Heterogeneity among human bone marrow-derived mesenchymal stem cells and neural progenitor cells

WICHARD VOGEL, FRANK GRÜNEBACH, CONRAD A. MESSAM, LOTHAR KANZ, WOLFRAM BRUGGER, HANS-JÖRG BÜHRING

Background and Objectives. Mesenchymal stem cells (MSC) and neural progenitor cells (NPC) are pluripotent cells. The former can give rise to myocytes, chondrocytes, adipocytes, and osteogenic cells, while the latter can give rise to astrocytes, neurons, and oligodendrocytes. The aim of this study was to analyze and compare the antigen expression patterns of MSC and NPC.

Design and Methods. Human bone marrow-derived MSC and NPC were analyzed by flow cytometry and immunocytochemistry using a variety of unique monoclonal antibodies (57D2, W4A5, W8B2) generated in our laboratory. In addition, the expression profile of CD antigens and intracellular differentiation markers was analyzed.

Results. We show for the first time that $CD10^+$, $CD13^+$, $CD61^+$, $CD90^+$, CD105 (endoglin)$^+$, $CD45^-$, $CD34^-$, and $CD133^-$ MSC also expressed CD109, CD140b (PDGF-RB), CD164, and CD172a (SIRPα). In addition, we found heterogeneity of MSC as demonstrated by the preferential expression of nestin and W8B2 antigen on distinct MSC subpopulations. Morphologically, these populations comprised small single cells and larger cells with polygonal appearance. NPC expressed high levels of CD56, CD90 and nestin and moderate levels of CD15, W4A5, and 57D2 antigens. In contrast, CD133 and CD172 were found only on NPC subpopulations.

Interpretation and Conclusions. Our data demonstrate nestin expression in most NPC as well as in immature MSC subpopulations. MSC and NPC subpopulations can now be distinguished using our novel antibodies W8B2, 57D2, and W4A5.

Key words: mesenchymal stem cells, heterogeneity, phenotypes.

Haematologica 2003;88:126-133
http://www.haematologica.org/2003_02/88126.htm

From the Department of Internal Medicine II, Division of Hematology, Oncology, Immunology, and Rheumatology, University of Tübingen, Tübingen, Germany (WV, FG, LK, WB, H-JB) and Laboratory of Molecular Medicine and Neuroscience, NINDS, NIH, Bethesda MD, USA (CAM).

Correspondence: Hans-Jörg Bühring, Ph.D., Department of Internal Medicine II, Division of Hematology and Oncology, University of Tübingen, Otfried-Müller-Str. 10, 72076 Tübingen, Germany.
E-mail: hans-joerg.buehring@med.uni-tuebingen.de

Human bone marrow contains hematopoietic cells and precursors of non-hematopoietic tissues.[1-3] Bone marrow plastic-adherent cells were first described as stromal cells, but more recently they have also been designated as mesenchymal stem cells (MSC) because of their capacity to differentiate into myogenic, osteogenic, chondrogenic, and adipogenic lineages.[4,5] In murine transplant models, bone marrow-derived MSC or hematopoietic progenitor cells were also able to differentiate into non-mesenchymal cells including liver cells, neuronal and glial cells.[6-8] More recently, it was shown that MSC from adult human bone marrow can differentiate into neural cells *in vitro*. In the presence of dimethylsulfoxide (DMSO) and β-mercaptoethanol (BME) MSC could be differentiated into neurofilament and neuron-specific enolase (NSE)-expressing cells.[9] Another report describes the differentiation of bone marrow stromal cells with epidermal growth factor (EGF) and brain-derived neurotrophic factor (BDNF) to neural cells that express nestin, glial fibrillary acidic protein (GFAP) as well as neuron-specific nuclear protein (Neu-N).[10]

Pluripotent stem cells with the capacity to differentiate into neural cells have also been described in the human central nervous system. These cells express nestin and are able to generate neurons, astrocytes, and oligodendrocytes via lineage-restricted precursors.[11] Pluripotent precursors were isolated from different adult human brain regions including the temporal and the frontal cortex, the amygdala, the hippocampus, and the ventricular zone.[12] Neural progenitor cells (NPC) derived from human fetal central nervous system tissue are $CD133^+$ and can be differentiated with EGF, basic fibroblast growth factor and leukemia inhibitory factor into neurons and astrocytes *in vitro*.[13] CD133 was originally found on hematopoietic stem and progenitor cells but more recent studies demonstrated that this marker is also expressed on a subset of both neural and skeletal muscle tissue.[14]

Most of the reports describe MSC and NPC as populations with homogeneous morphology and phenotype. The apparent homogeneity of their phenotypes may be explained by the limited number of the antigens studied. Using a larger panel of unique antibodies for analysis we extend the previous knowledge on MSC and NPC phenotypes and show that some markers are suitable for distinguishing MSC from NPC subpopulations.

Design and Methods

Isolation and cultures of MSC

Bone marrow (BM) cells of the iliac crest of volunteer donors (n = 10) were drawn after informed consent according to the guidelines of the local ethics committee. Mesenchymal stem cells (MSC) were generated from the interphase fraction of Ficoll gradient-separated BM cells (Biocoll separating solution, 1.077 g/mL, Biochrom KG, Berlin, Germany). The separated cells were cultured in RPMI 1640 medium supplemented with glutamax, 1% non-essential amino acids, 1% sodium pyruvate (GIBCOBRL, Paisely, Scotland), 1% penicillin/streptomycin (Biochrom KG) and 10% fetal bovine serum (PAA Laboratories, Linz, Austria). The cells were cultured at $2.0\times10^5/cm^2$ in tissue culture flasks (75 cm^2, Cellstar, Frickenhausen, Germany) and incubated at 37°C in a humidified atmosphere (5% CO_2). Two days after incubation the non-adherent cells were removed by replacing the medium. After confluency had been reached (usually within 14 days), the cells were detached by incubation with 0.25% trypsin/1 mM EDTA for 3 min at 37°C. MSC were maintained and subcultured until passage 5. For immunohistochemical and morphologic studies, first passage MSC were cultured at a density of $1.0\times10^4/cm^2$ in 8-well chamber slides (Falcon, Heidelberg, Germany) (n=6). The phenotype of MSC was determined by light and fluorescence microscopy of cells on chamber slides and by examination of cells in culture flasks using an inverted microscope.

Commercially available MSC (n=3) and NPC (n=3) were obtained from CellSystems, St. Katharinen, Germany. Normal human neural progenitor cells were obtained from cerebral cortex (or forebrain from cerebral hemisphere) by fragmentation of fetal nervous tissue (week 16-20). MSC and NPC were thawed after freezing according to the instructions of the manufacturer. NPC were either cultured in serum-free neural progenitor cell medium, astrocyte medium (both CellSystems), serum-free medium supplemented with BDNF (Sigma-Aldrich, Steinheim, Germany), or serum-containing RPMI 1640 medium. NPC grew as spheroids in serum-free hFGF-B (human recombinant basic fibroblast growth factor), hEGF (human recombinant epidermal growth factor) and NSF (neural survival factor-1) containing medium (Neural Progenitor Cell Maintenance Medium, CellSystems). The proportion of neurons and glial cells generated by these NPC was approximately 1:4 after cultivation of NPC in 5% serum (FBS).

Antibodies used in this study

The following monoclonal antibodies (mAbs) or antibody conjugates were used for microfluorimetric analysis: W4A5, W8B2, W8C3, and W12D1 (all IgG1) are unique mAbs with unknown specificity that were raised against the retinoblastoma cell line WERI-RB-1. They were selected for their non-reactivity with hematopoietic cells (from peripheral blood and bone marrow). Antibody W7C5 was also raised against WERI-RB-1 cells and was described to detect $CD34^+$ as well as $CD34^-$ bone marrow stem cells.[15] Very recently, we identified CD109 as the recognized antigen.[16] Antibody W6D3 was also raised against WERI-RB-1 cells and recognizes CD15 (BD Biosciences Pharmingen, unpublished results 2002). Antibody 57D2 (IgG1) was raised by immunizing mice with the TF-1 erythroleukemic cell line. It was selected for its non-reactivity with normal hematopoietic cells and its reactivity with neural progenitor cells. CD133-PE (clone W6B3C1),[17] CD167a (DDR1; clone 48B3, IgG3 subtype),[18] CD172a-PE (SIRPα: clone SE5A5),[19] and CD105-specific mAb 43A3[20] were also generated in our laboratory. Commercially available antibodies included CD10-PE, CD13-PE, CD34-PE, CD45-PE, CD56-PE, CD61-PE, CD117-PE (all from Becton Dickinson, Heidelberg, Germany). PE-conjugated mAbs with specificity for CD90, CD140b, and CD164 were obtained from Pharmingen (San Diego, USA). Monoclonal anti-nerve growth factor receptor antibody (NGFR) was purchased from Sigma (München, Germany). Non-conjugated antibodies were stained with isotype-matched PE-conjugated goat anti-mouse antisera (Southern Biotechnology Associates, Inc., Birmingham, USA).

For immunocytochemical analysis antibodies against vimentin (DAKO, Glostrup, Denmark, 1:25), neurofilament (NF) 70 kDa (Chemicon International, Inc., Temecula, USA, 1:25), glial fibrillary acidic protein (GFAP), (DAKO, 1:25), neuronal nuclei (NeuN), (Chemicon, 1:100), MAP-2 (ICN Biomedicals, Inc., Ohio, USA, 1:500), β-tubulin (Santa Cruz Biotechnology, Inc., Santa Cruz, USA, 1:100), fibronectin (Immunotech, Marseille, France, 1:50), and nestin (polyclonal) (21) were used. For staining, Alexa Fluor 488-conjugated goat anti-mouse IgG or Rhodamine Red 570 goat anti-rabbit IgG antisera (Molecular Probes, Göttingen, Germany, 1:100) were employed.

Staining of cells and flow cytometric analysis

For flow cytometric analysis, trypsin-treated MSC or commercially available NPC were incubated with 10 μL phycoerythrin-conjugated antibodies or 25 μL culture supernatant at 4°C for 20 minutes in 96-well microtiter plates. Non-conjugated mAbs were stained after washing in FACS buffer (PBS; 0.5% BSA; 0.1% NaN3) with goat anti-mouse IgG1-PE (1:100) or goat anti-mouse IgG3-PE (1:20) antisera. After washing, cells were analyzed on a flow cytometer (FACSCalibur, Becton Dickinson)

using the Cell Quest Software (Becton Dickinson).

For immunocytochemical analysis of intracellular antigens and extracellular matrix proteins, MSC or NPC cultured on 8-well chamber slides were fixed with acetone for 2 minutes and labeled with the primary antibody for 60 minutes followed by staining with Alexa 488-conjugated goat anti-mouse IgG or Rhodamine Red 570-conjugated goat anti-rabbit IgG antisera. For controls, cells were either labeled with an isotype-matched control antibody or with pre-immune rabbit serum. The fluorescence of cells was evaluated on a fluorescence microscope (Zeiss, Oberkochen, Germany).

Reverse transcriptase polymerase chain reaction (RT-PCR) analyses

Total RNA was isolated from cell lysates using the High Pure RNA Isolation Kit (Roche Molecular Biochemicals, Mannheim, Germany) according to the instructions of the manufacturer. This protocol includes a DNase incubation to digest contaminating DNA. Approximately 1 μg of total RNA was subjected to a 20 μL cDNA synthesis reaction using random primer (1st Strand cDNA Synthesis Kit for RT-PCR (AMV), Roche). Two microliters of cDNA were used for PCR amplification. To control the integrity of the RNA and the efficiency of the cDNA synthesis, 1 μL of cDNA was amplified by an intron-spanning primer pair for the β_2-microglobulin gene. The primer sequences for β_2-microglobulin were: 5'-GGGTTTCATCCATCCGACAT-3' and 5'-GATGCTGCTTACATGTCTCGA-3'. PCR profiles: 25 cycles and primer annealing at 55°C (GeneAmp PCR System 2400, Applied Biosystems, Weiterstadt, Germany). RT-PCR for vimentin, nestin, MAP-2, neurofilaments, and GFAP was performed as described by Shamblott *et al.*[22] Ten microliters of the PCR reactions were electrophoresed through a 3% agarose gel and stained with ethidium bromide for visualization under UV light.

Statistics

Statistical analyses were performed with the Instat, GraphPad Software, San Diego, USA.

Results

Immunophenotype of MSC

We compared the phenotype of commercially available MSC with MSC generated in our laboratory. In the latter case bone marrow cells from healthy donors were cultured in tissue culture flasks (75cm^2) at a density of 2.0×10^5 cells/cm^2 for 10 to 14 days in RPMI medium containing 10% FBS. As shown by light and fluorescence microscope analysis, the resulting adherent MSC population was heterogeneous and consisted of fibroblastoid cells in addition to small single cells and polygonal cells of different size. The immunophenotype of these MSC was analyzed at various passages (passage 1-5 = 14-61 days of culture). Flow cytometry analyses demonstrated that MSC of all passages, including commercially available MSC, expressed CD10, CD13, CD56 low, CD61, CD90,

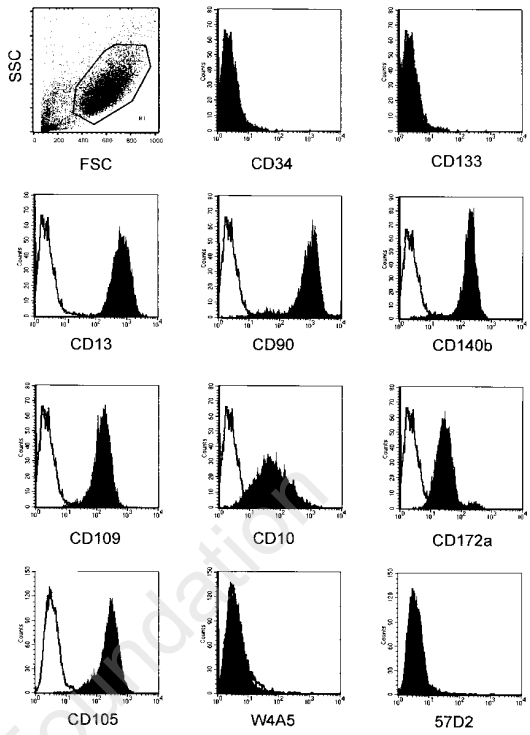

Figure 1. Immunophenotype of mesenchymal stem cells (MSC). Flow cytometry analyses demonstrate that MSC express CD13, CD90, CD140b, CD109, CD10, CD172a, and CD105. However, they are negative for CD34, CD133, 57D2, and W4A5.

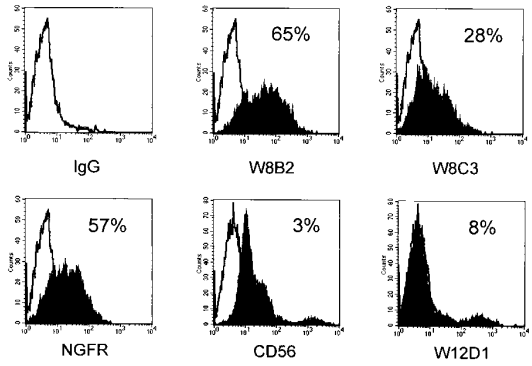

Figure 2. Selected markers that are heterogeneously expressed on MSC: W8B2, W8C3, nerve growth factor receptor (NGFR), CD56, and W12D1.

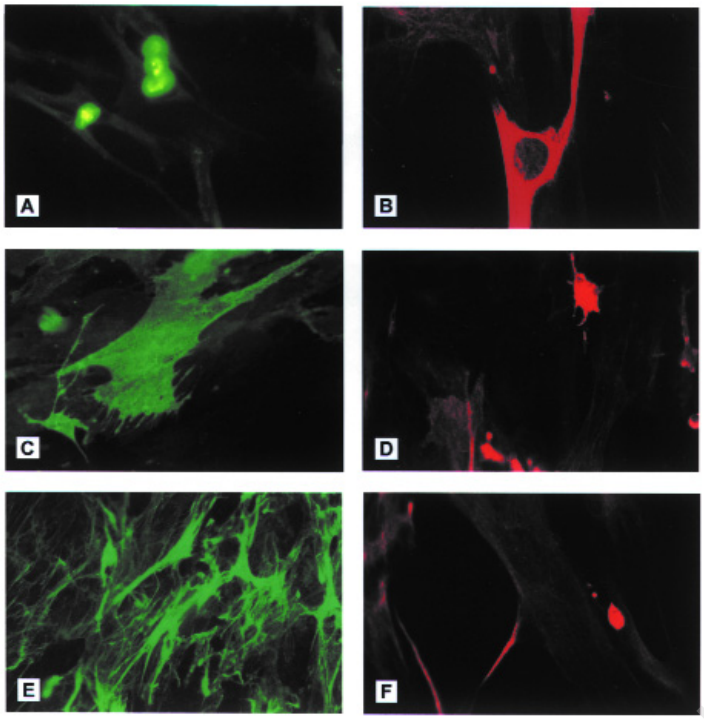

Figure 3. Alexa Fluor 488 or Rhodamine Red 570 immunofluorescence of MSC grown on chamber slides. (A) The mAb against the W8B2 antigen reacts with small round cells. (B) Note the strong intracellular staining of nestin in a fibroblastoid cell. (C) Staining of large fibroblastoid cells and cells with polygonal appearance with the W8B2 antibody. (D) Note the nestin reactivity of a polygonal, branched cell. (E) MSC strongly express the extracellular matrix component fibronectin. (F) Small cells also express nestin.

CD105 (endoglin), CD109, CD140b (PDGF-RB), CD164, and CD172a (SIRPα), as well as an antigen defined by our mAb W8B2, but were negative for CD15, CD45, CD34, CD117, CD133, CD167a, and 57D2 antigen (selected antigens are shown in Figure 1). In most cases, the expression patterns of commercial MSC and MSC generated in our laboratory were identical or similar (Table 1). The main difference was the exclusive reactivity of mAb W8B2 with laboratory-generated MSC and not to commercial MSC.

In individual cultures of our MSC preparations, we observed reactivity with mAbs W8B2, W8C3, and W12D1. In all cases mAb W8B2 reacted with one or two MSC subpopulations. Nerve growth factor receptor (NGFR) was expressed only in three MSC preparations and only one MSC preparation contained a small subpopulation expressing the surface markers CD56 and W12D1 antigen (Figure 2). We also observed a passage number-dependent heterogeneity of antigen expression on MSC. In the case of CD109 expression, a gradual decrease to about 10% of the original value was recorded from the first to the fifth passage (p=0.0026). In addition, the reactivity of mAb W8B2 with MSC decreased significantly with increasing passage number (p=0.0047). Thus, we demonstrated stage-specific expression as well as heterogeneity among MSC populations.

To analyze the expression of intracellular differentiation antigens and extracellular matrix proteins, immunofluorescence analysis was performed on MSC grown on chamber slides. The mAb against the W8B2 antigen reacted with small round cells

Table 1. Antigen expression on mesenchymal stem cells (MSC) and neural progenitor cells (NPC).

Antigen/Antibody	*MSC lab.*	*MSC com.*	*NPC com.*
CD13	+	+	P
CD15	–	–	+
CD34	–	–	S
CD45	–	–	–
CD56	S	S	+
CD90	+	+	+
CD105	+	+	–
CD109	+	+	–
CD117	–	–	S
CD133	–	–	+
CD140B	+	+	–
CD164	+	+	+
CD167	–	S	S
CD172a	+	+	+
W4A5	–	–	+
W8B2	+	–	–
W8C3	(+)	(+)	P
W12D1	(+)	-	-
57D2	-	-	+
NGFR	(+)	(+)	+

Abbreviations: lab.: laboratory; com.: commercial; – negative; (+) at least one analysis positive; S: low to undetectable expression; + positive: p cell population < 5%.

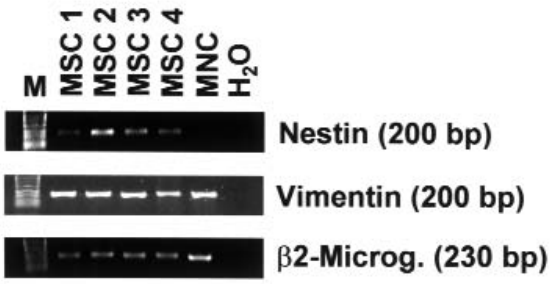

Figure 4. RT-PCR analysis of nestin and vimentin mRNA expression in MSC. Nestin and vimentin were expressed in four mesenchymal stem cell (MSC1-4) preparations. Mononuclear peripheral blood cells (MNC) and water were used as controls.

(Figure 3A) as well as with large fibroblastoid cells and cells with polygonal appearance (Figure 3C). Strong intracellular staining of nestin could be demonstrated in fibroblastoid cells (Figure 3B) and polygonal, branched cells (Figure 3D). We also observed that small cells frequently expressed nestin (Figure 3F). However, the W8B2$^+$ population was distinct from the nestin$^+$ cells in all studied cases (n=3). As shown in Figure 3E, MSC also strongly expressed the extracellular matrix component fibronectin. In contrast, the neuronal markers Neu-N, NF, GFAP, β-tubulin, and MAP-2 were negative (*not shown*).

m-RNA expression in MSC

Using RT-PCR we analyzed the expression of nestin, MAP-2, neurofilaments, and GFAP mRNA. In line with immunophenotypic analyses we detected nestin and vimentin mRNA (Figure 4). As expected, no neurofilaments, GFAP or MAP-2 mRNA was found in MSC (*not shown*).

Immunophenotype of NPC

We also studied the immunophenotype of commercial fetal neural progenitor cells (NPC). CD13, CD133, CD172a, and W8C3 antigen were heterogeneously expressed on NPC, whereas all NPC subsets expressed CD15, CD56, CD90, CD164, NGFR, and antigens defined by our unique mAbs 57D2 and W4A5. The cells were negative for CD45, CD105 (endoglin), CD109, and CD140b (PDGF-RB) as well as for W8B2 antigen (Figure 5).

NPC cultured on chamber slides in the presence of astrocyte growth medium (CellSystems) or FBS-containing medium expressed nestin, Neu-N, MAP2, neurofilaments, and W4A5 antigen (Figures 6A-F). NPC cultured in the presence of neural progenitor cell medium (CellSystems) gave rise to neurospheroids (*not shown*).

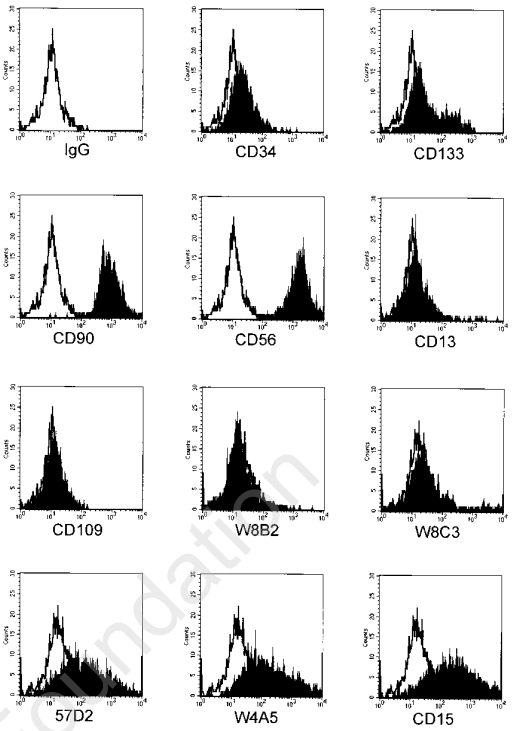

Figure 5. Immunophenotype of neural progenitor cells (NPC). NPC or NPC subpopulations express CD133, CD56, CD90, CD15, 57D2, and W4A5. Antibodies W7C5 (CD109) and W8B2 do not react with NPC. MAb W8C3 reacts only with a small NPC subpopulation.

In summary, comparative analysis of NPC and MSC immunophenotypes revealed that expression of CD34, CD45, CD117, and CD167 is low or absent in MSC and NPC (Table 1). In contrast to NPC, MSC express CD13, CD105, CD109, CD140b, and our novel monoclonal antibody-defined antigen W8B2. CD15, CD133, 57D2, and W4A5 antigen expression is exclusively found on NPC. Notably, CD133 expression is only detected on a NPC subset. CD90, CD164, and CD172a are expressed on both cell types (Table 1).

Discussion

The purpose of these studies was the phenotypic characterization of bone marrow-derived MSC as well as NPC. MSC were typically negative for CD45, CD34, CD133 and positive for CD10, CD13, CD61, CD90, and CD105 (endoglin). This immunophenotype is consistent over culture passages and is in accordance with published data.[23] Here we show for the first time that MSC also express CD109, CD140b (PDGF-RB), CD164, and CD172a (SIRPα). Interestingly, our novel antibody W8B2 (non-reactive with bone marrow cells) was able to fraction-

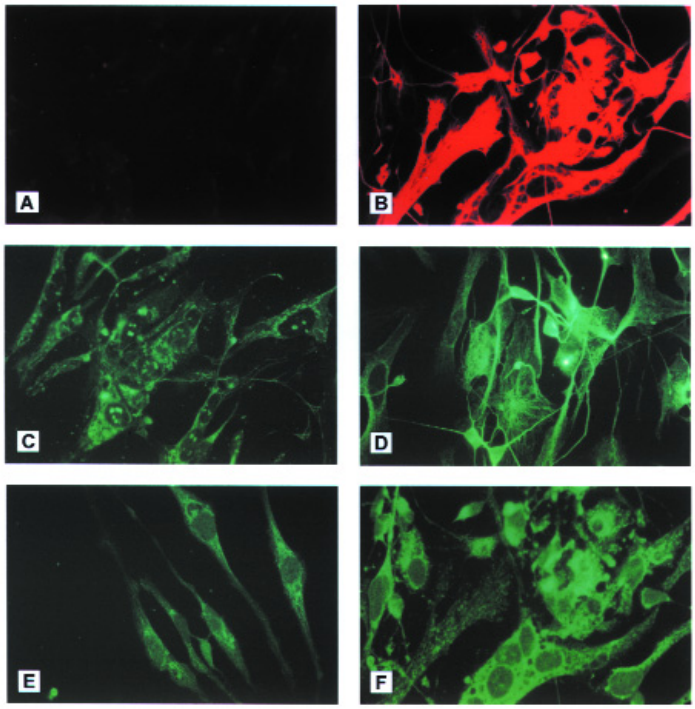

Figure 6. Alexa Fluor 488 or Rhodamine Red 570 immunofluorescence of cultured NPC: (A) Negative control with Rhodamine Red 570. (B) The majority of cultured NPC show a positive reaction with nestin. After cultivation for three days in astrocyte medium, NPC express the typical differentiation markers Neu-N (C), MAP-2 (D), NF (E), and W4A5 antigen (F).

ate the MSC cultures into subpopulations. Moreover, the stem cell antigen CD109 is decreasingly expressed on MSC passage cultures. After culture of primary cells, MSC are known to lose the surface antigens SH3, ICAM-1, and integrin β1 and to decrease their production of extracellular matrix molecules.[24] In contrast, CD109 and W8B2 antigen are first upregulated after culture of primary bone marrow cells into first passage MSC and then gradually downregulated after passages two to five. These molecules may, therefore, play an important role in the proliferative or differentiation potential of MSC. Interestingly, all studied cases of commercially available MSC were W8B2 negative. Most likely, the W8B2 antigen was downregulated in commercial MSC because of the higher number of passages these cells had gone through. This is also in line with the fact that commercial MSC expressed much lower levels of CD109 than did our laboratory-made first-passage MSC. Together, CD109 and W8B2 appear to represent markers of early MSC.

Stem cells derived from adult tissues retain some degree of plasticity and as a result, their differentiation capacity is less dependent on their primitive status but rather on their microenvironment.[25] Ferrari *et al.* described that lacZ-labeled bone marrow cells from donor mice could be incorporated into regenerating skeletal muscle of recipients.[26] Following bone marrow transplantation, donor-derived cells have been found in multiple non-hematopoietic tissues, including liver and astroglia in the brain.[27,28] These cells expressed the neural antigens Neu-N and nestin.[29]

In our studies we observed nestin expression also in bone marrow-derived MSC cultures. This intermediate filament was co-expressed with vimentin in MSC subpopulations. Nestin-positive MSC subpopulations with immature appearance consisted of small round cells and larger polygonal cells that were frequently branched. This demonstrates that MSC are morphologically and phenotypically heterogeneous. It has been reported that nestin is also found in neural precursors including neurospheres, neuron-restricted precursors, glial restricted precursors as well as in other developing cells including muscle and myocardial cells.[30,31] It was further demonstrated that nestin is associated with dividing and migrating cells and with cells rapidly changing their morphology.[32,33] The prominent expression of nestin in immature cells therefore suggests that the nestin-positive MSC populations represent more immature MSC precursors.

Recently, a novel mesenchymal progenitor cell termed multipotent adult progenitor cell (MAPC) or mesodermal progenitor cell (MPC) was described. This cell is able to expand and to differentiate into many mesenchymal cell lineages. Moreover, MAPC can also be induced to differentiate into cells with neuroectodermal and endodermal characteristics *in vitro*. The frequency of these rare cells in the adult bone marrow ranged between 1 in 10^7 to

10^8.[34-36] We did not find expression of classical neural markers such as NF and GFAP, even when first passage MSC were cultured with DMSO or retinoic acid or with serum-free culture medium supplemented with EGF or FGF, indicating that our MSC preparations did not contain cells committed to the neural lineage.

Because of their potential therapeutic use, stem cells of the central nervous system, such as NPC, represent an important basis for developing therapies for the reconstitution of the diseased or injured brain. Clonogenic central nervous system stem cells (NPC) can be isolated from adult and fetal brain. A major subpopulation of these cells is CD133-positive and negative for CD34, CD45, and CD24. In culture, these cells can differentiate into neurospheres.[37] To identify possible rare bone marrow populations with phenotypes consistent with neural cells, we compared the phenotypes of primary bone marrow cells with those of MSC and NPC. NPC were positive for CD15, CD56, CD90, CD133, W4A5, and 57D2 antigen, but negative for CD45, CD109, and W8B2 antigen. In contrast, MSC were positive for CD10, CD109, and W8B2 antigen, but negative for CD15, CD133, W4A5, and 57D2. Similar to NPC, small bone marrow subpopulations expressed CD15, CD56, CD90, and CD133. However, it is unlikely that any of these markers serve also as a marker for NPC. The most promising NPC markers, W4A5 and 57D2, are completely nonreactive with bone marrow populations.

Our data demonstrate individual morphologic and immunological heterogeneity of MSC and NPC. In MSC, the CD109 and W8B2 antigen expression profile was passage-dependent. Nestin expression was found in subpopulations of MSC as well as in the majority of NPC. The antigens defined by antibodies 57D2 and W4A5 represent two novel and promising surface markers with considerable specificity for NPC. The molecular characterization of these antigens is under way.

References

1. Caplan AI. Mesenchymal stem cells. J Orthop Res 1991;9:641-50.
2. Prockop DJ. Marrow stromal cells as stem cells for nonhematopoietic tissues. Science 1997;276:71-4.
3. Seshi B, Kumar S, Sellers D. Human bone marrow stromal cell: coexpression of markers specific for multiple mesenchymal cell lineages. Blood Cells Mol Dis 2000;26:234-46.
4. Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, et al. Multilineage potential of adult human mesenchymal stem cells. Science 1999;284:143-7.
5. Colter DC, Class R, DiGirolamo CM, Prockop DJ. Rapid expansion of recycling stem cells in cultures of plastic-adherent cells from human bone marrow. Proc Natl Acad Sci USA 2000;97:3213-8.
6. Lagasse E, Connors H, Al-Dhalimy M, Reitsma M, Dohse M, Osborne L, et al. Purified hematopoietic stem cells can differentiate into hepatocytes in vivo. Nat Med 2000;11:1229-34.
7. Kopen GC, Prockop DJ, Phinney DG. Marrow stromal cells migrate throughout forebrain and cerebellum, and they differentiate into astrocytes after injection into neonatal mouse brains. Proc Natl Acad Sci USA 1999;96:10711-6.
8. Brazelton TR, Rossi FM, Keshet GI, Blau HM. From marrow to brain: expression of neuronal phenotypes in adult mice. Science 2000;290:1775-9.
9. Woodbury D, Schwarz EJ, Prockop DJ, Black IB. Adult rat and human bone marrow stromal cells differentiate into neurons. J Neurosci Res 2000;61:364-70.
10. Sanchez-Ramos J, Song S, Cardozo-Pelaez F, Hazzi C, Stedeford T, Willing A, et al. Adult bone marrow stromal cells differentiate into neural cells in vitro. Exp Neurol 2000;164:247-56.
11. Rao MS. Multipotent and restricted precursors in the central nervous system. Anat Rec 1999;257:137-48.
12. Arsenijevic Y, Villemure JG, Brunet JF, Bloch JJ, Deglon N, Kostic C, et al. Isolation of multipotent neural precursors residing in the cortex of the adult human brain. Exp Neurol 2001;170:48-62.
13. Barami K, Grever WE, Diaz FG, Lyman WD. An efficient method for the culturing and generation of neurons and astrocytes from second trimester human central nervous system tissue. Neurol Res 2001;23:321-6.
14. Bhatia M. AC133 expression in human stem cells. Leukemia 2001;15:1685-8.
15. Giesert C, Almeida-Porada G, Scheffold A, Kanz L, Zanjani ED, Bühring HJ. The monoclonal antibody W7C5 defines a novel surface antigen on hematopoietic stem cells. Ann N Y Acad Sci 2001;938:175-83.
16. Giesert C, Marxer A, Sutherland DR, Schuh AC, Kanz L, Bühring HJ. Antibody W7C5 defines a CD109 epitope expressed on $CD34^+$ and $CD34^-$ hematopoietic and mesenchymal stem cell subsets. In: Orlic D, Brümmendorf TH, Sharkis SJ, Kanz L, eds. Hematopoietic stem cells 2002. Genetics and Function. Ann N Y Acad Sci 2002; in press.
17. Bühring HJ, Marxer A, Lammers R, Wissinger B. CD133 cluster report. In: Mason D, André P, Bensussan A, Buckley C, Civin C, Clark E, et al., eds. Leucocyte Typing VII. White Cell Differentiation Antigens. Oxford: Oxford University Press. 2002. p. 622-3.
18. Vogel W. CD guide on CD167a. http://www.ncbi.nlm.nih.gov/prow/guide/ 1986613159_g.htm.
19. Seiffert M, Cant C, Chen Z, Rappold I, Brugger W, Kanz L, et al. Human signal-regulatory protein is expressed on normal, but not on subsets of leukemic myeloid cells and mediates cellular adhesion involving its counterreceptor CD47. Blood 1999;94:3633-43.
20. Greaves MF, Titley I, Colman SM, Bühring HJ, Campos L, Castoldi GL, et al. Report on the CD34 cluster workshop. In: Schlossman SF, Boumsell L, Gilks W, Harlan JM, Kishimoto T, Morimoto C et al, editors. Leucocyte Typing V. White Cell Differentiation Antigens. Oxford: Oxford University Press; 1995. p. 840-6.
21. Messam CA, Hou J, Major EO. Coexpression of nestin in neural and glial cells in the developing human CNS defined by a human-specific anti-nestin antibody. Exp Neurol 2000;161:585-96.
22. Shamblott MJ, Axelman J, Littlefield JW, Blumenthal PD, Huggins GR, Cui Y, et al. Human embryonic germ cell derivatives express a broad range of developmentally distinct markers and proliferate extensively in vitro. Proc Natl Acad Sci USA 2001;98:113-8.
23. Deans RJ, Moseley AB. Mesenchymal stem cells: biology and potential clinical uses. Exp Hematol 2000;28:875-84.
24. Conget PA, Minguell JJ. Phenotypical and functional properties of human bone marrow mesenchymal progenitor cells. J Cell Physiol 1999;181:67-73.
25. Goodell MA, Jackson KA, Majka SM, Mi T, Wang H, Pocius J, et al. Stem cell plasticity in muscle and bone marrow. Ann N Y Acad Sci 2001;938:208-18.
26. Ferrari G, Cusella-De Angelis G, Coletta M, Paolucci E, Stornaiuolo A, Cossu G, et al. Muscle regeneration by bone marrow-derived myogenic progenitors. Science 1998;279:1528-30.
27. Petersen BE, Bowen WC, Patrene KD, Mars WM, Sullivan AK, Murase N, et al. Bone marrow as a potential source of hepatic oval cells. Science 1999;284:1168-70.
28. Eglitis MA, Mezey E. Hematopoietic cells differentiate into both microglia and macroglia in the brains of adult mice. Proc Natl Acad Sci USA 1997;94:4080-5.

29. Mezey E, Chandross KJ, Harta G, Maki RA, McKercher SR. Turning blood into brain: cells bearing neuronal antigens generated in vivo from bone marrow. Science 2000;290:1779-82.
30. Lendahl U, Zimmerman LB, McKay RD. CNS stem cells express a new class of intermediate filament protein. Cell 1990;60: 585-95.
31. McKay R. Stem cells in the central nervous system. Science 1997;276:66-71.
32. Sjoberg G, Jiang WQ, Ringertz NR, Lendahl U, Sejersen T. Colocalization of nestin and vimentin/desmin in skeletal muscle cells demonstrated by three-dimensional fluorescence digital imaging microscopy. Exp Cell Res 1994;214:447-58.
33. Sahlgren CM, Mikhailov A, Hellman J, Chou YH, Lendahl U, Goldman RD, et al. Mitotic reorganization of the intermediate filament protein nestin involves phosphorylation by cdc2 kinase. J Biol Chem 2001;276:16456-63.
34. Reyes M, Verfaillie CM. Characterization of multipotent adult progenitor cells, a subpopulation of mesenchymal stem cells. Ann N Y Acad Sci 2001;938:231-5.
35. Reyes M, Lund T, Lenvik T, Aguiar D, Koodie L, Verfaillie CM. Purification and ex vivo expansion of postnatal human marrow mesodermal progenitor cells. Blood 2001;98:2615-25.
36. Jiang Y, Jahagirdar BN, Reinhardt RL, Schwartz RE, Keene CD, Ortiz-Gonzalez XR, et al. Pluripotency of mesenchymal stem cells derived from adult marrow. Nature 2002;418:41-9.
37. Uchida N, Buck DW, He D, Reitsma MJ, Masek M, Phan TV, et al. Direct isolation of human central nervous system stem cells. Proc Natl Acad Sci USA 2000;97:14720-5.

Pre-publication Report & Outcomes of Peer Review

Contributions

WV: conduction and design of experiments; FG: conduction of mRNA expression experiments; CAM: design of nestin expression experiments, provided anti-nestin antibody; LK: general design of experiments; WB: general design of experiments; H-JB: conduction and design of experiments. WV and HJB; primarily responsible for the publication; WV and HJB; primarily responsible for Figures 1, 2, 3, 5 and 6; FG: primarily responsible for Figure 4.

The authors gratefully acknowledge the excellent technical assistance of Ms. Alexandra Wahl and Ms. Anke Marxer.

Funding

This work was supported by a grant from the Federal Ministry of Education and Research (BMFB) and the Interdisciplinary Clinical Research Center (IZKF) Tübingen: Cell biology in diagnostic and therapy of organ systems diseases (Fö.01KS 9602) and in part by a grant from the Else-Übelmesser foundation.

Disclosures

Conflict of interest: none.

Redundant publications: no substantial overlapping with previous papers.

Manuscript processing

This manuscript was peer-reviewed by two external referees and by Professor Mario Cazzola, Editor-in-Chief. The final decision to accept this paper for publication was taken jointly by Professor Cazzola and the Editors. Manuscript received July 15, 2002; accepted December 3, 2002.

In the following paragraphs, the Editor-in-Chief summarizes the peer-review process and its outcomes.

What is already known on this topic

Bone marrow-derived mesenchymal stem cells and neural progenitors cells are generally considered as populations with homogenous morphology and phenotype.

What this study adds

This study shows that the above populations can be distinguished using three monoclonal antibodies.