homing may allow targeted delivery of systemically administered MSCs to damaged articular cartilage, where, with their immunosuppressive actions [23, 24], they could suppress immune-mediated cartilage destruction while contributing to cartilage repair via their chondrogenic differentiation and paracrine stimulation of endogenous intrinsic repair processes.

To harness the potential of MSC homing, a thorough understanding of the mechanism is needed. It still remains unclear whether MSCs migrate to injured tissues via a mechanistic process, directed by soluble factors, or a passive, stochastic process. This article aims to review current knowledge of the control of MSC homing and shed more light on how this knowledge can be applied to MSC-based treatment approaches for OA and RA.

The mechanistic argument
MSCs express a broad range of chemokine [25, 26] and growth factor [27–30] receptors, which suggests that the soluble ligands of these receptors may play a role in MSC homing. Of particular interest with regard to MSC homing to damaged joints are CXC chemokine receptors 1 and 2 (CXCR1 and CXCR2), CXCR4, CC chemokine receptor 1 (CCR1), CCR2, vascular endothelial growth factor receptor 1 (Flt1), platelet-derived growth factor receptor x (PDGFR-α), PDGFR-β and their respective ligands IL-8, stromal cell-derived factor 1 (SDF-1), macrophage inflammatory protein 1α (MIP-1α), monocyte chemoattractant protein 1 (MCP-1), placenta growth factor (PIGF) and PDGF.

SDF-1
The CXCR4 ligand SDF-1 has been shown to have a dose-dependent effect on human and murine bone marrow-derived MSC (BM-MSC) migration via Boyden chamber and transwell chamber chemotaxis assays (using 8.0 μm pore membranes) [25, 31, 32] that is inhibited in the presence of a CXCR4 antagonist [31–33]. Kitaori et al. [32] showed that SDF-1 induces the migration of murine BM-MSCs in vivo and that the SDF-1-induced migration is mediated by CXCR4. The migration of intravenously administered bromodeoxyuridine (BrdU)-labelled culture-expanded BM-MSCs to a fracture site was significantly reduced in mice treated with a CXCR4 antagonist; 25.2% of chondrocytes in the resulting fracture callus were BrdU-positive in control mice compared with 15.4% in treated mice.

The reported migration of MSCs in an SDF-1 gradient in vivo and in vitro presents an argument for mechanistic control of MSC homing involving the SDF-1/CXCR4 axis, since SDF-1 is known to be up-regulated at sites of tissue damage, including arthritic joints. Kanbe et al. [34] demonstrated that synovial fibroblasts secrete significantly higher (P < 0.01) levels of SDF-1 in OA and RA [575 ng SDF-1/ml culture medium (s.d. 58) and 825 ng/ml (s.d. 89), respectively] compared with normal synovial fibroblasts [380 ng/ml (s.d. 40)], resulting in increased SDF-1 concentration in the SF of patients with OA and RA (3.57-fold and 10.71-fold, respectively) compared with normal controls. This raises the possibility of using the SDF-1 secreted in arthritic joints, and its action as an MSC chemoattractant, to direct MSC homing.

MIP-1α
MIP-1α is a chemokine that causes migration of pro-inflammatory cells to sites of inflammation and regulates their transendothelial migration [35]. Its expression is induced by inflammatory stimuli, mainly in cells involved in the immune response (e.g. macrophages, lymphocytes, dendritic cells), but also in other cells, including fibroblasts and epithelial cells [35]. Its levels are increased in the SF of patients with RA [36]. MSCs express CCR1, one of the receptors for MIP-1α [26].

In cell migration assays carried out by Boomsma et al. [37], the presence of 100 pg/ml MIP-1α increased the migration of culture-expanded murine BM-MSCs through a 3.0 μm pore polycarbonate membrane to 157.6% (s.d. 23.2) of the control (P < 0.05), corroborating the findings of the Boyden chamber and transwell migration assays carried out by Sordi et al. [26] and Takano et al. [38] (using 12 and 8 μm pore polycarbonate membranes, respectively), which showed that MIP-1α induces migration of culture-expanded human and murine BM-MSCs.

The ability of MIP-1α to cause BM-MSC migration [26, 37, 38], in addition to its increased expression at injured sites, suggests that it may be a soluble factor that is involved in a mechanistically controlled process of MSC homing. Its up-regulation in the joints of patients with RA may make it useful as a chemoattractant in targeted MSC-based therapy for arthritis. However, there is a need for more robust evidence on the role of MIP-1α in MSC chemotaxis. We are not aware of any experiments looking into MIP-1α-induced MSC chemotaxis after MSC pre-incubation with CCR1 inhibitor. Such experiments are needed to investigate whether MIP-1α-induced MSC chemotaxis occurs via CCR1 signalling. There is also a need to investigate MIP-1α-mediated MSC migration in vivo to provide evidence of a bona fide role in MSC homing.

IL-8
The expression of IL-8 (a chemokine involved in neutrophil accumulation during the inflammatory response) by
synovium is up-regulated in RA [39] and OA [40, 41]. Using transwell migration assays (with 8 μm pore polycarbonate membranes), Ringe et al. [25] demonstrated that culture-expanded human BM-MSCs show a dose-dependent chemotactic response to IL-8, the CXCR1/CXCR2 ligand. There was no cell migration after elimination of the concentration gradient, showing that MSC migration was a chemotactic response to an IL-8 gradient and not due to chemokinesis.

The known up-regulation of IL-8 at sites of tissue injury and the chemotactic response of MSCs to an IL-8 gradient shown by Ringe et al suggests that IL-8 may be involved in MSC migration to sites of tissue damage. Its up-regulation in arthritic joints may also allow directed MSC-based therapy to be targeted to arthritic joints; however, there is a need for further evidence, especially from experiments in vivo, to either support or disprove the role of the IL-8–CXCR1/CXCR2 axis in MSC homing. Further in vitro experiments need to be carried out using a CXCR1 or CXCR2 antagonist to show that the action of IL-8 on MSC migration is mediated by CXCR1/CXCR2 on MSCs, and in vivo experiments using MSCs pre-incubated with a CXCR1/CXCR2 antagonist would show whether IL-8 is specifically involved in the homing of systemically administered MSCs to sites of tissue damage.

MCP-1

MCP-1 is a chemokine whose expression is up-regulated at times of stress [42]. It signals via the chemokine receptor CCR2, which is expressed on MSCs [25, 29] and is primarily involved in the recruitment of monocytes to sites of inflammation. MCP-1 levels are up-regulated in the SF of patients with OA [43] and RA [44, 45], with the SF of patients with RA [mean 25.5 ng/ml (s.d. 8.1)] containing significantly higher levels of MCP-1 than that of patients with OA [mean 0.92 ng/ml (s.d. 0.08)] [45].

Ringe et al. [25] reported that although human BM-MSCs express CCR2, they do not migrate in response to MCP-1. Takano et al. [38] also reported that murine BM-MSCs do not express and have no significant migratory response to MCP-1. However, other groups have shown that MCP-1 induces the migration of human and murine BM-MSCs in transwell migration assays (3.0 and 8.0 μm pore polycarbonate membranes) [37, 46] and in vivo [47], which is inhibited by MSC pre-incubation with anti-CCR2 antibodies and blockage of downstream CCR2 signalling (via blockage of FROUNT signalling) [47].

The variation observed in MSC migration in an MCP-1 gradient demonstrates the need for further investigation of the role of MCP-1 in homing of human MSCs.

PIGF

PIGF is a protein, highly similar to VEGF [48], that stimulates endothelial cell proliferation [48, 49] and increases the production of inflammatory cytokines—TNF-α, IL-1, IL-8 and MCP-1—from cultured monocytes [50]. Park et al. [51] reported that BM-MSCs express the PIGF receptor (Flt-1). They also demonstrated, via chemotaxis assays using 8 μm pore polycarbonate filters, dose-dependent PIGF-induced BM-MSC migration. This PIGF-induced migration was inhibited in a dose-dependent manner by MSC pre-incubation with an anti-Flt-1 antibody, indicating that PIGF-induced BM-MSC migration is mediated by the PIGF–Flt-1 axis [51] and may be involved in MSC homing.

PIGF is produced by fibroblast-like synoviocytes and is up-regulated in the SF of patients with RA [52]. Its demonstrated ability to induce MSC migration suggests that it may play a role in MSC homing and may allow targeted MSC delivery in RA.

PDGF

PDGF is a regulatory peptide whose expression is up-regulated in the synovial tissue of patients with RA [53]. Boyden chamber assays (using 8 μm nuleopore filters) performed by Cipriani et al. [54] showed that PDGF induces human BM-MSC migration. They also reported that PDGF-induced BMSC migration is inhibited by anti-PDGF antibody [54]. Ponte et al. [29] and Lee et al. [30] reported that human BM-MSCs highly express PDGFRα. They showed that PDGF is a more potent MSC chemo-attractant than chemokines (including SDF-1 and MCP-1) in transwell migration and Boyden chamber assays (using 8 μm pore membranes), which indicates that MSC homing may be a mechanistic process involving PDGF. Interestingly though, Ponte et al. [29] showed that BM-MSCs pretreated with TNF-α have an enhanced migration capacity to chemokines but not to PDGF. The migration capacity of TNF-α pretreated BM-MSCs towards SDF-1 increased by 267% in transwell migration assays; however, there was no significant increase in migration in the presence of PDGF. Considering the lack of increased PDGF-mediated MSC migration in in vitro assays of mimicked inflammation (pretreatment with TNF-α), it can be argued that chemokines may be of greater importance in the mechanistic control of MSC homing to injured sites.

The stochastic argument

As discussed above, the literature shows that there is a variety of signalling molecules, which are up-regulated in arthritic joints and other damaged tissues, capable of causing MSC chemotaxis, suggesting that MSC homing to injured sites may be a mechanistic process (Table 1). However, the findings of several research groups suggest that other passive mechanisms may be involved. MSC homing may well be a stochastic event that could be explained by the vasodilatation and increased blood supply (and cells) to the injured/infamed areas of the body.

Endres et al. [55] showed that SF obtained from patients with RA displayed significantly reduced migration of culture-expanded human mesenchymal progenitors (in transwell migration assays using 8.0 μm pore polycarbonate membranes) compared with SF from normal subjects (P < 0.0001) and patients with OA (P = 0.0054). As has been discussed above, many signalling molecules purposed to play a role in MSC homing are up-regulated in the SF of patients with RA, so these data raise questions
**TABLE 1** Summary of the chemokines and growth factors proposed to be involved in mesenchymal stem cell homing

<table>
<thead>
<tr>
<th>Research group</th>
<th>Methods</th>
<th>Findings</th>
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<tr>
<td>SDF-1</td>
<td>Wynn et al. [31]</td>
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<td>Ringe et al. [25]</td>
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<td></td>
<td>Kitaori et al. [32]</td>
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<td>Belema-Bedada [47]</td>
<td>Intravenous administration of eGFP-labelled BM-MSCs in transgenic mice with specific expression of MCP-1 in the myocardium</td>
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<td>Park et al. [51]</td>
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<td>Lee et al. [30]</td>
<td>Boyden chamber assay using 8.0 µm pore polycarbonate membranes</td>
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BM-MSC: bone marrow mesenchymal stem cell; CCR2: chemokine (C-C motif) receptor 2; CXCR4: CXC chemokine receptor 4; eGFP: enhanced green fluorescent protein; FROUNT: an intracellular molecule that interacts with CCR2; MCP-1: macrophage chemoattractant protein 1; MIP-1α: macrophage inflammatory protein 1α; PDGF: platelet-derived growth factor receptor; PIGF: placenta growth factor; SDF-1: stromal cell-derived factor 1.
about the mechanistic control of MSC homing in vivo. However, an inflammatory milieu has a complex composition of cytokines and chemokines that individually could exert different and even contrasting effects on MSC migration. Hence a net reduction of chemoattractant properties of RA SF would not necessarily oppose a mechanistic basis for MSC homing.

MSC sequestration within the small-diameter capillaries of the lungs, liver and spleen [56–61] has been widely described after i.v. administration. Schrepfer et al. [58]
demonstrated that after i.v. administration of BM-MSCs in mice, MSC trapping in the small-diameter pulmonary capillaries was decreased by pretreatment with a vasodilator, sodium nitroprusside. This information suggests that a passive process of entrapment in injured tissue due to microvascular changes that occur as a consequence of tissue injury may play a role in MSC homing.

**Implications for arthritis**

As with leucocyte homing, MSC homing to injured/inflamed sites may involve both stochastic and mechanistic processes, including chemokine signalling and local microvascular changes. There is a need for further in vivo studies to characterize the mechanistic and/or stochastic processes involved in MSC homing before therapeutic use of systemically delivered MSCs can be considered.

Chemokines have been implicated in MSC migration (Fig. 1), providing avenues for the control of MSC homing in MSC-directed therapy. However, this also creates challenges for targeting exogenously administered MSCs to specific sites, as the chemokines in question are produced by various cell types located at various sites in the body. Another challenge that needs to be addressed for the therapeutic application of MSC homing is how expression of chemokine receptors can be maintained at a high enough level to allow optimal migration of exogenously delivered MSCs in a chronic disease such as arthritis.

The use of bioengineered scaffolds loaded with BM-MSCs genetically engineered to produce bone morphogenetic protein 2 (BMP-2) has shown promise in the treatment of bone defects in vivo [62–64]—sustained production of a BMP (an osteoinductive [65] growth factor with a short half-life) by BM-MSCs in the scaffold maintained the high BMP concentrations needed for bone repair. This concept could be applied to maintain the production of MSC chemoattractants for MSC-based therapy for arthritis.

Several chemokines, including SDF-1 and MCP-1, that have been shown to induce MSC migration and which are also up-regulated in the SF of arthritic joints, have also been shown to inhibit stem cell chondrogenesis and promote chondrocyte necrosis [34, 43, 66, 67]. This may also present challenges in the use of systemically delivered MSCs to specific sites for MSC-based therapy in arthritis.

RA patients who may require MSC-based therapy are likely to be on conventional therapy such as DMARDs or biologics. It is therefore important to consider how these medications could alter MSC function and the chemokine gradients required for MSC homing.

TNF-α is a key mediator in the development of inflammatory diseases such as RA [68] and is now one of the main targets of biologic agents for the treatment of RA. Experiments in vitro have shown that the addition of TNF-α (50 ng/ml) reverses the suppressive effect of MSCs on T cell proliferation [69, 70]. Therefore MSC-based therapy in addition to anti-TNF-α therapy may have a synergistic effect in diseases such as RA. We are not aware of any studies investigating the effect of anti-TNF-α on MSC homing, so further work is needed to see how anti-TNF-α therapy affects MSC homing.

In summary, migrating MSCs represent a source of multipotent cells that could be available for the repair of damaged tissues and organs. The levels of chemokine receptors at the site of injury or inflammation may not be sufficient to recruit the administered exogenous MSCs, raising issues of biodistribution of the MSCs and their long-term fate in the body. Genetic engineering of MSCs for targeted migration to a desired site of the body such as an arthritic joint could be envisaged, e.g. by MSCs expressing antibodies on their cell membrane that recognize epitopes specific to the damaged articular cartilage [71] or by bioengineered scaffolds that produce high levels of MSC chemoattractants at the damaged articular cartilage.

Culture-expanded MSCs appear to have immunomodulatory properties [72] and therefore, after systemic administration, could interact with immune cells and modulate their function during inflammation. Such interactions will likely affect the way MSCs contribute to the repair process [73, 74]. Several challenges remain to be addressed before systemic administration of MSCs becomes a feasible therapeutic approach for targeted delivery to arthritic joints.

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**Rheumatology key message**

- Chemokines released from arthritic joints modulate mesenchymal stem cell homing.

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Clinical vignette

Atypical femoral fractures as an incidental finding on dual X-ray absorptiometry

Atypical femoral fractures (AFFs) have been associated with long-term bisphosphonate use [1]. We present a case of AFF first identified with dual X-ray absorptiometry (DXA). A 64-year-old female attended for DXA with over 10 years of bisphosphonate use. DXA of the left hip showed a focal thickening of the lateral cortex (Fig. 1). Image appearances were drawn to the attention of the patient’s physician and bisphosphonates were discontinued. No further imaging studies were performed. The patient fractured her right proximal femur 95 days later, appearances consistent with AFF. Further imaging with X-rays and CT of the left femur showed focal cortical thickening with a transverse lucency, indicative of impending fracture. This correlated with the area of thickening seen on DXA. The patient underwent bilateral femoral nailing. Twelve months later, the patient is making a good recovery.

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