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In Search of the In Vivo Identity of Mesenchymal Stem Cells

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ABSTRACT

In spite of the advances in the knowledge of adult stem cells (ASCs) during the past few years, their natural activities in vivo are still poorly understood. Mesenchymal stem cells (MSCs), one of the most promising types of ASCs for cell-based therapies, are defined mainly by functional assays using cultured cells. Defining MSCs in vitro adds complexity to their study because the artificial conditions may introduce experimental artifacts. Inserting these results in the context of the organism is difficult because the exact location and functions of MSCs in vivo remain elusive; the identification of the MSC niche is necessary to validate results obtained in vitro and to further the knowledge of the physiological functions of this ASC. Here we show an analysis of the evidence suggesting a perivascular location for MSCs, correlating these cells with pericytes, and present a model in which the perivascular zone is the MSC niche in vivo, where local cues coordinate the transition to progenitor and mature cell phenotypes. This model proposes that MSCs stabilize blood vessels and contribute to tissue and immune system homeostasis under physiological conditions and assume a more active role in the repair of focal tissue injury. The establishment of the perivascular compartment as the MSC niche provides a basis for the rational design of additional in vivo therapeutic approaches. This view connects the MSC to the immune and vascular systems, emphasizing its role as a physiological integrator and its importance in tissue repair/regeneration. STEM CELLS *2008;26:2287–2299*

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

INTRODUCTION

Recent years have witnessed an exponential increase in the number of published papers dealing with stem cells (SCs) (Table 1). The number of publications on embryonic stem cells (ESCs) entered exponential growth phase 2 years after Thomson et al. derived the first human ESC line [1], reflecting the potential of using ESCs in cell-based therapies for humans [2]. ESCs alone were not responsible for the exponential increase in papers on SCs, since the number of publications on nonembryonic SCs also increased exponentially. Adult stem cells (ASCs), even though they are generally regarded as having restricted differentiation capabilities compared with ESCs [3], have been the focus of a large part of stem cell research.

In contrast to this increased interest in ASCs in recent years, their individual in vivo locations and activities are still poorly understood. ESCs can be reproducibly obtained from the inner cell mass of blastocysts, whereas ASCs cannot be reproducibly isolated, for example, by sorting on the basis of morphological characteristics or surface molecules, because ASC populations do not have unique markers. These cell surface markers are specific only in a particular context or are redundantly expressed by other SCs. Consequently, ASCs are usually defined on an operational basis: culture adherence or growth in suspension, and their ability to self-renew and to differentiate into at least one mature cell type. These functional assays are usually performed in vitro. Again, the anatomic location and in vivo functions have not been emphasized, whereas understanding the biology of ASCs in vivo is fundamental for the development of successful cell-based therapies [4].

In considering in vitro approaches, it is recognized that cells are subjected to conditions that are quite different from those found in their original anatomic location, in spite of efforts to mimic site-specific conditions. The success of this mimicry is dependent on several factors, including the cell type studied. ESCs, for example, retain their pluripotency when cultured, as shown by their ability to form teratomas when injected into immunodeficient mice [5]. ASCs, on the other hand, are altered by the culture conditions, and the results from functional assays performed in vitro may sometimes be difficult to interpret. The possible existence of ASCs as heterogeneous populations in vivo adds complexity to their study in vitro, since the isolation procedures or the culture conditions used may select for specific subsets of cells or cellular characteristics.

Avoiding stem cell cultivation is the logical alternative for the purpose of preserving the properties of the cell populations isolated. However, culture expansion may be necessary, since high absolute numbers of SCs are required and their in vivo frequencies are intrinsically low. Unlike ESCs, which proliferate at high rates during development, ASCs are supposed to be slow-cycling in vivo [6]. Consequently, ASC expansion in vitro may be contrary to their expected natural

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Table 1. Numbers of articles on stem cells published in a recent 10-year period (1997–2006)

Year	SC	ESC	HSC	MSC
1997	1,990 (418)	193 (23)	407 (126)	16 (2)
1998	2,164 (493)	213 (38)	412 (135)	23 (5)
1999	2,486 (510)	210 (34)	487 (149)	40 (10)
2000	2,905 (576)	254 (34)	591 (154)	60 (17)
2001	3,417 (819)	336 (64)	680 (212)	79 (15)
2002	4,156 (1,041)	438 (98)	945 (260)	148 (36)
2003	4,715 (1,147)	505 (107)	1,027 (266)	270 (48)
2004	5,605 (1,312)	736 (153)	1,082 (264)	411 (58)
2005	6,803 (1,583)	950 (199)	1,330 (342)	697 (94)
2006	7,676 (1,416)	1,210 (181)	1,332 (240)	890 (78)

The PubMed database (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?DB=pubmed) was queried using the following terms (quotation marks included): "stem cell" OR "stem cells" for SC; "embryonic stem cell" OR "embryonic stem cells" for ESC; "hematopoietic stem cell" OR "hematopoietic stem cells" OR "hemopoietic stem cell" OR "hemopoietic stem cells" for HSC; and "mesenchymal stem cell" OR "mesenchymal stem cells" for MSC. The queries were limited to Title/Abstract fields, in addition to publication years. Numbers in parentheses correspond to the number of review articles of the total yearly number.
Abbreviations: ESC, embryonic stem cells; HSC, hematopoietic stem cells; MSC, mesenchymal stem cells; SC, stem cells.

behavior, and expanding ASCs while still keeping their undifferentiated state is difficult [7].

With the above in mind, we focus on an ASC historically derived from bone marrow and called a mesenchymal stem cell (MSC), since MSCs have been shown to have a broad differentiation capability and reproducible culture methods are available that allow for their expansion in an apparently undifferentiated state [8]. These properties are comparable, to some extent, to those of ESCs, with the advantage of the absence of associated ethical problems or possibility of teratoma formation. These characteristics may explain the exponential increase in the number of papers published on MSCs in a recent 10-year period, whereas the increase in publications on hematopoietic stem cells (HSCs), the best-studied type of ASC, remained somewhat linear (Table 1).

Hypothesis

We focus here on MSCs and use this discussion to provide evidence for the following thesis: MSCs are situated throughout the body as pericytes. We would further state that their in vivo functions are to stabilize blood vessels and to contribute to tissue homeostasis [9]. With the disruption of blood vessels in or near injured tissue, the MSC pericyte is liberated; it divides and secretes bioactive factors that function to protect and repair or regenerate the injured tissue. The secretory products of MSCs at sites of injury strongly repress immune surveillance and inhibit the T- and B-cell-mediated destruction of the injury site. This immunoprotection serves to protect tissue from losing its "tolerance" and thus eliminates autoimmunity issues. This proposition may be extended to allow the inference that defects in these immunoregulatory capabilities of MSCs may result in diseases such as multiple sclerosis or type I diabetes. In addition, the secretory products have a powerful trophic effect [10] on the injury site that limits the field of injury (antiapoptosis), inhibits scarring, stimulates angiogenesis/vasculogenesis, and is mitotic to tissue-intrinsic progenitor cells. The implication of this hypothesis is that the perivascular zone in all tissue sites is the MSC niche. The corollary is that the decrease of vascular density in some tissues with age also results in a diminution of MSC titers.

Mesenchymal Stem Cells

MSCs may be defined as progenitor cells capable of giving rise to a number of unique, differentiated mesenchymal cell types [11]. First described as fibroblast precursors from bone marrow (BM) by Friedenstein et al. in 1970 [12], MSCs may be also referred to as fibroblast colony-forming units (CFU-Fs) or marrow stromal cells [13]. There is evidence that MSCs exist not only in bone marrow but in virtually all organs [9, 14, 15].

In the bone marrow, MSCs are an important component of the HSC niche. Although the HSCs are the best-characterized adult stem cells, their niche is still poorly understood. Recent studies have shown the existence of two types of HSC niches in the BM, an "endosteal" niche and a "perivascular" niche (reviewed in [16, 17]), which may be close to each other or may be interdigitated. MSC-derived cells, such as osteoblasts and fibroblasts, are mainly involved with the endosteal niche, located at the endosteum of trabecular bone (reviewed in [18]). Besides producing mature, specialized cells that interact with the HSCs, mesenchymal stem cells are also directly involved with regulation of the hematopoietic process [16]. Several molecules produced by niche cells can regulate HSC number and function through a complex paracrine signaling network (reviewed in [19, 20]). A recent study of the global transcriptional profile of murine MSCs, complemented by functional studies, identified a set of genes specifically involved in the HSC niche [21].

Although no unique markers are known for MSCs, their cell-surface antigen profile has been well explored (reviewed in [22]), and a comprehensive list of surface markers in MSCs and other cell types is shown in Table 2. As part of the minimal criteria proposed by the Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy to define human MSCs [23], cells must be positive for CD105, CD73, and CD90 and negative for CD45, CD34, CD14 or CD11b, CD79a or CD19, and HLA-DR. The identification of a definitive marker allowing the prospective isolation of MSCs from fresh tissue would be of the utmost importance. Stro-1, the best-known MSC marker, is not exclusive to these cells and is lost during culture (reviewed in [22]), so that it is not a general MSC marker. Recent studies have shown that stage-specific embryonic antigen 1 (SSEA-1) and SSEA-4 are markers for primitive mesenchymal cells in murine and human bone marrow [24, 25]. Fluorescence-activated cell sorting of human bone marrow mononuclear cells based on the expression of CD49b, CD105, CD90, CD73, CD130, CD146, CD200, and $\alpha V/\beta 5$ integrin allowed for 23-, 50-, 60-, 100-, 256-, 278-, 333-, and 1,750-fold enrichment of recovered CFU-Fs, respectively, and the expression of CD73, CD146, and CD200 was found to be downregulated during differentiation [26]. $CD200^+$ cells were shown to possess osteo-, chondro-, and adipogenic differentiation capabilities, suggesting the use of CD200 for the purification of bone marrow MSCs. CD200 is also expressed by thymocytes, B and T lymphocytes, and endothelial cells [27]; nevertheless, its use in combination other MSC markers shows promise for the isolation of pure bone marrow MSCs.

Cultured MSCs and their progeny exhibit a high degree of plasticity. For example, cultured human articular chondrocytes have been shown to be inducible into the osteogenic and adipogenic pathways [28], and cultured adipocytes into the chondrogenic or osteogenic pathways. This plasticity may not be limited to the mesenchymal phenotypes, when maintained in vitro or implanted in vivo [29–33], although these studies can be interpreted in other ways. For example, by using marked MSCs into in vivo situations that have experimentally produced tissue damage, the label is seen in nonmesenchymal cell types [34]. These observations may be explained, in most instances, by the fusion of the marked cell with host cells. Likewise, the effects of such injected MSCs may be due

Table 2. Markers expressed by MSCs or pericytes and other cell types

CD	Molecule	MSCs	Pericytes	vSMCs	ECs
	3G5 antibody-defined ganglioside	(+) UO	(+) [**178**, 179]	(−) [179]	(−) [180]
	Angiopoietin-1		(+) [181]		(−) [181]
	Angiopoietin-2		(−) [181]		(+) [181]
	Annexin A5		(+) [**182**]		(−) [**182**]
	Calponin	(+) [183]	(−) [**184**]	(+) [**184**]	
	Desmin	(+) [183]	(−) [**184**]	(+) [**184**]	
	Nestin	(+) [185, 186[c]]	(+) [**187**, **188**]		(+) [**188**[b]]
	NG2 proteoglycan, HMW-MAA	(+) UO	(+) [**189**, **190**, 165, 191]	(+) [191]	(−) [191]
	Sca-1 (mouse only)	(+) [**135**]	(+) [**182**]		(+) [**192**]
	Smooth muscle myosin		(−) [**184**]	(+) [**184**]	(−) [117]
	Stro-1	(+) [193]	(+) [111, 115]		(+) [111, 115]
	Tie-1		(−) [181]		(+) [181]
	Tie-2		(−) [181]		(+) [181]
	VEGFR1		(−) [181]; (+) [**194**,[a] **195**[a]]		(+) [181]
	Vimentin	(+) [185, 186]	(+) [**184**]	(+) [**184**]	(+) [180]
	vWF	(−) [43]			(+) [196]
	α-SMA	(+) [183, **9**]	(+) [**184**, 197]	(+) [**184**, 197]	(−) [179]
4		(−) [43]			
9		(+) [43]			
10		(+) [185]			
11a	Integrin αL chain	(−) [43]			
11b	Integrin αM chain	(−) [**135**]			
13	Aminopeptidase N	(+) [185]	(+) [**187**]	(+) [**187**]	(+) [198]
14		(−) [43]			
15		(−) [43]			
18	Integrin β2 chain	(−) [43]			
25	IL-2R	(−) [43]			
29	Integrin β1 chain	(+) [43]			
31	PECAM	(−) [43]	(−) [**199**]		(+) [196]
34		(−) [43]; (−)/(+) [**9**]			(+) [196]
36					(+) [**192**]
44	Hyaluronan receptor	(+) [43]	(+) [**199**]		
45		(−) [43]			
49a	Integrin α1 chain	(+) [43]			
49b	Integrin α2 chain	(+) [43]			
49c	Integrin α3 chain	(+) [43]			
49d	Integrin α4 chain	(−) [43]; (+) [**135**]			
49e	Integrin α5 chain	(+) [43]			
50	ICAM-3	(−) [43]			
51	Integrin αV chain	(+) [43]			(+) [**192**]
54	ICAM-1	(+) [43]			(+) [**192**]
58	LFA-3	(+) [43]			
61	Integrin β3 chain	(+) [43]			(+) [**192**]
62E	E-selectin	(−) [43]			(+) [**192**]
62L	L-selectin	(+) [43]			
62P	P-selectin	(−) [43]			(+) [**192**]
71	Transferrin receptor	(+) [43]			
73	Ecto-5′-nucleotidase	(+) [43]			(+) [**192**]
90	Thy-1	(+) [43]	(+) [**199**]		
102	ICAM-2	(+) [43]			(+) [**192**]
104	Integrin β4 chain	(+) [43]			
105	Endoglin; TGFβRIII	(+) [43, 200]	(+) [201]		(+) [201]
106	VCAM-1	(+) [43]	(+) [**121**]		(+) [**192**]
109		(+) [185]			
117	c-KIT; SCFR	(−) [185], (−)/(+) [**9**]			
w119	IFNγR	(+) [43]			
120a	TNFIR	(+) [43]			
120b	TNFIIR	(+) [43]			
121a	IL-1R	(+) [43]			
123	IL-3Rα	(+) [43]			
124	IL-4R	(+) [43]			
126	IL-6R	(+) [43]			
127	IL-7R	(+) [43]			
133	AC133	(−) [185]			
140a	PDGF-R; PDGFRα	(+) [185]			
140b	PDGFRβ	(+) [185]	(+) [**189**]		
144	VE-cadherin	(−) [43]			(+) [**192**]
146	MUC18, S-endo	(+) [93]	(+) [17]		(+) [**192**], [202]
164		(+) [185]			
200	OX2	(+) [26]			(+) [27]
172a		(+) [185]			
249	Aminopeptidase A		(+) [**187**]	(+) [**187**]	
271	p75, NGFR		(+) [158]		

This table incorporates results from different species, and there may be species-dependent variations. References in boldface denote results from studies using species other than human. (+) and (−) indicate presence and absence of marker, respectively.

[a]Under hypoxic conditions.

[b]In neocapillaries.

[c]In subsets.

Abbreviations: CD, cluster of differentiation number; ECs, endothelial cells; IFNγR, interferon (gamma) receptor; MSCs, mesenchymal stem cells; NG2, nerve/glial antigen 2; PDGF, platelet-derived growth factor; PDGFRα, platelet-derived growth factor receptor (alpha); PDGFRβ, platelet-derived growth factor (beta); SMA, smooth muscle actin; UO, unpublished observations; VEGFR1, vascular endothelial growth factor-receptor-1; vSMCs, vascular smooth muscle cells; vWF, von Willebrand factor.

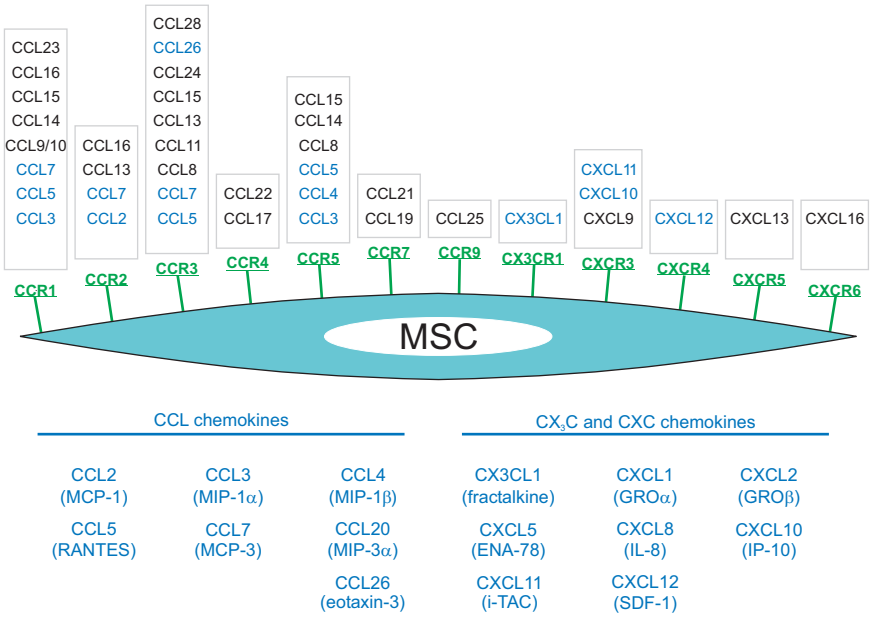

Figure 1. Expression of chemokines and chemokine receptors by cultured MSCs. MSC chemokine receptors are represented in green. Chemokines secreted by MSCs are shown in blue. Chemokines able to stimulate MSCs, including those that can act in a paracrine way (in blue), are contained in the boxes directly above their respective receptors (based on [203–207]). Chemokine receptor nomenclature and ligands are described in detail elsewhere [208]. Abbreviations: ENA-78, epithelial neutrophil-activating protein 78; IL-8, interleukin 8; IP-10, interferon-inducible protein-10; i-TAC, interferon-inducible T-cell alpha chemoattractant; MCP-1, macrophage chemoattractant protein 1; MIP, macrophage inflammatory protein; MSC, mesenchymal stem cell; RANTES, regulated upon activation, normal T-cell expressed and secreted; SDF-1, stromal-derived factor-1.

to secretory products rather than the differentiation of the MSC into a nonmesenchymal phenotype.

MSCs have shown promising results in preclinical and clinical studies for a number of conditions (reviewed in [35–37]), such as bone or cartilage defects, cardiac disorders, central nervous system or spinal cord injury, and lung diseases. The use of MSCs for tissue engineering also shows promise, and to date bioengineered structures with a defined shape can be produced by combining these cells with appropriate biomaterials and bioactive factors [38]. Injected MSCs have also hastened hematopoietic recovery after bone marrow transplantation, and their immunoregulatory properties facilitate engraftment of transplanted organs and reduce graft-versus-host disease [39]. Again, MSCs appear to exert paracrine trophic effects through the secretion of bioactive molecules [10, 40] and hold great promise as tools for cell-based therapies.

Protocols for the isolation of MSCs involve the selection of plastic-adherent cells [41–43]. Our lack of understanding of the basic biology of MSCs is reflected in the different culture systems and nomenclature used: nonequivalent cell populations have been named MSCs, marrow stromal cells, marrow-isolated adult multilineage inducible cells (MIAMI), recycling SCs (RS-1, RS-2), and multipotent adult progenitor cells [MAPCs] (reviewed in [44]). An attempt to clarify the nomenclature for MSCs has recently been put forward by the International Society for Cellular Therapy [45] and discussed at several international meetings, such as Adult Mesenchymal Stem Cells in Regenerative Medicine (MSC 2007, http://www.msc2007.net).

MSCs have been studied extensively in culture; our inability to prospectively identify MSCs in their natural location in the organism has the result that "MSC biology, at the present time, remains biology out of context" [46]. Even though several cell populations derived from bone marrow or other tissue locations from human and other species have been reported to differentiate into mesenchymal cell phenotypes in vitro (as discussed below), it is important to recall that there is evidence that cells that have a mature phenotype in vivo are able to dedifferentiate to a more primitive phenotype when cultured [47] and to differentiate into other cell types in vitro [28, 48]. Consequently, MSCs defined operationally following in vitro culture on the basis of their ability to self-renew and to differentiate into mesodermal cell lineages might represent experimental artifacts. In vitro differentiation of MSCs into ectodermal [30] and endodermal [32] cell lineages could, likewise, be a consequence of cell culture rather than representing an intrinsic MSC in vivo differentiation potential.

Overall, even though culture conditions may be created that are complex and provide a medium composition very similar to that found in vivo, they are not able to supply all the extrinsic factors [49] provided by the stem cell niche. A stem cell out of its original environment may not, and probably will not, behave in the same way it would if it remained in its natural habitat. Hence, cultured MSCs should not be considered equivalents to MSCs under physiological conditions in vivo.

MSC Paracrine Effects

The reproducible isolation and differentiation capabilities of MSCs suggested their use as building blocks for tissue engineering [50]. The use of MSCs for cell therapy is also feasible, as they display multisite engraftment when systemically injected in vivo [51] and exhibit an intrinsic ability to home to injured sites [52–54]. The assumption that MSCs present in bone marrow aspirates would differentiate into mature cell types after transplantation into children with osteogenesis imperfecta [55, 56] is an example of their use that takes advantage of their differentiation potential. Accordingly, cultured MSCs were infused into children with osteogenesis imperfecta who had previously received bone marrow transplantation. The beneficial treatment effects, as judged by the patients' growth velocity, decreased to baseline levels at around 6 months after MSC infusion [57].

Experiments using transplantation of cultured MSCs into animals led to the realization that MSCs' therapeutic effects could not be explained by differentiation into tissue-specific cells alone [58–61]. Indeed, transplanted MSCs exert beneficial effects through the secretion of bioactive factors [62–66]. Similar findings have been recently observed for neural SCs [67]. Bioactive factors secreted by MSCs have angiogenic and antiapoptotic properties that serve to limit the extent of tissue damage at the injured sites and to re-establish blood supply. Furthermore, systemically injected MSCs reduce fibrosis in animal models of drug-induced tissue injury, indicating that they also secrete antifibrotic factors [68, 69]. The MSC paracrine effects mentioned above have been referred to as trophic effects [10]. In addition to having trophic properties, MSCs secrete

chemoattractant molecules (Fig. 1); one example is monocyte chemoattractant protein-1, which recruits circulating monocytes to injured sites, where they can differentiate into endothelial cells [64, 70]. Lastly, paracrine action of MSCs over immune system cells is at least partially responsible for their immunoregulatory properties (described below).

MSC Immunoregulatory Properties

The immunosuppressive effects of MSCs on multiple components of the immune system are now well documented (reviewed in [71, 72]). The effect was initially described for T cells, in in vitro studies that demonstrated that MSCs were able to suppress T lymphocyte activation and proliferation in response to alloantigens and nonspecific mitogens [73–75]. Inhibition of lymphocyte proliferation has not been associated with the induction of apoptosis but rather with inhibition of cell division. With regard to the T helper cell compartment, MSCs suppress mainly Th1 cells, so that the secretion of interferon-γ is reduced [76]. The proliferation of antigen-primed cytotoxic T cells is also inhibited by MSCs [77]. MSCs may also suppress immune responses by increasing the proportion of regulatory $CD4^+CD25^+$ T cells, which have potent suppressor activity [78, 79].

MSCs were also shown to prevent the proliferation of resting natural killer (NK) cells, but they only partially inhibited the proliferation of activated NK cells [80, 81]. Antigen stimulation of B cells was similarly inhibited by MSCs, which blocked B-cell proliferation in the G0/G1 phase of the cell cycle, with no apoptosis [82]. Finally, MSCs were shown to inhibit the differentiation and maturation of antigen-presenting cells [83]. Dendritic cells generated in the presence of MSCs have impaired response to maturation signals and no expression of costimulatory molecules, resulting in reduced ability to induce activation of T cells [76, 84]. The effect is dose-dependent and, interestingly, is stimulatory (rather than inhibitory) at low concentrations of MSCs [75].

The limited in vivo data available seem to support the immunosuppressive capacities of MSCs. Animal studies show that allogeneic MSCs engraft and fail to induce an immune response in immunocompetent adult rodent, porcine, and baboon experimental models (reviewed in [71]), and they may prevent the rejection of allografts in immunocompetent baboons [85] and mice [78]. Clinical studies in humans seem to indicate that MSCs can be tolerated when transplanted across MHC barriers [86].

The mechanisms responsible for these effects are not yet fully understood, but evidence shows the involvement of cell-to-cell interactions and, probably more important, of soluble factors such as transforming growth factor-β1, hepatocyte growth factor [73], prostaglandin E2 [76], indoleamine dioxygenase-mediated tryptophan metabolites [87], and nitric oxide [88]. The therapeutic applications of this immunosuppressive potential involve the prevention of acute graft-versus-host disease [39, 89], allogeneic rejection of transplanted tissues [85, 90], and the treatment of autoimmune disorders [91].

Where Are the MSCs?

Adult, tissue-specific SCs are found in specialized niches in their corresponding tissues of origin [49, 92]. Hematopoietic SCs can be found in the bone marrow (reviewed in [93]), epidermal SCs in mammalian hair follicles [94], neural SCs in the subventricular zone [95], and so on. Where are the MSCs to be found?

The main differentiated cell types that originate from MSCs, osteoblasts, chondrocytes, and adipocytes, are present throughout the entire organism from head to toe. The location of these SCs in the organism must allow for their progeny and therapeutic effects to reach most or all tissues and organs. Considering this, three possibilities could be envisioned. In the first, MSCs are located in only one specific tissue or organ, from which they exit and circulate to other sites to replenish cell populations that undergo apoptosis through physiological turnover processes, or necrosis in case of lesions. The considerable difficulty in establishing conventional MSC cultures from peripheral blood under physiological conditions or stimulation by cytokines [9, 96, 97] argues against this possibility. Experiments using rats exposed to low-oxygen conditions suggest that MSCs may be specifically mobilized into peripheral blood as a consequence of hypoxia [98]. However, the origin(s) of the mobilized cells remains unclear.

The second possibility is based on the fact that postnatal MSCs have been isolated from different tissues, in addition to bone marrow: these include adipose tissue [99], periosteum [100–102], tendon [103], periodontal ligament [104], muscle [105, 106], synovial membrane [107], skin [108], and lungs [109]. MSC cultures with very similar morphologic, immunophenotypic, and functional properties have been established from brain, spleen, liver, kidney, lungs, bone marrow, muscle, thymus, and pancreas of mice, even when the animals had their blood washed out from their vessels by perfusion prior to cell isolation [9]. These findings document that cultured MSC-like cells can be derived from a variety of tissue locations, suggesting that different tissue-intrinsic stem cells might behave as MSCs when characterized in vitro.

The third possibility, which constitutes the main theme of this article, has been suggested previously and involves a relationship between MSCs and perivascular cells [9, 110–115]. This suggestion would adequately explain why MSCs can be isolated from all tissues. Association of these mesenchymal progenitor cells with the vasculature would allow them to function as a source of new cells for physiological turnover and be available for the repair or regeneration of local lesions. In this scenario, the damage to any tissue would release the MSC from its perivascular site; it would divide, and in this activity state it would secrete immunoregulatory and trophic bioactive factors. The establishment of MSC-like cultures from blood vessels alone, including decapsulated glomeruli [9], argues favorably for this hypothesis. Consequently, the pericyte emerges as a possible in vivo source for locally therapeutic MSCs.

The Pericyte

Pericytes, also referred to as periendothelial cells or Rouget cells, are mural cells that lie on the abluminal side of blood vessels, immediately opposed to endothelial cells (ECs) [116–118]. Specialized pericytes called Ito cells, hepatic stellate cells, or hepatic lipocytes exist in the liver [119]; another specific pericyte, the mesangial cell, is found in the kidney glomerulus [120]. In bone marrow, cells exhibiting pericytic characteristics are referred to as adventitial reticular cells (ARCs) [121] or myoid cells, as they express α-smooth muscle actin [122–124].

Pericytes are defined morphologically on the basis of their location in relation to ECs, especially in microvessels (arterioles, capillaries, and venules). There is evidence, however, that pericytes are also present in large vessels: by staining tissue sections with the 3G5 antibody, a pericyte marker, pericytes were identified adjacent to ECs in both small and large vessels, suggesting that pericytes form a continuous subendothelial network that spans the entire human vasculature [125]. (Further information is given in the supplemental online data.)

Pericyte-Endothelial Cell Interactions

Pericytes and ECs exhibit an interdependent relationship, wherein soluble factors and physical interactions synergistically

contribute to blood vessel structure, both their formation and maintenance [126, 127] (supplemental online data). The intimate relationship between pericytes and endothelial cells is often not considered when defining a pericyte. It is possible that what ultimately distinguishes the pericytes from other cell types is the nature of their interaction with ECs. To be considered a pericyte, a given cell (a) should establish physical contact with endothelial cells by means of gap junctions, (b) should express at least one marker attributed to pericytes, and (c) should not express pan-endothelial cell markers. Likewise, when this interaction is interrupted and the pericyte is liberated, we would suggest that it be considered an MSC.

The Pericyte as a Local Source of Mature Cell Types

In 1982, Richardson et al. suggested the role of pericytes as progenitors for adipocytes during tissue injury after retrospective analysis of sections from thermally injured fat pads of rats [128]. In that study, cells adjacent to the lesion site seemed to be liberated from their anatomical site 6 hours after the lesion and to become activated, as judged by their change in morphology and increase in RER content, 4 hours later. Five days after the lesion, new blood vessels and multilocular adipocytes that appeared to arise from fibroblast-like cells were observed in the wound site. Unilocular adipocytes were visible 7 days after the lesion. The authors concluded that activated pericytes, in addition to tissue-resident fibroblasts, differentiated into mature adipocytes during the healing process [128].

A role of pericytes as progenitors for cartilage and bone has also been suggested [129, 130]. The fate of vascular and perivascular cells labeled with monastral blue B was tracked during periosteal bone healing and in grafted perichondrium. Mature osteocytes and chondrocytes displaying cytoplasmic inclusions of the dye were observed by means of light and electron microscopy. The authors of these studies concluded that pericytes can give rise to osteocytes and chondrocytes in vivo.

The early behavior of periosteal vascular and perivascular cells in response to bone fracture was studied by light and electron microscopy [131]. At 24 hours after the fracture, both endothelial cells and pericytes were hypertrophic, and at 48 hours, putative pericytes had divided and formed layers of stacked cells. Five days after the fracture, chondroblasts that retained remnants of the basal lamina were observed in close proximity to the hypertrophic pericytes. At 6 days postfracture, hypertrophic chondrocytes, some of them showing signs of degeneration, could be observed in the proximal zone of the inner layer of the periosteal callus. At this time, woven bone was being replaced by lamellar bone in the distal zone of the inner layer. On the 7th day, woven bone had been almost completely replaced by lamellar bone in the distal zone of the inner layer of the callus, whereas endochondral bone formation was observed in the proximal zone. These results were interpreted to indicate that pericytes gave rise to chondrocytes and that osteocytes arise subsequently.

In an animal model of drug-induced Leydig cell death, pericytes were also shown to give rise to Leydig cells [132], which are testicular cells that secrete androgens such as testosterone. Using immunohistochemistry, the authors found cells positive for the Leydig cell marker cytochrome P450 side chain cleavage enzyme (CytP450) completely disappeared 3 days after the injection of the drug; $CytP450^{+}$ cells were visible again approximately 14 days after the injection, in the paravascular region of intertubular vessels [132]. Perivascular cells became proliferative 2 days after drug administration, as assessed by incorporation of bromodeoxyuridine injected 2 hours before euthanasia. These cells coexpressed nestin and α-smooth muscle actin (αSMA), indicative of their perivascular phenotype. In addition, newly formed Leydig cells expressed the pericyte markers NG2 and platelet-derived growth factor β, in agreement with a perivascular origin.

Whereas the studies quoted above detected SCs in perivascular locations using retrospective analysis, others have identified SCs around blood vessels by means of label-retaining cell (LRC) methodologies, in which the slowly proliferating stem cells are identified by retention of a nucleotide label incorporated into the DNA of cells during DNA synthesis. Perhaps the earliest evidence of SC association with blood vessels using an LRC assay comes from the analysis of periodontal ligaments of 6-week-old mice that received ^{3}H-thymidine [133]. In this study, slow-cycling cells were observed more frequently within a distance of 10 μm from the blood vessels, whereas proliferating cells were often more distant. The results also indicated that a small fraction of the perivascular cells enter cell cycle and migrate to a paravascular location, where they undergo proliferation. A likely interpretation is that SCs reside in a perivascular location; at times, some of them divide perpendicularly in relation to the blood vessel, giving rise to progenitor cells that take up a paravascular site. There, the perivascular-born progenitors proliferate to provide differentiated progeny.

Recently, two populations of SCs were detected in murine endometrium using the LRC methodology [134]. One of the approaches used involved bromodeoxyuridine injections in 3-day-old female mice for 3 days; a second experiment consisted of the implantation of an osmotic pump containing bromodeoxyuridine into prepubertal female mice. In both cases, labeled nuclei were detected immunohistochemically in the subsequent weeks. As a result, LRCs were detected in the epithelium and around blood vessels. Perivascular LRCs did not express CD31 or CD45, and some of them were positive for αSMA, indicating that these cells were pericytes. Importantly, a fraction of perivascular LRCs were shown to bear α-estrogen receptors and to proliferate in vivo in response to estrogen injection, confirming their role as SCs in endometrium.

A Perivascular Niche for MSCs

Specialized niches for different types of ASCs are characterized by the complex interactions between surrounding cells, extracellular matrix molecules, and soluble factors [6]. It is of interest that cultured marrow-derived MSCs retain their self-renewal and differentiation capabilities under rather simple in vitro conditions: culture medium supplemented with aliquots from competent lots of fetal bovine serum [41–43, 135]. The ability of these artificial culture conditions to support retention of some degree of stemness by MSCs does not necessarily mean that they perfectly mimic the "true" in vivo MSC niche.

Some studies indicate that ARCs are the cells that best resemble the in vivo correspondents of cultured MSCs in bone marrow [17, 136]. ARCs have been shown to maintain the HSC pool by providing HSCs with stromal-derived factor 1, also known as CXCL12, in postnatal life [136]; during development, hematopoiesis becomes established in long bones only after the appearance of myoid cells on the abluminal surface of the vasculature that invades the pre-existing cartilage [122]. The physiological support provided by ARCs to HSCs in vivo can be transposed to an in vitro context where MSCs, viewed here as cultured ARCs, show the ability to support HSC survival and differentiation [135, 137]. This view is further supported by data demonstrating the functional reconstitution of a human hematopoietic environment by direct injection of human MSCs into murine bone marrow [138].

In addition to the data suggesting a periendothelial location for MSCs in bone marrow, a number of reports suggest a

widespread perivascular niche for MSCs. Conventional MSC cultures have been established from artery or vein walls [9, 139, 140]. Furthermore, cells with cell-surface marker profiles similar to MSCs (positive for Stro-1 and CD146) were observed lining blood vessels in bone marrow and dental pulp [115]. In spite of their ability to support hematopoiesis, pericytes/MSCs distributed along the vasculature do not define the HSC niche, and adherence of HSCs to osteoblastic cells in vivo [141, 142] would explain why these ASCs are confined to bone marrow and not spread throughout all perivascular locations. A recent report demonstrated that bone marrow-derived $CD146^+$ ARCs can transfer the hematopoietic environment to heterotopic sites in mice when mixed with hydroxyapatite/tricalcium phosphate, embedded in fibrin, and transplanted subcutaneously [17]. Although these cells are not osteoblasts, a proportion of them is expected to differentiate into osteoblasts because of contact with the osteogenic ceramics, as described for MSC-loaded ceramic implants [143], making way for the adherence of circulating HSCs brought in by the newly formed vasculature.

Further functional overlap between MSCs and pericytes comes from studies demonstrating that pericytes are able to differentiate into mesenchymal cell types in vitro [112–114] and in situ, giving rise to adipocytes [128], chondrocytes [129, 131], and osteoblasts [130, 131]. In this context, vascular smooth muscle cells (vSMCs) have been shown to differentiate into the mature mesenchymal cell types, osteoblasts, chondrocytes, and adipocytes (reviewed in [144]). Distinguishing vSMCs from other cell types, such as fibroblasts and pericytes, is very difficult, especially in culture, as vSMCs are prone to dedifferentiation during in vitro cultivation, as described above. In fact, vSMC phenotypical change from a contractile to a synthetic phenotype [145] may reflect this phenomenon. On the other hand, pericytes may assume a phenotype that resembles that of vSMCs, as seen during the conversion of hepatic perisinusoidal (stellate) cells into myofibroblasts [146]. Nonetheless, pericytes can be distinguished from vSMCs on the basis of some molecules, such as the 3G5-defined ganglioside, calponin, desmin, and smooth muscle myosin (Table 2). Recently, serial analysis of gene expression performed on different cultured cell types allowed for the clustering of retinal pericytes and MSCs from bone marrow and umbilical vein in a single group, in close proximity to hepatic stellate cells [147]. In the same study, skin fibroblasts, smooth muscle cells, and myofibroblasts constituted a distinct group, confirming that these cell types differ from pericytes.

Partly on the basis of the evidence described above, we have previously suggested a model in which MSCs have a perivascular niche and lie in/on the basement membrane, opposed to endothelial cells [9]. According to this model, cues provided by the niche (cells, extracellular matrix, and signaling molecules [22]) coordinate a gradual transition of the SCs to progenitor and mature cell phenotypes. Differentiation of MSCs/perivascular SCs into tissue-specific cells in vivo is inferred not only because of the studies suggesting differentiation of pericytes in situ, but also because of the apparent innate propensity of cultured MSCs to acquire tissue-specific characteristics when cocultured with mature cell types or tissue extracts [31, 148, 149].

According to our model, cells operationally characterized as MSCs by functional assays in vitro would be the progeny of perivascular cells. The inverse that all perivascular cells are MSCs is not necessarily correct since highly differentiated vSMCs may not be able to exhibit full plasticity and differentiate in all mesenchymal phenotypes. It is possible that some perivascular cells are local progenitors in ectodermal or endodermal tissues. For example, pericytes in the brain bear a neural stem cell potential [150], and hepatic stellate cells display properties of hepatic progenitor cells [151]. The term perivascular stem cell would describe the mixture of SCs associated with the perivascular compartment all over the vascular network; a subset of these might be MSCs. Conversely, it may be that all MSCs are pericytes.

Finally, MSC ontogeny and developmental origin is still poorly understood (reviewed in [46]). The view of MSCs as a subset of postnatal perivascular SCs has been suggested, with their embryonic origins as descendants of cells from the embryonic dorsal aorta called mesoangioblasts [152]. Given a common embryonic origin, the association of postnatal SCs with a perivascular location suggests that the different types of ASCs are similar and could thus explain the observed plasticity (reviewed in [3]). Other data suggest a neuroectodermal origin for MSCs. Takashima et al. [153] have found evidence that a population of MSCs arises from $Sox1^-$ platelet-derived growth factor receptor α $(PDGFR\alpha)^+$ trunk neural crest cells derived from $Sox1^+$ neuroepithelial cells during embryonic development. Foster et al. [154] reported that neural crest-derived cells enter the thymus before embryonic day 13.5, where they remain through adulthood as pericytes and vSMCs; a neural crest origin for pericytes and vSMCs of the head has also been demonstrated [155, 156]. Although trunk and cranial neural crest cells are not equivalent, the results above allow for the speculation of an early neuroepithelial/neural crest origin for MSCs; this would adequately explain the successful isolation of MSCs in bone marrow by means of positive selection for cells expressing the low-affinity nerve growth factor receptor [157] and also why MSCs exhibit neural differentiation capabilities. Importantly, ARCs are selectively stained by anti-nerve growth factor antibodies [158], indicating that these are the MSCs isolated by Quirici et al. [157], and further suggesting that neural crest-derived cells persist in postnatal life as pericytes. Whether this is in agreement or disagreement with the view that postnatal MSCs are derived from mesoangioblasts still requires further experimentation. Since mesoangioblasts can be derived from the dorsal region of aorta, which is close to the neural tube, at embryonic day 9.5, a time when $Sox1^+$ neuroepithelial cells are the only cells displaying MSC characteristics when cultured [153], the possibility that the former descend from the $Sox1^+$ neural crest cells or their derivatives cannot be discarded.

A Role for the MSC in Immune Homeostasis

As stated above, MSCs must replenish expired mesenchymal cells in connective and skeletal tissues all over the body. Nevertheless, do they exert any other role in the organism? Their immunosuppressive effects suggest that they do.

Immune tolerance, critical for the prevention of autoimmunity and maintenance of immune homeostasis, is now believed to depend fundamentally on active mechanisms that operate in the periphery (i.e., outside the central immune organs) [159]. These mechanisms involve the participation of a number of elements, such as regulatory T cells and dendritic cells (reviewed in [160]). We propose here that MSCs are one more important player in the self-tolerance capacity, as they are distributed throughout the periphery because of their association with blood vessels, and they have immunomodulatory properties. According to this hypothesis, the immunosuppressive effect of MSCs on T lymphocytes and dendritic cells, observed in vitro and in vivo as detailed above, would operate to prevent self-responses in both physiological and pathological conditions (Fig. 2). Furthermore, MSC effects on immune system cells would be important in tissue repair (described below). Participation of MSCs/pericytes in self-tolerance maintenance by exerting immunosuppressive effects would adequately explain why autoimmune reactions are not frequent in spite of the

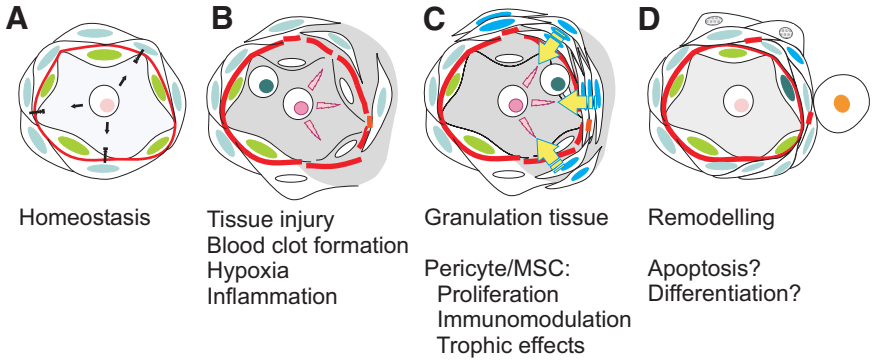

Figure 2. Proposed model of the MSC role in the tissue repair process. **(A):** Under normal circumstances, pericytes/MSCs (represented as cells bearing bluish nuclei) contribute to tissue maintenance [9], contributing to immune system homeostasis by avoiding unnecessary immune cell responses (arrows). **(B):** With tissue damage, the harmonious interactions between pericytes/MSCs and endothelial cells (represented as cells bearing green nuclei) are disturbed; the basement membrane (represented by a red line) is disrupted, allowing for blood extravasation into the tissue. A blood clot forms, wherein platelets release a multitude of bioactive molecules. Immune system cells (represented as cells bearing a pink nucleus) are attracted to the wound site, where they start immunosurveillance and an immune response (as represented by pink arrows). Circulating progenitors (dark green nucleus) are likewise recruited to the lesion area. **(C):** The events described in **(B)** lead to migration of adjacent pericytes/MSCs to the wound area, which become activated (as represented by a change to a strong blue nucleus) and undergo intensive proliferation. Pericytes/MSCs secrete several bioactive molecules that exert antiapoptotic effects, and some of these factors counteract (as represented by yellow arrows) the immune response. **(D):** By the end of this process, local endothelial cells, in concert with circulating progenitors, have re-established the endothelium and its associated basement membrane. Some pericytes/MSCs resume a pericytic phenotype (as represented by the change in their nuclei color back to bluish) as they function to stabilize the blood vessel. Some of the pericytes that had lost contact with the basement membrane undergo apoptosis (fragmented nuclei). Some of the pericytes/MSCs (blue nucleus) retain their progenitor status, remaining between the pericytes and the tissue-specific cells. Differentiation (as represented by the change in one nucleus color to orange) may occur, particularly in mesenchymal tissues, such as bone or muscle. Abbreviation: MSC, mesenchymal stem cell.

existence of self-reactive lymphocytes [161]. Likewise, some autoimmune diseases may reflect an intrinsic or genetic impairment of this immunosuppressive capacity of MSCs/pericytes.

A Role for the MSC in Tissue Repair

Tissue repair consists of a complex sequence of events [162]. Following tissue injury, a blood clot forms in a matter of minutes; platelets become activated and release a number of growth factors. An inflammatory process involving mainly innate immune system cells takes place within hours after injury, paving the way for a subsequent adaptive immune response [163]. During the subsequent days, different cell types migrate into the lesion site, local cells undergo proliferation, fibroblasts deposit extracellular matrix extensively, and myofibroblasts arise locally and exert a contractile action. A scar tissue forms later, and a remodeling process starts.

The origins of fibroblasts involved in tissue repair are still debated, and it is possible that they derive from different cell types [164]. A connection between perivascular cells and fibrosis was established by studies demonstrating that PDGFRb$^+$, HMW-MAA$^+$ pericytes are present in large numbers in tissue samples from patients with systemic sclerosis [165] and in cases of excessive scarring [166]. Extensive fibrosis due to pericyte proliferation is an exception rather than the rule. In rats, hepatic stellate cells proliferate vigorously after injury induced by CCl_4; after activation and proliferation, apoptosis takes place because of upregulation of both CD95 and CD95L 96 hours after the injury, limiting fibrotic effects [167]. Hence, pericytes are expected to proliferate in response to injury but not to cause fibrosis. Another possible fate for the proliferative pericytes during tissue repair is differentiation into tissue-specific cells, as inferred from the studies showing pericyte differentiation in situ. Recently, mesodermal differentiation has been shown to be increased in murine embryoid bodies lacking basement membranes [168]. Hence, it is possible that loss of contact between pericytes/MSCs and the basement membrane during wound healing allows for their differentiation in mesenchymal tissues. Evidence supporting a role for pericytes in neural tissue repair comes from studies where brain ischemia was experimentally

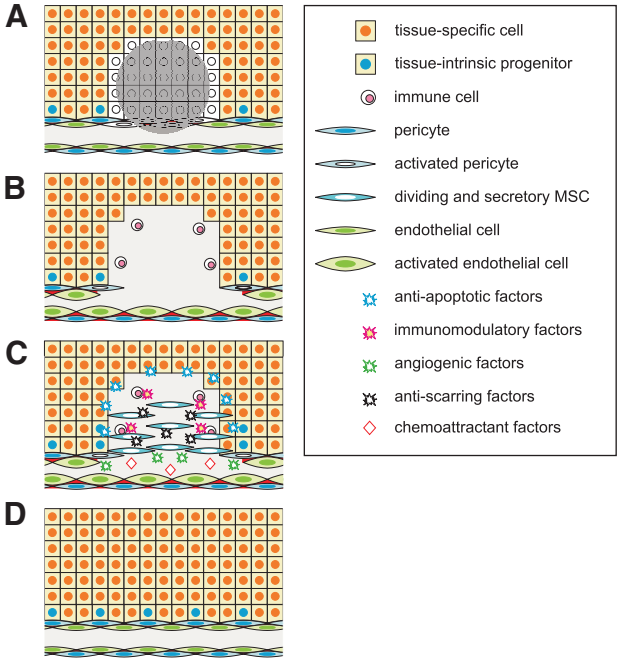

Figure 3. Paracrine effects and trophic action of MSCs during tissue repair. **(A):** Representation of a focal lesion leading to the death of tissue-specific cells, and part of a blood vessel. **(B):** Endothelial cells become activated, immune system cells are attracted to the necrotic area, and pericytes/MSCs become activated. **(C):** Activated pericytes/MSCs migrate into the lesion site and proliferate. The proliferating MSCs secrete bioactive molecules that will exert (a) antiapoptotic effects on tissue-specific cells, (b) immunomodulatory effects on immune system cells, (c) angiogenic effects, (d) antiscarring effects near the wound site, and (e) chemoattractant effects on other cells. **(D):** MSC paracrine effects described in **(C)** led to stimulation of tissue-intrinsic progenitors to regenerate the damaged tissue area, modulation of immune response and consequent maintenance of self-tolerance, and re-establishment of blood supply. Abbreviation: MSC, mesenchymal stem cell.

induced. Yamashima et al. [169] have shown that pericytes and other pericyte-like cells termed adventitial cells give rise to neurons in the subgranular zone and to glial cells in the dentate gyrus of hippocampus in monkeys following experimentally induced ischemia. Ohab et al. [170] have shown the expression of stromal-derived factor-1 and angiopoietin-1 by the vascular niche close to the infarct areas and related the presence of these molecules to guidance and survival of tissue-intrinsic progenitors in the subventricular zone of mice after focal cortical stroke.

On the basis of the above evidence, a model for the MSC/pericyte role during tissue repair was conceived and is depicted in Figure 2. According to this model, pericytes/MSCs proliferate in response to loss of contact with ECs and the basement membrane. Proliferation increases pericyte/MSC numbers in the injured site and, consequently, the number of molecules they secrete. These exert trophic and immunosuppressive effects on the surrounding tissue, minimizing the extent of damage and leading to a reduction in the inflammatory response (Fig. 3). Cultured MSCs would correspond to proliferative pericytes at this stage in tissue repair. Interestingly, cultured MSCs upregulate the expression of immunomodulatory molecules in response to stimulation by interferon-γ, a potent proinflammatory cytokine [171–173]. The use of cultured MSCs in tissue repair would accelerate healing because they are trophic and immunomodulatory, and they can be directly delivered to damaged areas in large numbers or home to injured sites after systemic infusion due to the expression of specific extracellular matrix [174, 175] and chemokine (Fig. 1) receptors (further information on possible homing mechanisms for MSCs is given in the supplemental online data). Homing to injured sites followed by exertion of local trophic and immunomodulatory effects could thus be the most important functions of cultured MSCs when they are systemically delivered. These therapeutic properties can be further augmented by genetic modification [176, 177].

CONCLUSION

Data showing the existence of SCs in perivascular locations in vivo in addition to the successful isolation and culture of MSC-like cells from different tissues support the hypothesis that cultured MSCs are derived from a perivascular niche. The ability of these cells to differentiate into several cell types in vitro probably reflects the efficacy of the niche in the maintenance of their stemness. Although not all perivascular SCs are expected to act as MSCs in vivo, they probably behave as such in mesenchymal tissues. Evidence thus far indicates that the cellular component of the perivascular compartment that is more likely to function as a stem cell in the postnatal organism is the pericyte.

We believe that experimental evidence is consistent enough to establish the concept that MSCs isolated from the bone marrow, adipose tissue, and any other organ or tissue are actually derived from blood vessel walls. The view of cultured MSCs as descendants of periendothelial cells suggest that pericytes play an important role in the tissue repair process, in addition to their role in maintaining blood vessel integrity and contributing to tissue homeostasis. We emphasize, furthermore, the importance of MSCs/pericytes in immune system homeostasis, besides their regenerative functions.

Finally, the MSC concept discussed here calls for focusing future experimental approaches on the perivascular compartment to bring about new insights in ASC biology in vivo and to provide data for the design of new therapeutic approaches. These may include the use of vascular fractions or pericytes from different tissues as a starting material for cell-based therapies, of DNA vectors targeting the perivascular compartment to treat genetic disorders, and of genetically modified cultured MSCs to treat relevant injuries.

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DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST

The authors indicate no potential conflicts of interest.

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