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Impact of individual platelet lysates on isolation and growth of human mesenchymal stromal cells

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Abstract

Background aims. Culture medium for mesenchymal stromal cells (MSC) is frequently supplemented with fetal calf serum (FCS). FCS can induce xenogeneic immune reactions, transmit bovine pathogens and has a high lot-to-lot variability that hampers reproducibility of results. Several studies have demonstrated that pooled human platelet lysate (HPL) provides an attractive alternative for FCS. However, little is known about the variation between different platelet lysates. *Methods.* We compared activities of individual HPL on initial fibroblastoid colony-forming units (CFU-F), proliferation, *in vitro* differentiation and long-term culture. These data were correlated with chemokine profiles of HPL. *Results.* Isolation of MSC with either HPL or FCS resulted in similar CFU-F frequency, colony morphology, immunophenotype and adipogenic differentiation potential. Osteogenic differentiation was even more pronounced in HPL than FCS. There were significant differences in MSC proliferation with different HPL, but it was always higher in comparison with FCS. Cell growth correlated with the concentration of platelet-derived growth factor (PDGF) and there was a moderate association with platelet counts. All HPL facilitated expansion for more than 20 population doublings. *Conclusions.* Taken together, reliable long-term expansion was possible with all HPL, although there was some variation in platelet lysates of individual units. Therefore the use of donor recipient-matched or autologous HPL is feasible for therapeutic MSC products.

Key Words: *colony-forming units, cytokine profile, fetal calf serum, long-term culture, mesenchymal stromal cells, platelet lysate*

Introduction

Mesenchymal stromal cells (MSC) are precursors for mesodermal cell lineages such as osteocytes, chondrocytes and adipocytes. They can be isolated from many tissues by plastic-adherent growth under various culture conditions. MSC are heterogeneous and comprise different cell types; so far reliable markers for definition of the pure multipotent subset of 'mesenchymal stem cells' remain to be elucidated (1–4). Nevertheless, the ease of isolation, possibility of culture expansion to very high cell numbers as well as absence of critical side-effects in recent clinical trials raise hopes for several therapeutic applications (5–7).

Isolation and culture expansion of MSC is commonly performed in media supplemented with fetal calf serum (FCS). FCS is isolated from clotted blood of unborn bovine fetuses and has been shown to be low in antibodies and rich in growth factors. FCS has evolved to the most widely used growth supplement for cell culture media, and a huge arsenal of different preparations is available commercially. For clinical application, cell products have to be generated under good manufacturing practice (GMP) conditions and therefore FCS needs to be pre-tested and sterile filtered or irradiated. Because of bovine spongiform encephalopathy (BSE), clinical-grade FCS usually comes from Australia or New Zealand. Nevertheless, the risk of xenogene contamination and immunologic consequences, such as anti-FCS antibodies, remains (8,9). Furthermore, high lot-to-lot variability can hamper reproducibility and consistency of results from cell culture experiments. Therefore, regulatory

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guidelines for cellular products aim to reduce FCS in favor of human cell culture supplements (10).

Human serum has been suggested as a substitute for FCS for the isolation and expansion of MSC (11–14). However, the activity of human serum for proliferation of MSC is often rather low. Therefore, this method has not been implemented for culture expansion of MSC (15,16). Platelets contain several growth factors, such as platelet-derived growth factor (PDGF) which has been shown to enhance proliferation of MSC (17,18). This has fuelled research in alternative supplements, such as platelets, platelet lysate and platelet-released factors (16,19).

Human platelet lysate (HPL) can be generated from common platelet units by a simple freeze–thaw procedure. Several recent studies have demonstrated that HPL is very effective for the expansion of MSC and protocols have been implemented for their therapeutic application (20–26). It has been anticipated that there is donor-specific variation between lysates of platelet units. Therefore pooled HPL of about 40–50 donations per batch has been used to minimize donor-specific effects (16,21). On the other hand, it might be advantageous to use donor–recipient matched or even autologous HPL for therapeutic applications to minimize even further the risk of immunologic side-effects and viral infections. Therefore, we compared the variation between lysates of individual platelet units and analyzed which compounds support the expansion of MSC.

Methods

Generation of human supplements

HPL were generated from platelet units that were harvested from individual healthy donors by apheresis using the Trima Accel collection system (CaridianBCT, Garching, Germany). Such thrombocyte concentrates have a platelet content of 2.0–4.2 $\times$ 10^{11} platelets in 200 mL plasma supplemented with acid–citrate–dextrose (ACD) (1:11 v/v). If indicated in the text, alternatively we have used thrombocyte concentrates of four AB Rh^+ buffy coats to prepare pooled human platelet lysate (pHPL; kindly provided by Professor Reinhard Henschler, German Red Cross Blood Donation Service, University Frankfurt, Germany). Five days after harvesting, the platelet units were distributed in aliquots of 45 mL, and twice frozen at –80°C and thawed at 37°C. To remove membrane fragments, the lysates were centrifuged at 2600 ***g*** for 30 min and the supernatant was filtered through 0.2-µm GD/X PVDF filters (Whatman, Dassel, Germany). HPL was stored at –80°C until use and 2 U/mL heparin were added to the media to avoid gelatinization.

For some experiments we separated the plasma and platelet fractions prior to lysis to determine the effects of these subfractions: platelet units were centrifuged at 400 ***g*** for 10 min and the plasma fraction was harvested carefully. The pellet of platelets was washed with phosphate-buffered saline (PBS) and resuspended in the same volume of culture medium. Both fractions were twice frozen at –80°C, sterile filtered and supplemented with heparin as described above.

Isolation of MSC from human bone marrow

MSC were isolated from the caput femoris upon hip fracture after written consent using guidelines approved by the ethic committee of the University of Aachen (Aachen, Germany). Bone fragments were flushed with PBS and mononuclear cells (MNC) subsequently isolated by density gradient on Biocoll (Biochrom AG, Berlin, Germany). For analysis of fibroblastoid colony-forming units (CFU-F) frequency and morphology, we used aspirates from human bone marrow (10 mL) supplemented with 0.5 mL heparin anti-coagulant (Becton Dickinson, San Jose, CA, USA; BD), after written consent using the guidelines approved by the ethic committee on the use of human subjects at the University of Heidelberg (Heidelberg, Germany). Erythrocytes were eliminated by red blood cell (RBC) lysis with ammonium chloride as described elsewhere (27,28).

Cells were cultured in fibronectin-coated cell culture dishes (Greiner, Kremsmünster, Austria) in Dulbecco's modified Eagle medium–low glucose (DMEM-LG; PAA, Pasching, Austria) with 2 mM L-glutamine (Sigma Aldrich, St Louis, MO, USA; Sigma) and 100 U/mL penicillin/streptomycin (pen/strep; Lonza, Basel, Switzerland). The culture medium was supplemented with FCS (HyClone, Bonn, Germany) or HPL as indicated in the text. For comparison, we also used the culture medium-M1 that was first described by Reyes *et al.* (29) and has been used in our previous work (30,31). Medium-M1 consists of DMEM-LG supplemented with 40% (v/v) MCDB201 (Sigma), 2 mM L-glutamine (Sigma), 100 U/mL pen/strep (Lonza), 1% (v/v) insulin transferrin selenium (Sigma), 1% (v/v) linoleic acid albumin from bovine serum albumin (Sigma), 10 nM dexamethasone (Sigma), 0.1 mM L-ascorbic acid-2-phosphate (Sigma), homodimer of PDGF subunit B (PDGF-BB) and epidermal growth factor (EGF) (both 10 ng/mL; PreproTech, Rocky Hill, NJ, USA) and 2% (v/v) FCS (HyClone). Culture medium was always changed twice per week. When reaching 80% confluence, cells were trypsinized, counted in a Neubauer counting chamber (Brand, Wertheim, Germany) and replated at 10^4 cells/cm^2. Cumulative population doublings were calculated from the first passage onwards, as described previously (31,32).