Mesenchymal stem cells display coordinated rolling and adhesion behavior on endothelial cells

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To explore the initial steps by which transplanted mesenchymal stem cells (MSCs) interact with the vessel wall in the course of extravasation, we studied binding of human MSCs to endothelial cells (ECs). In a parallel plate flow chamber, MSCs bound to human umbilical vein ECs (HUVECs) similar to peripheral-blood mononuclear cells (PBMCs) or CD34+ hematopoietic progenitors at shear stresses of up to 2 dynes/cm². This involved rapid extension of podia, rolling, and subsequent firm adhesion that was increased when ECs were prestimulated with TNF-α. MSC binding was suppressed when ECs were pretreated with function-blocking anti–P-selectin antibody, and rolling of MSCs was induced on immobilized P-selectin, indicating that P-selectin was involved in this process. Preincubation of HUVECs with anti–VCAM-1 or of MSCs with anti–VLA-4 antibodies suppressed binding of MSCs to HUVECs but did not enhance inhibition by anti–P-selectin, indicating that both P-selectin and VCAM-1 are equally required for this process. Intravital microscopy demonstrated the capacity of MSCs to roll and adhere to postcapillary venules in vivo in a mouse model in a P-selectin–dependent manner. Thus, MSCs interact in a coordinated fashion with ECs under shear flow, engaging P-selectin and VCAM-1/VLA-4.

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Materials and methods

Isolation and characterization of cells

Bone marrow samples were obtained from patients undergoing hip joint replacement after informed consent in accordance with the Declaration of Helsinki. Then, 10 to 30 mL of aspirate from the femur cavity was anticoagulated with 500 IU/mL heparin. The light-density mononuclear-cell fraction was prepared by density gradient centrifugation (yield, 2 × 10^7–7.2 × 10^8 cells), seeded in T25 or T80 tissue culture plastic flasks at 1 × 10⁶ to 3 × 10⁶ cells/cm² in low-glucose DMEM medium (PAA, Cölbe, Germany) supplemented with 20% tested FCS (PAN Biotech, Aidenbach, Germany) and 25 ng/mL basic FGF (Tebu-Bio, Offenbach, Germany). After 48 hours of incubation at 37°C and 5% CO₂, nonadherent cells were removed and the remaining cells were cultured for another 7 to 10 days until reaching 70% to 80% confluence. The uniform spindle-shaped cell fraction was prepared by density gradient centrifugation (yield, 2 × 10⁷–7.2 × 10^8 cells), seeded in T25 or T80 tissue culture plastic flasks at 1 × 10⁶ to 3 × 10⁶ cells/cm² in low-glucose DMEM medium (PAA, Cölbe, Germany) supplemented with 20% tested FCS (PAN Biotech, Aidenbach, Germany) and 25 ng/mL basic FGF (Tebu-Bio, Offenbach, Germany). After 48 hours of incubation at 37°C and 5% CO₂, nonadherent cells were removed and the remaining cells were cultured for another 7 to 10 days until reaching 70% to 80% confluence. The uniform spindle-shaped cells were passaged by trypsinization and their multipotency was confirmed by successful induction of osteoblastic, chondrogenic, and adipogenic differentiation under the conditions published. MSCs used for the assays.
were harvested when they had reached approximately 80% confluence, corresponding to 1.5 × 10^6 to 2 × 10^6 MSCs/tissue flask. MSC populations could be passaged 14 to 20 times; to avoid acquisition of unfavorable genetic alterations, 22,23 MSCs used for the experiments were expanded for maximally 9 passages. PBMCs and mobilized CD34+ progenitor cells were isolated from voluntary donors after they gave informed consent in accordance with the Declaration of Helsinki as described. 24 For flow cytometric analysis, MSCs were trypanosed using trypsin-EDTA (0.5% trypsin, 6.8 mM EDTA in PBS; Invitrogen, Karlsruhe, Germany) at 37°C for 5 minutes, suspended as single cells in PBS, and incubated with fluorescence-labeled anti-CD24 (clone ALB9, Beckman Coulter, Krefeld, Germany), anti-CD34 (clone 581), anti-CD45 (clone H130), anti-CD73 (clone AD2), anti-CD117 (clone YB5.B8), anti-VEA-4 (clone 9F10), anti-β1 integrin (clone HUTS-21), anti-β2 integrin (clone L130), anti-CXCR4 (clone 12G5; all 13 cells from BD Pharmingen, Heidelberg, Germany); anti-PSSL1 (clone KPL-1; MoBiTec, Göttingen, Germany); and anti-L-selectin (clone FMC46; Dako Cytomation, Hamburg, Germany). MSCs were analyzed in a Coulter Epics XL MCL flow cytometer (Beckman Coulter). To control for a potential loss of adhesion molecules by trypsin treatment, expression of adhesion receptors was determined flow cytometrically after either mechanical disruption by rubber policeman, incubation with 6.8 mM EDTA in PBS for 5 minutes at 4°C, or trypsinization as described. This yielded comparable percentages of cells positive for CXCR4 and αα, αβ, and ββ integrins (data not shown). Expression of CXCR4 was also determined after passage of MSCs (10^6 in 1 mL) over glass slides in the parallel plate flow chamber system described below (see “Flow chamber assay”). After passage through the chamber, MSCs were immediately collected in HEPES-buffered salt solution (HBSS; Invitrogen) buffer at 4°C and analyzed by flow cytometry within 1 hour. Control cells were stored analogously and kept at no agitation for the period of the assay.

Detection of mRNA transcripts for adhesion molecules was performed by reverse transcription-polymerase chain reaction (RT-PCR). Total RNA was extracted from MSCs using TRIzol (Invitrogen). RNA integrity was checked by gel electrophoresis, and 1 μg RNA of intact samples was reverse transcribed by using the Omniscript Reverse Transcription Kit (Qiagen, Hilden, Germany) at 42°C for 50 minutes. PCR amplification of the resulting cDNA was performed by using the Taq PCR core kit (Qiagen). Primers (Biospring, Frankfurt, Germany) and corresponding annealing temperatures (Tm) were as follows: GAPDH: sense 5'-GAAGATGGTGATGG-GATTTC-3', antisense 5'-GAAGATGGTGATGG-GATTTC-3' (Tm 62°C); eIF2α: sense 5'-GGCATGCTTCTTACATTG-3', antisense 5'-ATGTATCATACTTACAGACAG-TGC-3' (Tm 60°C); CXCR4: sense 5'-CTGAGAAAGATCGACGAGCAATTCAG-3', antisense 5'-CAACGCTTCTTGCGCTGCTGACT-3' (Tm 66°C); PSL1: sense 5'-AGTGGCT TGCAACTCCTCCTC-3', antisense 5'-GTCTGACGATGTCGACGTTG-3' (Tm 61°C); L-selectin: sense 5'-TTGACGATCAGTCTACAGTGATG-3' (Tm 60°C); β2 integrin: sense 5'-ACAGCACCGAGGAGCAGT GT-3', antisense 5'-CTGCTCTTGGAT- GCACCTGCTG-3' (Tm 59°C); αα integrin: sense 5'-AGGAGACATGAGTGTTG-3', antisense 5'-TCAGTCTGCTGTTGATACAGG-3' (Tm 57°C); CD24: sense 5'-CTCTACCCAAGCGAGATTATTC-3', antisense 5'-GAAGTGGACGACGAGAACAGAC-3' (Tm 59°C); CD44: sense 5'-TGGGCGTCTTTGCACAGGTA-3', antisense 5'-TGGGCCCACTGGATGACA-3' (Tm 59°C). Expression was validated using RNA from the mesenchymal tumor cell line HO/S (American Tissue Type Culture Collection [ATCC], Manassas, VA) or the leukemic cell line KG-1 (ATCC) as positive controls and RNA from the untransplanted MSC sample as negative control. PCR products were separated on a 2% agarose gel and stained with ethidium bromide, and images were recorded.

**Flow chamber assay**

Glass slides precoated with 0.1% (wt/vol) gelatin (Sigma, Taufkirchen, Germany) were seeded with human umbilical vein ECs (HUVECs; Cambrex Bio Science, Verviers, Belgium) in EGM-2 medium (BulletKit, Cambrex Bio Science, Cambridge, United Kingdom). In some experiments, glass slides were left uncoated or coated with 10 μg/mL human P-selectin (R&D Systems, Wiesbaden, Germany) as previously described. 25 HUVECs were used when they reached more than 90% confluency. In part of the experiments, HUVECs were preincubated with TNF-α (10 ng/mL) for 4 to 6 hours prior to the assay. MSCs were trypsinized within 1 to 2 hours before use in the assay, resuspended at 10^5/mL in HBSS supplemented with 1% human plasma and 25 mM HEPES, and incubated for 30 minutes at 37°C, 5% CO2. In some experiments, function-blocking antibodies were added to HUVECs (10 μg/mL anti–P-selectin clone AK4; 10 μg/mL anti–VCAM-1 clone 1G11; both from Immunotech, Marseille, France) or MSCs (anti–PSGL-1: 10 μg/mL clone KPL-1 from BD Pharmingen; anti–VLA-4: 10 μg/mL clone HP2/1 from Immunotech; anti–β2 integrin: 10 μg/mL clone 7E4 from Immunotech; anti–β1 integrins: 10 μg/mL clone Lαι/2 from Immunotech) 30 minutes prior to analysis in PBS/1% BSA. For some experiments, MSCs were also preincubated for 1 hour at 37°C with 50 μM/mL α-L-fucosidase (Sigma) or 25 μg/mL O-sialoglycoprotein endopeptidase (Biozol, Eching, Germany) dissolved in PBS/1% BSA. Efficiency of fucosidase treatment was controlled by staining of fucosidase-treated and, as a control, neuraminidase-treated MSCs with FITC-conjugated *Ulex europaeus* agglutinin I (Sigma) and subsequent flow cytometric analysis, leading to a 5- to 10-fold reduction in fluorescence intensity after fucosidase, but no change after neuraminidase-treatment of MSCs. A 35-mm circular parallel plate flow chamber containing a 5-mm wide and 0.01-inch high channel (GlycoTech, Gaithersburg, MD) was mounted on top of glass slides with pregrown confluent HUVECs, precoated P-selectin, or VCAM-1. Using a perfuser pump connected to the inlet port via a 1/16-inch diameter tube, a uniform laminar flow was applied, allowing regulation of calculated shear stresses between 0.1 and 4 dynes/cm^2^. Prefixed MSCs, PBMCs, or CD34+ cells (5 × 10^6/mL) were flushed over HUVECs at 0.1 dynes/cm^2 for 10 minutes. In some experiments, medium at room temperature was used. The flow rate was then increased to yield a shear stress of 2 dynes/cm^2 for a further 10 minutes. Video recordings were performed using a CCD camera (D-73431; Sony, Cologne, Germany) mounted on an inverted-stage microscope (Axiovert 135; Zeiss, Oberkochen, Germany) equipped with a ×10 objective (Zeiss). In addition, numbers of adherent cells were recorded in 3 representative fields after each flow phase (0.1 and 2 dynes/cm^2). Rolling velocities were recorded using real-time video imaging and a defined space grid in microscopic fields. Analysis of video sequences (for rolling) and single frames (for adhesion) used windows sized to correspond to an area of 15 μm^2; MSCs were considered noninteracting when they moved at the velocity of the flow, whereas cells moving at lower velocities were defined as rolling.

**Homing assay for MSCs and intravital microscopy**

MSCs were fluorescence-labeled using preincubation with PKH-26 (Sigma) or carboxyfluorescein diacetate succinimidyl ester (CFSE; Molecular Probes, Eugene, OR) according to the manufacturer’s instructions as described. 26 For homing assays, 1 × 10^6 MSCs were injected into the tail veins of NOD/SCID mice preirradiated with 2.5 Gy x rays. After 2, 6, or 24 hours, cell suspensions were prepared from tissues or blood of killed mice by mincing, filtered through 100-μm pore filters, and analyzed by flow cytometry. 27 Briefly, volumes and live-cell numbers were recorded, and from a subtraction of cells diluted in PBS/2% FCS, 100 000 events were analyzed within a live-cell gate for the presence of PKH+ (or CFSE+) cells. Control animals that received no cells were included. Absolute numbers of homed MSCs per organ were calculated according to the formula n = m × m × m × m × m × m/100 000/x, with m indicating total mass of the organ analyzed; m, mass of the analyzed proportion of the organ; x, total volume in which the entire organ-cell suspension was contained; y, volume of sample aspirated/analyzed; z, number of positive events acquired per 100 000 events as described. 26 Extravasation of transplanted MSCs was analyzed in mice injected with 5 × 10^6 MSCs, using frozen sections of spleens and lungs. Cryosections were prepared and stained with FITC-conjugated monoclonal anti-murine CD31 antibody MEC13.3 (Becton Dickinson, Heidelberg, Germany) and mounted using Vectashield medium (Vector Laboratories, Burlingame, CA). PKH, FITC, and nuclear (DAPI) fluorescence were visualized using a fluorescence microscope and imaging software (Zeiss). For intravital microscopy, wild-type or P-selectin−/− mice (in C57/BL6 background; kindly provided by Dr Lubor Borsig, Institute of Physiology, University of Zurich, Switzerland) were anesthetized by an intraperitoneal injection of ketamine (Schwabe-Curamed, Karlsruhe, Germany) and xylazine (Bayer, Leverkusen, Germany) and placed on a
homothemric blanket. The right carotid artery was prepared microsurgically, and a catheter was inserted for injection of MSCs (5 × 10^6/mL in PBS/1% BSA). The left ear of the mouse was placed gently on a microscope slide and covered with glycerol and a coverslip. Vascular architecture and labeled cells were visualized during their passage through vessels under fluorescent epi-illumination using a multiband filter system (XF 53; Omega Optical, Brattleboro, VT). The microcirculation was continuously recorded using a 1/3-inch DSP 3-CCD camera (DXC-390; Sony) mounted on a modified Zeiss microscope (Axioptech Vario 100 HD; Zeiss) equipped with a ×10 immersion proof objective (Nikon, Düsseldorf, Germany). Images were digitally stored using Media Studio Pro 7.0 (Ulead, Kaarst, Germany) for later off-line analysis. Migration behavior of MSCs was determined in individual vessel segments of either wild-type or P-selectin–deficient mice. Cells were considered non-interacting when they moved at the velocity of the mean blood flow, whereas lower velocities were defined as rolling.28

Cells were considered interacting if they crossed the vessel structure. In spleen, 12 of 19 identified PKH labeled cells were found clearly outside the vessel structure. In liver, 16 of 16 detected PKH^+ cells were found clearly outside the vessel structure. In spleen, 12 of 19 identified PKH^+ cells had extravasated, 4 remained intravascular, and for 3 this remained difficult to determine. These data indicate that the transplanted MSCs circulate in the blood after transplantation and are capable of extravasating into tissue.

### Results

#### Expression of characteristic surface markers and homing receptors by MSCs

By flow cytometric analysis, MSCs were unequivocally negative for the hematopoietic markers CD45 and CD34, but expressed both the CD73/SH3 and CD105/SH2 antigens and, in part, c-kit (Figure 1A). MSCs did not express detectable levels of the adhesion molecules PSGL-1, L-selectin, the alternative P-selectin ligand CD24 or β2 integrin on their surface, but stained positive for α4, α5, and β1 integrins and CD44 (Figure 1B). Furthermore, a small percentage of MSCs expressed CXCR4 on the cell surface. RT-PCR analysis confirmed the absence of or only barely detectable expression of PSGL-1, CD24, and β2 integrin, and the presence of c-kit (Figure 1C). Interestingly, however, expression of L-selectin and CXCR4 was clearly detectable by RT-PCR. To investigate the possibility that shear stress may induce cell-surface expression of CXCR4 or L-selectin, MSCs were passed through parallel plate flow chambers at a calculated shear force of 0.5 dynes/cm². Whereas the percentage of MSCs expressing L-selectin, α4 integrin, or c-kit remained largely constant, the percentage of CXCR4^+ MSCs increased by approximately 2-fold after exposure of MSCs to shear stress (Figure 1D). Taken together, these data show that MSCs express several adhesion receptors shown to function as homing receptors in hematopoietic cells but lack expression of several selectins or their ligands.

#### Homing of MSCs in mice

To investigate whether MSCs can undergo a regulated homing process in vivo, we transplanted MSCs into immunodeficient NOD/SCID mice. Flow cytometric analysis of organ-cell suspensions demonstrated time-dependent changes in the distribution of the MSCs. MSCs were detected predominantly in blood and lungs at 2 and 6 hours, and, at decreasing levels, at 24 hours after transplantation (Figure 2A-B). Whereas relatively low numbers of MSCs were detected in femoral bone marrow, increasing amounts of MSCs were detected in spleen, liver, and brain. No MSCs were detected in muscle tissue in 6 of 6 mice in preparations of the muscularis quadriceps femoris (data not shown). Immunohistochemical analysis of lung, liver, and spleen sections was used to visualize the prelabeled transplanted cells by their PKH^+ red fluorescence and blood vessels by staining with anti-CD31–FITC (Figure 2C). Whereas in lung, 25 of 25 identified PKH^+ cells appeared to be contained within CD31^+–stained vessel structures, in liver, 16 of 16 detected PKH^+ cells were found clearly outside the vessel structure. In spleen, 12 of 19 identified PKH^+ cells had extravasated, 4 remained intravascular, and for 3 this remained difficult to determine. These data indicate that the transplanted MSCs circulate in the blood after transplantation and are capable of extravasating into tissue.

#### MSCs interact with ECs under flow

To investigate how MSCs interact with ECs under shear flow, a parallel plate flow chamber system was used with HUVECs as an endothelial-cell layer. As shown in Figure 3A, numbers of rolling MSCs were dependent on the applied shear force. Individual MSCs rolled on ECs with different velocities at low shear force (Figure 3B). When shear force was up-regulated, the frequency of rolling MSCs decreased, and their rolling velocity increased from approximately 100 μm/s to more than 500 μm/s. Rolling MSCs appeared as round cells, rapidly elaborating and retracting numerous podia that contacted the EC layer (Figure 3C; Video S1, available on the
blood, and spleen were derived from organs of mice given
planted PKH-labeled MSCs. Cryosections from lung,
8 (24 hours) mice. (C) Microscopic analysis of trans-
ods. Values are means
were determined as described in “Materials and meth-
trols. (B) Absolute numbers of MSCs homed per organ
were labeled with the vital dye PKH-26 or CFSE and
intravenously injected. At 2, 6, or 24 hours after transplan-
tions with $1 \times 10^5$ MSCs, mice were killed, organs were
minced, and single-cell suspensions were prepared. (A)
The incidence of labeled cells was determined by flow
cytometry by determination of the number of fluorescing
cells per 100 000 cells, including non-transplanted con-
trasts (red: indicated by arrowheads), and nuclei were
stained by DAPI (blue). Original magnification, $\times$ 200.
MSCs used for these experiments were from passages 6
or 7.

Blood website; see the Supplemental Video link at the top of
the online article). We observed that only rolling MSCs eventually
adhered. We then assessed adhesion of MSCs, which rolled on
HUVECs at 0.1 dynes/cm², and determined their capability to
remain adherent both at low (0.1 dynes/cm²) and at increased (2
dynes/cm²) shear stress. Numbers of adherent cells were similar
for all 3, MSCs, freshly isolated PBMCs, and hematopoietic CD34
progenitor cells (Figure 4A). Notably, however, TNF-α–induced
adhesion was more pronounced in MSCs at 2 dynes/cm² compared
with PBMCs or CD34⁺ cells, with only few adherent MSCs
detaching at 2 dynes/cm². Preculture of MSCs in the presence
or absence of bFGF did not influence their adhesion behavior (Figure
4B). This also did not affect the ability of the MSCs to differentiate
into chondrogenic, osteogenic, or adipogenic cells (data not
shown). Binding of MSCs to TNF-α–pretreated HUVECs was not
different at 37°C and room temperature in this assay (107.7 ± 10.2
versus 105.0 ± 17.7 cells binding at 0.1 dynes/cm², and 98.0 ± 17.7
versus 93.3 ± 9.4 cells binding at 2 dynes/cm² at room temperature
versus 37°C, respectively). In contrast to MSCs, PBMCs, or
CD34⁺ cells, no adhesion to the EC layer was observed with
human erythrocytes or trypsinized HUVECs (data not shown).
Thus, MSCs displayed coordinated, shear stress-dependent rolling
and adhesion behavior on ECs in vitro, similar to peripheral-blood
leukocytes or HPCs.

Involvement of adhesion molecules in the interaction of MSCs
with ECs

We next analyzed the influence of blocking antibodies against
adhesion receptors expressed on MSCs or ECs. Preincubation of
HUVECs with anti–P-selectin function-blocking antibody de-
creased numbers of MSCs bound to ECs to approximately 50% of
cells (Figure 5A). Moreover, MSCs also rolled on immobilized
P-selectin (Figure 5B). Interaction of MSCs with P-selectin was
dependent on the presence of divalent cations, because the numbers
of rolling cells decreased to 44.2% ± 13.5% of controls when the
assay was performed in the absence of Ca²⁺ and Mg²⁺. Whereas
preincubation of MSCs with function-blocking antibody against
the P-selectin ligand PSGL-1 efficiently inhibited binding of
CD34⁺ hematopoietic progenitors to ECs, it did not suppress
binding of MSCs to ECs, in agreement with the absent expression
of PSGL-1 on MSCs (Figure 5C). When MSCs were pretreated
with either O-sialoglycoprotein endopeptidase or fucosidase,
numbers of MSCs binding to HUVECs prestimulated with TNF-α
were suppressed 2.7-fold (0.1 dynes/cm²) or 1.7-fold (2 dynes/cm²)
compared to controls after O-sialoglycoprotein endopeptidase, and
54.4-fold (0.1 dynes/cm²) or 21.9-fold (2 dynes/cm²) after fucosi-
dase. This indicates that MSCs may bind to P-selectin using a
ligand that contains fucose and sialic acid residues, but that is
different from PSGL-1.

Figure 3. MSCs roll on HUVECs under shear flow. Analysis of numbers of rolling
cells (A) and rolling velocities (B). MSCs (10⁵, passage 9) were flushed at different
shear stresses over a HUVEC layer in the parallel plate flow chamber, and rolling
cells were determined as described in “Materials and methods.” Values are means
± SD; n = 7–9. (C) Lifted frames of a video clip with 2 moving cells and one stationary
adherent MSC. Values in top left corners indicate time from video start in seconds; the
white horizontal scale bar represents 50 μm.
Figure 4. MSCs adhere to ECs under shear flow. (A) MSCs from passage 9, PBMCs, or CD34+ HPCs (10⁷ analysis) were flushed over a HUVEC monolayer in the parallel plate flow chamber and analyzed at a calculated shear stress of 0.1 dynes/cm² (●) or 2.0 dynes/cm² (□). Numbers of adherent cells were determined in 3 representative fields. (B) MSCs (passage 8), pregrown in medium in the presence of bFGF for 7 days, were analyzed as described in panel A. The analyses were performed either with or without prestimulation of HUVECs with 10 ng/mL TNF-α for 4 to 6 hours as indicated. Values are means ± SD; n = 3.

Discussion

In this study, we have demonstrated that bone marrow–derived MSCs interact with ECs under shear flow, involving rolling with rapid extension of podia, and activation of both selectin–dependent and integrin–dependent binding.

Both culture-expanded as well as freshly isolated adult tissue–derived MSCs have so far been shown to circulate in the blood for limited time periods and to distribute to various organs including lung, liver, and spleen. Previous work has analyzed MSC homing at early time points (24 and 48 hours) mostly using PCR, or imaging techniques based on ¹¹¹In labeling of injected cells, which cannot determine whether MSCs have egressed from the bloodstream. Our data show extravasation of transplanted MSCs into tissue as early as 24 hours after injection. Anjos-Afonso et al have analyzed the tissue distribution of transplanted murine culture-expanded MSCs. They analyzed histologic sections at 4 weeks after transplantation and found that MSCs can be trapped intravascularly in the lungs where they caused damage. Similar to our observations, these authors found that murine MSCs homed to liver, spleen, and brain, but that very few or none homed to muscle. With regard to brain homing of MSCs, additional or different mechanisms may be operative. Further histologic analysis will be required to demonstrate that MSCs can pass the blood–brain barrier, as

Figure 5. MSCs display P-selectin–dependent rolling and adhesion behavior on ECs. (A-B) P-selectin–dependent interaction of MSCs under flow. (A) MSCs (10⁷) were preincubated or not with anti–P-selectin antibody and analyzed in the parallel plate flow chamber at a calculated shear stress of 0.1 dynes/cm² (●) or 2.0 dynes/cm² (□). Pretreatment of MSCs with anti–P-selectin antibody was performed as indicated. (B) Influence of VCAM-1/VLA-4 on adhesion of MSCs to HUVECs. MSCs were pretreated or not with anti–VLA-4 antibody, HUVECs with anti–P-selectin or anti–VCAM-1 or both antibody for 30 minutes prior to analysis as indicated. Subsequently, 10⁷ MSCs were flushed through a parallel plate flow chamber and numbers of adherent cells were recorded after application of a calculated shear stress of 0.1 dynes/cm² (●) or 2.0 dynes/cm² (□). Numbers of rolling events were determined in 3 representative microscopic fields. (C) MSC binding to HUVECs is not blocked by anti–PSGL-1. MSCs or CD34+ cells were pretreated or not with anti–P-selectin antibody, HUVECs with anti–P-selectin or anti–VCAM-1 (or both) antibody for 30 minutes prior to analysis as indicated. (D) Influence of VCAM-1/VLA-4 on adhesion of MSCs to HUVECs. MSCs were pretreated or not with anti–VLA-4 antibody, HUVECs with anti–P-selectin or anti–VCAM-1 (or both) antibody for 30 minutes prior to analysis as indicated.
has been suggested by recent data showing that transfused MSCs ameliorate experimental encephalomyelitis. Our results show that detectable numbers of MSCs can be found in blood and in different organs as early as 2 hours after transplantation. The transfused MSCs were detected at frequencies of between 10 and 200/100,000 cells, which is in the same range as HPCs, and they were found to distribute with similar kinetics to tissues as HPCs.

Compared with circulating leukocytes or HPCs, MSCs only partially expressed adhesion molecules, which are known to serve as homing receptors in circulating hematopoietic cells. Although MSCs did not express PSGL-1, they clearly bound to ECs in a P-selectin–dependent manner. From our data it can be assumed that MSCs possess 1 or more functional P-selectin ligands, which are different from PSGL-1 and CD24. Dimitroff et al have reported that CD44 can mediate binding to E-selectin of hematopoietic progenitors. Although MSCs expressed CD44, we found that pretreatment of HUVECs by function-blocking anti–E-selectin antibody did not suppress binding of MSCs to ECs (B.R. and R.H., unpublished data, July 2005). L-selectin was expressed in MSCs, whereas anti–L-selectin antibody did not suppress binding of MSCs to ECs (B.R. and R.H., unpublished results, August 2004).

We have used an intravital microscopy system to directly observe transplanted MSCs after intra-arterial injection. When focusing on postcapillary venules of mice constitutively expressing SDF-1, CXCR4. Our data showing that a subpopulation of MSCs express CXCR4 correspond to the findings of Wynn et al. The data suggest that MSCs can use this receptor for organ homing. However, the fraction of MSCs adhering to ECs in our experiments was higher than the fraction of MSCs detectably expressing CXCR4. Moreover, we observed that MSCs express CCR6 and that they are chemo-attracted by the CCR6 ligand, MIP-3α (B.R. and R.H., unpublished results, August 2004). Recently, Sordi et al observed expression of chemokine receptors CXCR4, CX3CR1, CXCR6, CCR1, and CCR7 on an MSC population that is able to migrate into pancreatic islets of mice. Similar to our study, chemokine receptors were expressed only in subpopulations of between 1.8% and 26% MSCs. Honczarenko et al have described the expression of only a limited number of chemokine receptors on MSCs, preferably at earlier passages, and have confirmed functionality of CXCR4 by the induction of specific signal transduction pathways. The data indicate that chemokines that are present on the luminal surface of ECs can activate cognate chemokine receptors on MSCs, thus enhancing stimulation of integrin receptors and subsequent firm adhesion of MSCs to ECs.

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Acknowledgments

Figure 6. MSCs interact with endothelium in vivo. MSCs (passages 6-9) were fluorescence-marked with PKH-26 as described in “Materials and methods,” injected intra-arterially into wild-type or P-selectin−/− mice and followed microscopically in an ear window. (A) Representative views of a fluorescing cell, rolling on a microvessel. Numbers represent time points in seconds after start of analysis. (B) The rolling fraction of all cells in murine skin. Representative views of a fluorescing cell, rolling on a microvessel. Numbers represent time points in seconds after start of analysis. (B) The rolling fraction of all cells in murine skin.
References


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