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Functional potentials of human hematopoietic progenitor cells are maintained by mesenchymal stromal cells and not impaired by plerixafor

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Abstract

Background aims. Mesenchymal stromal cells (MSCs) resemble an essential component of the bone marrow niche for maintenance of stemness of hematopoietic progenitor cells (HPCs). Perturbation of the C-X-C chemokine receptor type 4 (CXCR4)/stromal cell-derived factor-1α (SDF-1α) axis by plerixafor (AMD3100) mobilizes HPCs from their niche; however, little is known about how plerixafor affects interaction of HPCs and MSCs *in vitro*. *Methods.* We monitored cell division kinetics, surface expression of CD34 and CXCR4, migration behavior and colony-forming frequency of HPCs on co-culture with MSCs either with or without exposure to plerixafor. *Results.* Co-culture with MSCs significantly accelerated cell division kinetics of HPCs. Despite this, the proportion of $CD34^+$ cells was significantly increased on co-culture, whereas the expression of CXCR4 was reduced. In addition, co-culture with MSCs led to significantly higher colony-forming capacity and enhanced migration rate of HPCs compared with mono-culture conditions. The composition of MSC subpopulations—and conversely their hematopoiesis supportive functions—may be influenced by culture conditions. We compared the stromal function of MSCs isolated with three different culture media. Overall, the supporting potentials of these MSC preparations were quite similar. Perturbation by the CXCR4-antagonist plerixafor reduced the cell division kinetics of HPCs on co-culture with MSCs. However, the progenitor cell potential of the HPCs as reflected by colony-forming capacity was not affected by plerixafor. *Conclusions.* These results support the notion that the CXCR4/SDF-1α axis is critical for HPC-MSC interaction with regard to migration, adhesion and regulation of proliferation but not for maintenance of primitive progenitor cells.

Key Words: *CXCR4/SDF-1α, hematopoietic progenitor cells, mesenchymal stromal cells, plerixafor, stem cell niche*

Introduction

Mesenchymal stem cells are defined as plastic adherent spindle-shaped cells with the ability to differentiate into adipogenic, osteogenic and chondrogenic cells. Because the resulting cell population is heterogeneous, and only a few cells actually possess stem cell capabilities, many authors recommend calling them mesenchymal stromal cells (MSCs) (1–3). Various different protocols for preparation and cultivation of MSCs are used in laboratories worldwide, hampering the comparison of experimental data. Although criteria for defining MSC preparations have been delineated by the International Society for Cellular Therapy (4), these parameters represent the lowest common denominator. We have demonstrated that even slight variations in culture conditions—especially in the composition of expansion medium—can significantly alter the resulting cell populations (5–8). Previous studies clearly demonstrated the capability of MSCs to maintain stemness of hematopoietic progenitor cells (HPC) under co-culture conditions (9–14). However, the role of the C-X-C chemokine receptor type 4 (CXCR4)/stromal cell-derived factor-1α (SDF-1α) interaction and the influence of the applied MSC expansion media on this effect are still unclear.

We hypothesize that the interaction between HPCs and MSCs is significantly mediated by the

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CXCR4/SDF-1α axis. We analyzed whether the differentiation potential of human HPCs in a co-culture setting with MSCs could be altered by the CXCR4-antagonist plerixafor. To discern the impact of different culture conditions of MSCs on their hematopoiesis supportive function, we conducted all co-culture experiments with three different MSC populations, which had been cultured before in different expansion media.

Methods

Isolation of human HPCs

HPCs were gained from umbilical cord blood after obtaining informed consent using guidelines approved by the Ethics Committee on the Use of Human Subjects at Heidelberg University. HPCs were isolated as described previously (15). Briefly, mononuclear cells were isolated by density gradient centrifugation on Ficoll-Hypaque technique (Biochrom KG, Berlin, Germany). $CD34^+$ cells were purified by positive selection with a monoclonal anti-CD34 antibody using magnetic micro-beads on an affinity column with a MACS CD34 isolation kit (Miltenyi Biotec, Bergisch-Gladbach, Germany). Purification of isolated cells was quantified by flow cytometry (fluorescent activated cell sorting) analyses (>90% for all experiments).

Isolation and culture of human MSCs

MSCs were derived from aspirated bone marrow of healthy voluntary donors after obtaining informed consent and according to guidelines approved by the Ethics Committee on the Use of Human Subjects at Heidelberg University. Bone marrow aspirates (10−30 mL) were collected in a syringe containing 10,000 IU heparin to prevent coagulation. The mononuclear cell fraction was isolated by density gradient centrifugation on Ficoll-Hypaque (density =1.077 g/cm^3) and seeded in tissue culture flasks at a density of 1×10^6 cells/cm^2 (Nunc 75 cm^2-flasks; Nalge Nunc, Naperville, IL, USA) for 2o days.

MSC Expansion

MSCs were expanded by using three culture conditions (M1−M3). MSC M1 were cultivated following the protocol described by Jiang *et al.* (16). Cells were seeded in tissue flasks coated with 10 ng/mL fibronectin (Sigma-Aldrich, Taufkirchen, Germany) at a density of 1×10^4 cells/cm^2. The culture medium M1 consisted of 58% Dulbecco's Modified Eagle's Medium-Low Glucose (DMEM-LG; Cambrex, Apen, Germany) and 40% MCDB201 (Sigma-Aldrich), 2% fetal calf serum (FCS; HyClone, Bonn, Germany), supplemented with 2 mmol/L L-glutamine (PAA Laboratories), 100 U/mL penicillin/streptomycin (PAA Laboratories, Cölbe, Germany), 1% insulin transferrin selenium, 1% linoleic acid bovine serum albumin, 10 nmol/L dexamethasone, 0.1 mmol/L L-ascorbic acid-2-phosphate (all from Sigma), human platelet-derived growth factor-bb and epidermal growth factor (10 ng/mL each, R&D Systems, Minneapolis, MN, USA).

MSC M2 were cultivated in commercially available Osiris medium (MSCGM; Lonza, Basel, Switzerland) with 10% FCS following the manufacturer's instructions; 5000 cells/cm^2 were plated in tissue flasks without any pre-coating. MSC M3 were cultured according to the protocol of Bieback *et al.* (17), using 90% basal medium (DMEM with 2% L-glutamine and 1% penicillin/streptomycin), 100 U/mL heparin and 10% pooled human platelet lysate as a substitute for FCS.

Culture medium was always changed twice per week. After reaching 80% confluence, MSCs were trypsinized, counted with a Neubauer counting chamber (BRAND, Wertheim, Germany), and re-seeded at 10^4 cells/cm^2 for further expansion. If not indicated otherwise, we used sub-confluent MSCs feeder layer (70−80%) of passages 4−6 in this study.

Culture conditions and expansion of HPCs

In all experiments, HPCs were expanded in 24-well plates in long-term bone marrow culture (LTBMC) medium consisting of Iscove's modified Dulbecco's medium (Gibco, Life Technologies Ltd., Paisley, UK) with 12.5% FCS, 12.5% horse serum (Terry Fox Laboratories, Vancouver, Canada), 2 mmol/L L-glutamine (Gibco, Life Technologies Ltd.), penicillin 100 U/mL, streptomycin 100 U/mL (Gibco, Life Technologies Ltd.) and 10^{-6} mol/L hydrocortisone. $CD34^+$ cells were cultivated either without stromal feeder layer or on a confluent feeder layer of MSCs M1−M3 irradiated with 20 Gy in 24-well plates 24 h before use.

Analyses of cell division kinetics

$CD34^+$ cells were stained with carboxyfluorescein diacetate *N*-succinimidyl ester (CFSE; Sigma-Aldrich) to monitor cell division kinetics for several days of (co-)culture, as described previously (18). Briefly, cells were washed at day 0 in phosphate-buffered saline (PBS) with 0.1% FCS and stained with CFSE at a final concentration of 2.5 µmol/L for 10 min at 37°C. Staining reaction was stopped with 6 mL ice cold Roswell Park Memorial Institute medium with 10% FCS for 5 min followed by three

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