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Evolving paradigms for repair of tissues by adult stem/progenitor cells (MSCs)

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Abstract

In this review, we focus on the adult stem/progenitor cells that were initially isolated from bone marrow and first referred to as colony forming units-fibroblastic, then as marrow stromal cells and subsequently as either mesenchymal stem cells or multipotent mesenchymal stromal cells (MSCs). The current interest in MSCs and similar cells from other tissues is reflected in over 10,000 citations in PubMed at the time of this writing with 5 to 10 new publications per day. It is also reflected in over 100 registered clinical trials with MSCs or related cells (http//www.clinicaltrials.gov). As a guide to the vast literature, this review will attempt to summarize many of the publications in terms of three paradigms that have directed much of the work: an initial paradigm that the primary role of the cells was to form niches for haematopoietic stem cells (paradigm I); a second paradigm that the cells repaired tissues by engraftment and differentiation to replace injured cells (paradigm II); and the more recent paradigm that MSCs engage in cross-talk with injured tissues and thereby generate microenvironments or 'quasi-niches' that enhance the repair tissues (paradigm III).

Keywords: mesenchymal stem cells • multipotent mesenchymal stromal cells • bone marrow • anti-inflammatory • anti-apoptotic • TSG-6 • STC-1

Paradigm I: the haematopoietic niche

Early attempts to culture bone marrow revealed that a small fraction of the cells that adhered to culture dishes were not haematopoietic precursors (Fig. 1). Some investigators were struck by the morphological similarity of the non-haematopoietic cells to the spindle-shaped cells that formed the stroma of marrow [1–4]. Therefore, they developed the paradigm that the cells formed niches for the propagation of haematopoietic stem cells. The paradigm proved extremely useful in that the confluent cultures of mesenchymal stromal cells (MSCs) were found to be effective feeder layers for the culture of haematopoietic stem cells [5, 6]. The niche role of MSCs was directly demonstrated by the observation that islands of haematopoiesis were formed within ceramic cubes that were seeded with human MSCs and then inserted under the skin of immunodeficient mice [7]. Also, the niche role of MSCs was indirectly supported by clinical trials in which the cells were shown to hasten the recovery of the haematopoietic system after bone marrow transplants [8].

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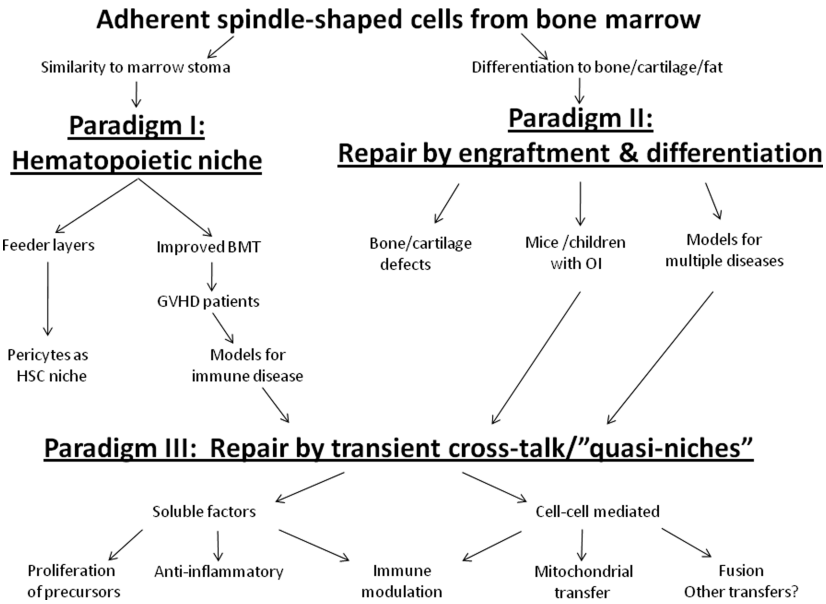

Fig. 1 Schematic summarizing three evolving paradigms for the repair of tissues by MSCs. The morphology of a small number of adherent cells from bone marrow suggested the paradigm that the cells served as a niche for haematopoietic cells (paradigm I). The ready differentiation of the cells in culture suggested that the cells could repair tissues by engrafting and differentiating (paradigm II). Clinical trials using the cells to improve bone marrow transplants unexpectedly demonstrated that they improved graft-*versus*-host diseases in a few patients and thereby drew attention to their immune modulatory properties. Functional improvement without significant engraftment in animal models and a few patients suggested that MSCs enhanced repair by forming microenvironments or 'quasi-niches' (paradigm III).

Paradigm II: engraftment/differentiation

Early investigators studying cultures of bone marrow were impressed with the facility with which the adherent, spindle-shaped cells differentiated into distinct cellular phenotypes. In particular, Friedenstein and others [1] demonstrated that the cells readily became mineralizing cells or chondrocytes both in culture and after implantation in diffusion chambers *in vivo*. These observations suggested the paradigm that MSCs might repair injured tissues by engraftment and differentiation (Fig. 1). The paradigm had broad implications for medical therapies in part because of the ease with which the cells could be isolated from a small sample of human bone marrow and then rapidly expanded in culture through 30 or more population doublings [9–11].

Early observations on engraftment and differentiation

Repair by paradigm II was supported by early observations that local administrations of MSCs improved bone repair [12]. The potential therapeutic implications of the paradigm were expanded by the observation that after systemic infusions of MSCs containing a mutated human gene into irradiated young mice, the mutated gene was detected in multiple tissues of the mice [13]. Also, further support for the therapeutic potentials was provided by the observation that infusions of MSCs from wild-type mice produced small but significant improvements in the bones of a transgenic mouse model for osteogenesis imperfecta [14]. The potential therapeutic implications were expanded still further by the observation that, after BrdU-labelled MSCs were injected into the cerebral ventricles of newborn mice, the cells migrated throughout the brain, and a few of the cells became astrocytes [15].

These early observations prompted a clinical trial in which children with severe osteogenesis imperfecta first received bone marrow transplants from a haplotype-matched normal donor and then were treated, several years later, with intravenous infusions of a large number of MSCs from the same donors [16]. The therapy produced a transient but significant improvement in the clinical course of the children. Most importantly, there was only one adverse event: one of the children developed a mild allergic reaction to foetal calf serum in which the MSCs were expanded. The results were followed by a clinical trial in which administration of MSCs produced encouraging results in children with severe lysosomal storage diseases [17]. These initial observations raised the possibility that paradigm II might provide new therapies for a broad spectrum of human diseases.

Technical challenges in testing paradigm II

The early efforts to test the paradigm encountered a series of technical challenges: (1) No endogenous markers for MSCs were available that could be used to track the cells *in vivo* [18]. Exogenous markers such as dyes or transduced genes were employed instead, but most produced unexpected artefacts [19–21]. (2) Only a small number of antibodies and other markers were available to follow differentiation of the cells *in vivo*. Also, the microscopes and algorithms to overcome some of the artefacts of immunohistochemistry were not commonly available. (3) Species differences in MSCs created a significant experimental barrier. Cultures of human MSCs were relatively easy to purify from haematopoietic precursors by simply re-plating the cells. Cultures

of mouse MSCs remained contaminated by haematopoietic precursors through several passages. Also, as was observed much earlier with mouse fibroblasts [22], cultures of mouse MSCs expanded slowly until they underwent 'crisis' during which a few cells were transformed and then expanded rapidly [23]. Rat MSCs initially resembled human MSCs but at a later stage also underwent crisis and transformation [24, 25]. (4) MSCs were not readily transplanted into marrow ablated mice and therefore presented a further limitation in the use of transgenic mice. (5) Most importantly, tissue repair is a highly complex biological process that varies with the type of injury and the tissue injured [26]. Also, there are marked species differences in inflammatory and immune responses [27] and as a result many experimental animals, especially rodents, repair tissues much more efficiently than human beings. In effect, there were several serious barriers to definitive experiments to test paradigm II.

The impetus to test the paradigm II in clinical trials

Despite these technical challenges, there continues to be great interest in testing the medical implications inherent in paradigm II. The paradigm has been pursued against the history that discoveries of new therapies in medicine have rarely been linear processes. Initial tests of a potential therapy *in vitro* are rarely as convincing as one would like, because of the limitations of experiments with purified molecular components and the artefacts inherent in culturing cells. The data from animal experiments are usually even more limited because of the difficulty of mimicking human diseases. The history of medicine is replete with examples of therapies that failed in the patients despite the extensive basic and preclinical research. However, the history of medicine also includes examples of therapies that were not fully developed or whose beneficial effects were not understood until after they were first tested in patients [28]. The examples include discovery of the anti-thrombotic effects of aspirin [29, 30], the need of HLA typing in bone marrow transplants [31], the revised rationale and design of bisphosphonates for therapy of bone diseases [32] and the failure of sildenafil (Viagra) as a therapy for angina despite the Nobel prize research that led to its development [33, 34] (see Supporting Information).

Tests of the paradigm II with local administrations

Engraftment and differentiation of MSCs, as predicted by paradigm II, were seen in several settings. In models for bone and cartilage defects, a series of reports demonstrated that direct implantation of MSCs themselves or MSCs embedded in scaffolds enhanced repair [35–38]. There is a consensus that some of the administered cells differentiated into osteoblasts or chondrocytes. However, most reports indicated the MSCs disappeared in several weeks [36, 39], and most of the differentiated cells seen in long-term grafts are host cells, at least in part because of the normal turnover of the tissues.

In models of cardiac defects, several reports indicated that locally implanted MSCs engrafted and differentiated into cardiomyocytes [40, 41]. However, it has not been conclusively established that locally administered MSCs provide a sufficient number of fully integrated cardiomyocytes to account for the improvements in ventricular function observed in many experiments [42].

In the central nervous system, some experiments indicated that MSCs injected into the ventricles of embryos or of newborn pups migrated throughout the brain and differentiated as the organ developed [15, 43, 44]. In one series of experiments, quantitative PCR assays indicated that the number of MSCs or MSC-derived cells increased as much as 30-fold in a few days after male MSCs were injected into the ventricles of newborn female mice [43]. The possibility of neural differentiation was supported by the observation that some preparations of MSCs differentiated in culture into dopaminergic-like neurons with the appropriate electrophysiological properties [45]. However, it was difficult to establish differentiation of MSCs into functional neural cells *in vivo* [46, 47].

In contrast to transplants into embryonic brains, very few MSCs injected into the brains of adult rodents survived more than 1 or 2 weeks [21, 48, 49]. Surprisingly, the rate of disappearance was about the same with human MSCs injected into the hippocampi of both immunodeficient and wild-type mice [49].

In models for spinal cord injury, local administration of MSCs produced improved motor function but few, if any, of the cells engrafted for prolonged periods or differentiated into neural cells [50, 51]. One initial impression was that the cells formed a scaffold for regeneration of nerve tracts in the cord [51]. A recent study suggested that the therapeutic benefits were explained by anti-inflammatory effects of the cells [52].

Tests of paradigm II with systemic infusion

Tests of paradigm II with systemic infusions of the cells proved problematic. Numerous reports described functional improvements after systemic infusions of MSCs in models for human diseases that included osteogenensis imperfecta [53]; stroke [54]; myocardial infarction [55]; acute kidney injury [56] and diabetes [57, 58]. The initial interpretations of the data were based on paradigm II and assumed that the cells had homed to injured tissues, engrafted and differentiated to replace injured cells. However, it was difficult to demonstrate extensive engraftment of the cells. Also, the interpretations were not intuitively consistent with several reports about the fate of systemically infused MSCs: Observations with whole body imaging techniques indicated that most MSCs were trapped in the lungs after intravenous infusions into rodents, the route used in most of the experiments [59–61]. Therefore, the functional improvement of distal organs after intravenous infusions of the cells was paradoxical.

To explore the paradox, we recently employed quantitative PCR assays for human MSCs infused into mice [62], a strategy introduced earlier by Phinney and associates for tracking MSCs infused into the brain [43]. (Previous data developed from gel-based PCR assays probably overestimated engraftment of MSCs after systemic infusion [13].) An improved protocol for quantitative PCR

assay of human Alu sequences demonstrated that after i.v. infusion of the human MSCs, essentially all of the cells were cleared from the circulation within 5 min. [62]. Most of the human cells were recovered in the lungs. The cells in the lungs disappeared with a half-life of about 24 hrs but only trace amounts were recovered in the six other tissues that were assayed. Therefore, the results questioned whether paradigm II could account for the functional improvement observed after intravenous infusions of MSCs in animal models for diseases of distal organs. In addition, paradigm II could not account for reports that conditioned medium from cultures of MSCs was as effective in some disease models as the cells themselves [63–65].

Paradigm III: transient 'quasi-niches'

The accumulating evidence that MSCs could repair injured tissues without significant engraftment and differentiation called for a new paradigm that required re-examination of some of the early observations on cultures of the cells and more detailed examination of their effects *in vivo* (Fig. 1).

Unusual features of MSCs in culture

The early observations that confluent and non-propagating MSCs provided effective feeder layers for cultures of haematopoietic cells were explained in part by the cells secreting paracrine factors [5, 6, 66, 67]. However, the effectiveness of MSCs as feeder layers was not entirely explained by secretion of soluble factors; cell to cell contact was also required for reasons that were not apparent [5, 6].

Unusual features of MSCs in culture were also apparent from observing the cells after they were plated at clonal densities. The cells expanded as single-cell derived colonies but the properties of the cells changed as the colonies expanded. In the many of the colonies that formed, distinct inner and outer regions were apparent. The outer regions consisted of rapidly self-renewing cells and the inner regions consisted of slowly replicating cells that were partially differentiated [68]. Moreover, the cells displayed a remarkable plasticity in that the cells from both the inner and outer regions generated single-cell derived colonies with the same characteristics if they were lifted and re-plated at low density. Therefore, the MSCs expanded at clonal densities appeared to reversibly create their own microenvironments or 'quasi-niches' in culture in a manner that paralleled their ability to provide niches for haematopoietic stem cells.

Cross-talk with injured tissues

Although MSCs in culture secreted many cytokines [66, 67], it was not initially apparent that MSCs responded to injured tissues by being activated to express high levels of additional therapeutic proteins. In effect, there was cross-talk in which signals from injured cells activated MSCs to alter expression of large families of genes. At the same time signals from the activated MSCs both up-regulated and down-regulated large families of genes in the injured cells.

One of the first examples of cross-talk was observed between MSCs and multiple myeloma cells [69]. Co-culture experiments demonstrated that signals from the myeloma cells stimulated the MSCs to increase secretion of interleukin (IL)-6 and this IL-6 in turn, increased the proliferation of the myeloma cells. At the same time, the myeloma cells secreted high levels of Dkk-1, an inhibitor of Wnt signalling, that kept the MSCs in cell cycle and inhibited them from differentiating into osteoblasts. The cross-talk provided an explanation for why patients with multiple myeloma develop osteolytic lesions in which the cancer cells proliferate but osteoblasts are not recruited to fill the lesions [69].

A second example of cross-talk was encountered in experiments in which human MSCs were injected into the hippocampi of mice following transient cerebral ischemia. The human MSCs reduced neuronal death and improved the neurological deficits [49]. Assays of RNA from the hippocampus with human-specific mRNA/cDNA microarrays demonstrated that in the ischemia injured brain, the human MSCs increased expression of genes that modulated immune and inflammatory responses. Assays of the same RNA on mouse-specific microarrays demonstrated that the presence of the human MSCs modulated expression of mouse genes involved in immune responses to the ischemic environment.

A similar example of cross-talk was obtained by using species-specific mRNA/cDNA microarrays to survey the lungs of mice a few hours after intravenous infusions of human MSCs [62]. By producing microemboli, the human cells altered expression of hundreds of mouse genes in the lung. At the same time, signals from the mouse cells altered expression of hundreds of genes in the human MSCs. In parallel with these observations, reports from several laboratories demonstrated that the expression of potentially therapeutic cytokines was markedly increased by exposing MSCs to cytokines typically released by injured tissues [70, 71].

Modulation of inflammation in paradigm III

The experiments in which human MSCs were infused intravenously into mice with myocardial infarcts provided a clue to how they enhanced tissue repair. One of the most interesting genes up-regulated in human MSCs that were trapped in the lung after intravenous infusion [62] was tumour necrosis factor (TNF)-α stimulated gene/protein-6 (TSG-6) [72, 73]. Extensive previous research demonstrated that TSG-6 had remarkable anti-inflammatory properties in a number of experimental settings, including in both wild-type and transgenic mice [72, 73]. Experiments with recombinant TSG-6 and siRNAs demonstrated that the secretion of TSG-6 by MSCs trapped in the lung largely accounted for previous reports that intravenously administered MSCs improved mice with myocardial infarcts [55, 74–76]. TSG-6 decreased

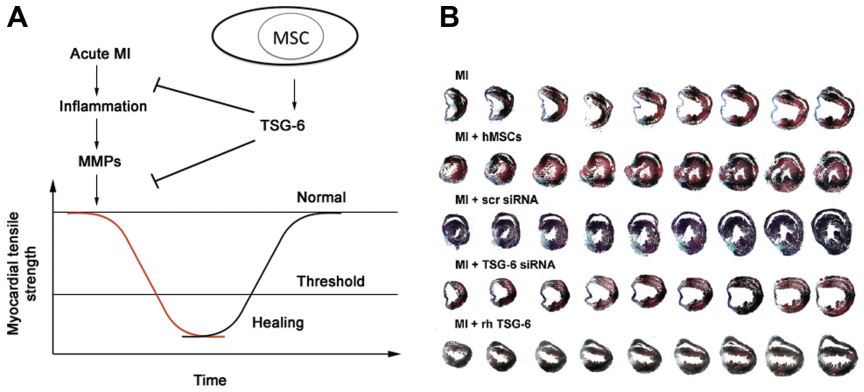

Fig. 2 Effects of human MSCs and recombinant TSG-6 in mice (NOD/scid) with myocardial infarcts (MI). (**A**) Schematic illustrating the progressive damage to the myocardium following myocardial infarction. The ischemia triggers invasion by inflammatory cells. The inflammatory cells and the matrix metalloproteinases they release accentuate damage to the myocardium. TSG-6 synthesized by MSCs or recombinant TSG-6 limits the injury and thereby enhances repair. Reproduced with permission and modified from [100]. (**B**) Protective/reparative properties of MSCs and TSG-6 in MI. Three weeks after permanent ligation of the anterior descending coronary artery in mice to produce MI, each heart was cut from the apex through base into over 400 sequential 5 μm sections and stained with Masson Trichrome. Every 20th section is shown. Symbols: Normal, naïve mice; –, MI only; hMSCs, 2×10^6 hMSCs infused intravenously (i.v.) 1 hr after MI; scr siRNA, 2×10^6 hMSCs transduced with scrambled siRNA infused i.v. 1 hr after MI; TSG-6 siRNA, 2×10^6 hMSCs transduced with TSG-6 siRNA infused i.v. 1 hr after MI; rhTSG-6, 30 μg recombinant TSG-6 protein infused i.v. 1 hr and again 24 hrs after MI. Reproduced with permission from [62].

activation of the inflammatory network of proteases in the heart and decreased monocyte and granulocyte infiltration. The TSG-6 thereby decreased the damage to cardiomyocytes and the size of the myocardial scar that subsequently formed (Fig. 2).

Modulation of apoptosis in paradigm III

Several reports indicated that one of the potential therapeutic effects of MSCs was to decrease apoptosis [77, 78]. Co-culture experiments demonstrated that MSCs decreased apoptosis in two model systems in part by being activated to express stanniocalcin-1(STC-1), a calcium regulatory protein [79]. The effects of STC-1 on apoptosis were apparently explained by its uncoupling of oxidative phosphorylation and suppression of reactive oxygen species [80]. Suppression reactive oxygen species also explains the recent observation the STC-1 has anti-inflammatory properties [81].

Modulation of immune reactions

Preliminary observations made in clinical trials to improve bone marrow transplants with MSCs provided an unexpected observation: In a few patients, the MSCs improved the effects of graft-*versus*-host disease [82]. These and related observations led to experiments that demonstrated intravenous infusions of MSCs reduced neurological deficits in the experimental autoimmune encephalitis (EAE) model for multiple sclerosis (see [83]). The findings spurred extensive efforts to define the mechanisms whereby MSCs modulated the immune system. The results have provided several different scenarios. Here we will focus on four recent accounts. (For more complete reviews, see [83, 84].)

Shi and associates [70] demonstrated that the immunosuppressive effects of murine MSCs were triggered by the cells being stimulated by interferon-γ together with any one of three other pro-inflammatory cytokines (TNF-α, IL-1α or IL-1β). The stimulated MSCs expressed several cytokines and inducible nitric oxide synthase. The chemokines attracted T cells to the MSCs and then the T cells were suppressed by nitric oxide from the MSCs. They subsequently found a marked species difference in that human and monkey MSCs did not synthesize nitric oxide under similar conditions. Instead, the MSCs suppressed T cells by secreting indoleamine 2,3-dioxygenase that depleted tryptophan in the medium or generated toxic concentrations of kynurenine and other metabolites to suppress T cells [85].

Galipeau and associates [86] examined the effects of MSCs on activated $CD4^+$ T cells in the EAE model for multiple sclerosis. They found that the MSCs inhibited activation of the T cells by secreting both CCL2 (monocyte chemotactic protein-1 or MCP-1) and matrix metalloproteinases-9 that cleaved the CCL2 into an antagonistic derivative. The role of the soluble factors was confirmed by the demonstration that conditioned medium from MSCs inhibited activation of $CD4^+$ T cells from EAE mice and that the effects of MSCs were not observed in $CCL2^{-/-}$ EAE mice. The same laboratory also demonstrated that MSCs can stimulate immune and inflammatory responses. They found that MSCs can cross-present exogenous antigen and induce an effective $CD8^+$ T-cell immune response [87]. They can also be activated through Toll-like receptors to recruit inflammatory and immune cells [71].

Mahon and associates [88] suggested that MSCs might exert their immune regulatory effects by enhancing T regulatory cells. They demonstrated that allogeneic MSC induced expression in $CD4^+$ T cells of forkhead box $P3^+$ and $CD25^+$, both markers of T regulatory cells. Their results supported a sequential process in which a first step required direct contact between MSCs and $CD4^+$ T cells followed by secretion of transforming growth factor-β_1 and prostaglandin E2 by the MSCs to drive differentiation of T cells to T regulator cells.

Uccelli *et al.* [83] offered a more pleiotrophic account of the effects of MSCs on the immune system. They suggested that

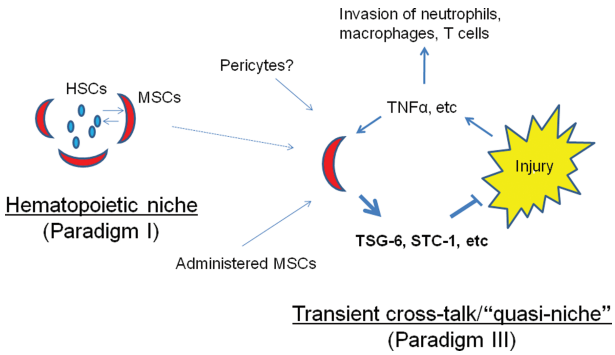

Fig. 3 Schematic for MSCs providing a niche for haematopoietic stem cells as in paradigm I and modulating excessive inflammatory and immune responses as in paradigm III. One effect of administered MSCs is to introduce a negative feedback into the excessive responses of tissues to sterile injury. They may also enhance repair by increasing propagation and differentiation of tissue endogenous stem/progenitor cells (not shown). Some of the therapeutic effects of MSCs may require direct cell-to-cell contact and transfer of components such as mitochondria.

MSCs produced a variety of effects such as (1) decreased proliferation, cytotoxicity and cytokine production by NK cells; (2) impaired maturation and antigen presentation by dendritic cells; (3) decreased proliferation of T cells and impaired T helper cells and (4) decreased proliferation and antibody production by B cells.

At the moment, it is not clear which of the proposals best accounts for the immune modulatory effects of MSCs *in vivo*.

Paradigm III and the similarities to paradigm I

The experiments in which MSCs enhance tissue repair without significant engraftment suggest that there is a complex series of interactions between the MSCs and the injured tissues. One of the key interactions is a sequence in which TNF-α and other signals from injured tissues activate the MSCs to secrete TSG-6, STC-1 and probably other soluble factors that decrease the production of TNF-α and other inflammatory signals from the injured tissues (Fig. 3). In effect, the MSCs introduce a negative feedback loop into excessive responses by tissues that frequently occur in injuries not accompanied by invading organisms. Such excessive inflammatory and immune responses are now recognized to contribute to the pathoetiology of many diseases, including diabetes and artherosclerosis [89, 90]. Secretion of soluble factors probably explains the therapeutic effects of intravenous infusion of MSCs or conditioned medium from MSC cultures in some animal models. However, some of the therapeutic effects, such as in models for immune diseases, may require direct cell-to-cell contact between MSCs and target cells. Also, in addition to modulating inflammatory/immune reactions, MSCs may enhance repair of tissues by stimulating the proliferation and differentiation of tissue endogenous stem/progenitor cells as was observed with infusion of MSCs into the hippocampus of mice [48]. Many of the effects of MSCs on tissue repair are transient 'hit and run' events (paradigm III) but they have some similarities to the ability of the cells to provide a niche for haematopoietic cells (paradigm I).

Conclusions/perspectives

Our knowledge of MSCs has evolved largely by serendipity, beginning with the first efforts to culture cells from bone marrow. Although our knowledge continues to expand at a rapid pace, a number of important questions still need to be addressed. Some examples include:

Why is administration of MSCs beneficial?

Bone marrow, fat and many other tissues contain MSCs or MSC-like cells. Therefore, it is not apparent why adequate numbers are not normally mobilized in response to tissue injury. One possibility is that the isolation of the cells from tissues or their expansion in culture may activate therapeutic properties of the cells that are otherwise latent. Another is that the normal mechanisms for mobilizing MSCs are simply not adequate to modulate the excessive inflammatory and immune responses to sterile tissue injuries.

Better assays for the potency of MSCs?

A major barrier to progress in the field is lack of an *in vivo* potency assay for MSCs. What is needed is an assay equivalent to the marrow ablated mouse that was key to essentially all the progress in the study of haematopoietic stem cells. Data on the transcriptomes or proteomes of cultured MSC are not adequate since they are simply snapshot pictures of the cells. Instead, what is needed is an assay of the potential of MSCs to respond to environmental factors such as signals from injured tissues. Unfortunately, current *in vitro* assays of differentiation or clonogenicity continue to disappoint. Given the multiple modes of action of MSCs, a battery of *in vivo* potency assays may be required.

Are MSCs pericytes?

Recent reports have provided convincing data for earlier suggestions (see [9]) that MSCs share many of the features of pericytes [91–93]; cells that have fascinated investigators since they were first described by Rouget in 1873 (see [94]). The similarities between MSCs and pericytes are impressive, including the sharing of several epitopes and the ability of pericytes to differentiate into multiple cellular phenotypes such as fibroblasts, osteoblasts, adipocytes, chondrocytes and endothelial cells. However, the overlap in properties is not complete. For example, pericytes from different vessels vary but most display contractility and myogenic

properties not observed with isolated MSCs. Also, pericytes propagate much more slowly than MSCs, *i.e.* initial population doubling rates as slow as 162 hrs [92] *versus* 12 to 20 hrs for MSCs. Therefore, pericytes and MSCs clearly have similar but perhaps not identical properties.

Therapies with recombinant proteins?

Recent observations suggest that therapies with some of the proteins produced by MSCs could replace therapies with the cells themselves. Use of the proteins has many attractions, but MSCs may provide major advantages in many situations by their responsiveness to the particular injury and their ability to deliver factors in high local concentrations. Also, as suggested by paradigm III, some of the therapeutic benefits of MSCs may require cell-to-cell contact for transfer of vesicles or other components such as mitochondria [95] that have not yet been defined.

Additional questions in developing therapies with MSCs

A number of additional questions need to be resolved to develop therapies with MSCs. Although no significant adverse events have been reported from clinical trials to date, all interventional therapies have some inherent risks and questions about the potential risks of therapies with MSCs must be carefully weighed against the potential benefits to patients. One question about the potential risks is whether MSCs, like embryonic stem cells or induced pluripotent stem cells, can cause tumours and malignancies [96]. The risk cannot be ignored, particularly since MSCs were observed to enhance the growth of some tumours [97]. However, MSCs in culture differ from embryonic stem cells and induced pluripotent cells in that they are not immortal cells and undergo senescence when expanded in culture. (A recent report indicated that a previous observation of malignant transformation of human MSCs during expansion in culture was explained by contamination of the cultures by small numbers of malignant cells [98].) Another question still under debate is whether autologous MSCs should be used or whether therapies with heterologous MSCs from 'universal donors' can be employed, a strategy currently embraced by several biotech companies. We all await the data from carefully conducted clinical trials and from additional basic research to resolve these and other remaining questions about MSCs.

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Conflict of interest

The authors confirm that there are no conflicts of interest.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Examples of Medical Therapies Developed after First Trials in Patients.

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