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STEM CELLS

Effects of a defined xeno-free medium on the growth and neurotrophic and angiogenic properties of human adult stem cells

MARIA BROHLIN[1], PEYMAN KELK[2], MIKAEL WIBERG[2,3] & PAUL J. KINGHAM[2]

[1]*Division of Clinical Immunology and Transfusion Medicine, Tissue Establishment, Cell Therapy Unit, Department of Laboratory Medicine, Umeå University Hospital, Umeå, Sweden,* [2]*Department of Integrative Medical Biology, Anatomy, Umeå University, Umeå, Sweden, and* [3]*Department of Surgical and Perioperative Sciences, Hand and Plastic Surgery, Umeå University, Umeå, Sweden*

Abstract

Background. The growth properties and neurotrophic and angiogenic effects of human mesenchymal stromal cells (MSCs) cultured in a defined xeno-free, serum-free medium (MesenCult-XF) were investigated. *Methods.* Human MSCs from adipose tissue (ASCs) and bone marrow (BMSCs) were cultured in Minimum Essential Medium-alpha (α-MEM) containing fetal calf serum or in MesenCult-XF. Proliferation was measured over 10 passages and the colony-forming unit (CFU) assay and expression of cluster of differentiation (CD) surface markers were determined. Neurite outgrowth and angiogenic activity of the MSCs were determined. *Results.* At early passage, both ASCs and BMSCs showed better proliferation in MesenCult-XF compared with standard α-MEM–containing serum. However, CFUs were significantly lower in MesenCult-XF. ASCs cultured in MesenCult-XF continued to expand at faster rates than cells grown in serum. BMSCs showed morphological changes at late passage in MesenCult-XF and stained positive for senescence β-galactosidase activity. Expression levels of CD73 and CD90 were similar in both cell types under the various culture conditions but CD105 was significantly reduced at passage 10 in MesenCult-XF. *In vitro* stimulation of the cells enhanced the expression of brain-derived neurotrophic factor (BDNF), vascular endothelial growth factor (VEGF-A) and angiopoietin-1. Stimulated ASCs grown in MesenCult-XF evoked the longest neurite outgrowth in a neuron co-culture model. Stimulated BMSCs grown in MesenCult-XF produced the most extensive network of capillary-like tube structures in an *in vitro* angiogenesis assay. *Conclusions.* ASCs and BMSCs exhibit high levels of neurotrophic and angiogenic activity when grown in the defined serum-free medium indicating their suitability for treatment of various neurological conditions. However, long-term expansion in MesenCult-XF might be restricted to ASCs.

Key Words: *adipose, bone marrow, clinical cell culture, mesenchymal stromal cells, neurotrophic factors*

Introduction

Mesenchymal stromal cells (MSCs), which can be derived from many different tissue sources including bone marrow and fat, have the potential for the treatment of a broad range of clinical conditions [1–6]. According to The International Society for Cellular Therapy (ISCT), MSCs are characterized by their (i) plastic adherence, (ii) expression of specific cell surface markers (CD73, CD90 and CD105) and (iii) their ability to differentiate *in vitro* into chondrocytes, adipocytes and osteoblasts [7]. More recently another joint statement article from the International Federation for Adipose Therapeutics and Science (IFATS) and the ISCT extended the characterization to stem cells isolated from adipose tissue [8]. In addition to their differentiation properties, MSCs secrete a plethora of factors that have immunomodulatory and growth-promoting functions. The MSCs' secretome thus plays a major role in the ability of the cells to elicit regeneration and repair of damaged tissue systems [9]. Our research has focused on using MSCs for peripheral nerve repair and our previous studies have shown that MSCs stimulated *in vitro* with a mixture of growth factors release high levels of neurotrophic molecules and enhance *in vitro* neurite outgrowth and axon

Correspondence: **Maria Brohlin**, PhD, Division of Clinical Immunology and Transfusion Medicine, Tissue Establishment, Cell Therapy Unit, Department of Laboratory Medicine, Umeå University Hospital, 907 85 Umeå, Sweden. E-mail: maria.brohlin@vll.se

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regeneration *in vivo* [10–12]. MSCs produce both angiogenic and neurotrophic factors including vascular endothelial growth factor (VEGF), angiopoietin-1, nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF) and glial-derived neurotrophic factor (GDNF) [13–16].

Today the standard protocol for culturing MSCs *in vitro* includes addition of fetal calf serum (FCS) to the medium that provides the essential factors for cell survival and growth [17]. However, adding serum from a xenogeneic source creates potential risks, such as contamination with prions, viruses and bacteria [18], and batch-to-batch variation presents a problem [19]. To minimize the risk for contamination and proceed toward clinical application of cultured cells, new defined and safe protocols are needed. Although there are a number of xeno- and serum-free replacement products commercially available, the challenge remains to find a standard protocol and a medium that is fully defined and can sustain the native character (biological trophic and immunomodulatory paracrine activity) of the cells over time to ensure safety in cell-based therapies. This also requires further optimization of the defined media under Good Manufacturing Practice (GMP) conditions [2,5,20–22]. In addition to xeno- and serum-free media formulations, various other possibilities have been suggested including the addition of autologous/allogenic human blood–derived platelet lysate, plasma or serum and umbilical cord blood serum [19,23–28].

Although the proliferation and differentiation of stem cells has been studied in defined xeno- and serum-free culture, there are few reports describing the trophic activity of the cells under these conditions. Therefore, in this study we have investigated the growth properties and neurotrophic and angiogenic effects of human adipose tissue–derived stem cells (ASCs) and human bone marrow–derived stem cells (BMSCs) cultured in a commercially available defined xeno-free, serum-free medium (MesenCult-XF).

Methods

Isolation of stem cells

Human adipose tissue samples were obtained from abdominal fat of three healthy patients undergoing elective surgery (mean age, 41 ± 4 years). Samples of human bone marrow were obtained from the iliac crests of three healthy donors during reconstructive surgery (mean age, 43 ± 8 years). Procedures were approved by the Local Ethical Committee for Clinical Research at Umeå University (No. 03–425 and 2013-276-31M). Adipose tissue was minced, digested with 0.15% weight/volume (w/v) type I collagenase and centrifuged as previously described [15]. The final stromal fraction pellet containing stem cells (ASCs) was resuspended in standard growth medium consisting of Minimum Essential Medium-alpha (α-MEM), 10% (v/v) FCS and 1% (v/v) penicillin/streptomycin (Invitrogen Life Technologies/Fisher Scientific) and plated (per 2 g initial tissue) onto 75 cm^2 culture flasks (Nunc). The flasks were washed with Hank's balanced salt solution every 24 h for 3 days to eliminate non-adherent hematopoietic cells, after which fresh growth medium was added. The BMSCs were isolated with modification of a previously described protocol [10]. Briefly, bone marrow samples were rinsed thoroughly with standard growth medium (as above). The cell suspension was centrifuged at 400*g* for 5 min and the cell pellet was filtered through a 70 μm nylon mesh (BD Falcon) and plated onto 75 cm^2 tissue culture flasks and incubated at 37°C, 5% CO_2. After 24 h in culture, the supernatant containing non-adherent cells was removed and discarded and fresh medium added. This was repeated for the next 2 days. Before beginning further experiments, the multi-potency of the ASCs and BMSCs was demonstrated by differentiation into osteogenic and adipogenic lineages as described previously [10].

Cell expansion and colony-forming unit assay

Cells were expanded under two different conditions. Upon reaching approximately 80% confluence, cells were enzymatically detached from the flasks using 0.25% (v/v) trypsin/ethylenediaminetetraacetic acid solution (Invitrogen Life Technologies/Fisher Scientific) and seeded in standard growth medium (as above, used for the isolation of the stem cells). Alternatively, the MesenCult-SF culture kit (Stem Cell Technologies) was used. This consists of MesenCult-XF Medium and MesenCult-SF Attachment Substrate and the kit was used in combination with the MesenCult-ACF Dissociation Kit. The complete system was used according to the manufacturer's instructions. The cells were reseeded at a density of 1000 cells/cm^2 and re-plated every week for proliferation in the α-MEM containing 10% FCS (v/v) or MesenCult-XF medium for a period of 10 weeks. From the weekly cell counts, population doubling (PD) times were calculated according to the calculation of doubling time (in h) = h*ln(2)/ln(c2/c1) and cumulative PDs were also calculated. At passages two and 10 the colony-forming unit (CFU) assay was performed with minor modification of previously published protocols [29]. Cultures were established as duplicates for each patient sample and experimental condition and incubated to 14 days. The initial cell seeding density varied from approximately 50 cells/cm^2 for cells at early passage in medium containing FCS to approximately 500 cells/cm^2 for early and late passage cells in MesenCult-XF (accounting for poor viability at the lower seeding densities) and

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