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In vitro immunologic properties of human umbilical cord perivascular cells

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Background

It has been shown recently that human umbilical cord perivascular cells (HUCPVC) are bio-equivalent to bone marrow-derived mesenchymal stromal cells (BM-MSC) in their mesenchymal differentiation and marker expression. HUCPVC populations provide high yields of rapidly proliferating mesenchymal progenitor cells. The question we wished to address, in two independent laboratory studies, was whether HUCPVC exhibit a similar in vitro *immunologic phenotype to that of BM-MSC.*

Methods

HUCPVC were isolated by physical extraction of umbilical vessels followed by enzymatic digestion of the perivascular cells, and lymphocytes were obtained from heparinized human peripheral blood. Experimental evaluations were lymphocyte proliferation in HUPCVC or BM-MSC co-cultures with peripheral blood lymphocytes (PBL), mixed lymphocyte cultures (MLC) containing BM-MSC or HUCPVC, CD25 and CD45 expression in co-cultures containing HUCPVC, and finally lymphocyte proliferation in TransWell MLC with HUCPVC.

Results

Both HUCPVC and BM-MSC showed no significant increase in proliferation of lymphocytes when co-cultured. The addition of 10% HUCPVC or BM-MSC significantly reduced proliferation of PBL in one-way MLC. Upon inclusion of HUCPVC with activated T-cell lines, the expression of both CD25 and CD45 showed a significant decrease. HUCPVC were able to reduce lymphocyte cell numbers significantly when separated with a membrane insert.

Discussion

HUCPVC are not alloreactive and exhibit immunosuppression in vitro. *Lymphocyte activation is significantly reduced in the presence of HUCPVC, and the immunosuppressive effect of HUCPVC is due, in part, to a soluble factor. Thus HUCPVC shows a similar immunologic phenotype to BM-MSC.*

Keywords

immunosuppression, mesenchymal stromal cells, perivascular, umbilical cord, MSC.

Introduction

The perivascular tissue of the human umbilical cord contains a rich source of mesenchymal precursor cells that we have called human umbilical cord perivascular cells (HUCPVC) [1]. In fact, the perivascular niche has recently been shown to be the source of mesenchymal progenitors in many organs [2] and bone marrow-derived multipotent mesenchymal stromal cells (BM-MSC; also known as mesenchymal stem cells) are themselves considered perivascular in origin [3]. The connective tissue matrix of the human umbilical cord has emerged as a promising cell source since McElreavey *et al.* [4] first extracted fibroblast-like cells. Following this, cells have been extracted using several methodologies, resulting in populations capable of differentiating into neural [5–8], cartilage [9,10], muscle [11] and heart leaflet [12] cells. HUCPVC express the markers 3G5 and CD146, indicating their pericytic derivation, which is further demonstrated by the geographic exclusivity of these markers to the perivascular regions of the cord [13]. HUCPVC have been shown to be bio-equivalent to BM-MSC in both their marker expression profile (including a lack of telomerase) and their lineage differentiation capacity [1,14,15]. However, HUCPVC populations provide higher yields of

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rapidly proliferating colony-forming cells at harvest [14,16], which has enabled their designation as mesenchymal stromal stem cells based on single-cell seeded clonal self-renewal and multilineage differentiation assays [16].

In addition to the multilineage differentiation capacity of MSC, which makes them a powerful tool for tissue repair therapies, much recent attention has been focussed on their immunoregulatory functions. MSC, from human and other species, suppress many T, B and natural killer (NK) cell functions and may also affect dendritic cells (DC) [17,18]. Their intermediate expression levels of human leukocyte antigen (HLA) class I [19,20] and lack of expression of the co-stimulatory molecules CD80 and CD86 [21] may, individually or synergistically, play a role in them being poorly recognized by HLA-incompatible hosts and mitigating an active immune response [22]. These phenomena of immunoprivilege and immunomodulation/suppression have been demonstrated using *in vitro* assays, based upon mixed lymphocyte cultures (MLC) [20,23,24], *in vivo* experiments in several species [25–29] and human clinical applications [30,31].

Although BM-MSC are both immunoprivileged and immunomodulatory, which make them ideal for their use in HLA-mismatched allogeneic cell therapies, their harvesting for such therapies requires an invasive and elective procedure; and it is known that the number and maximal life span of BM-MSC declines with increasing age of the donor [32,33]. Thus alternative MSC sources, suitable for large-scale expansion for clinical applications, have been sought in other tissues, such as the stromal cells derived from adult dermolipectomies, which have also been shown to be immunomodulatory [34,35].

Because of their similarity to BM-MSC in both phenotype and differentiation, the question we wished to address, in two independent laboratory studies, was whether HUCPVC exhibit a similar *in vitro* immunologic phenotype to that of BM-MSC. We used different *in vitro* co-culture methodologies, but with the same outcome: HUCPVC do not induce proliferation of allogeneic lymphocytes but suppress alloreactivity *in vitro*.

Methods

HUCPV cells

Ethical consent for this research was obtained from the University of Toronto as well as Sunnybrook & Women's College Health Sciences Centre. Umbilical cords were aseptically collected from Cesarean births of full-term babies, upon obtaining informed consent from the parent(s). The cords were immediately transported to the University of Toronto, where cells were extracted from the perivascular area under sterile conditions, as reported previously [1]. Briefly, 4-cm sections of cord were cut and the epithelium was removed. The vessels were then extracted, including their surrounding Wharton's jelly, tied in a loop to reduce blood cell contamination, and digested overnight in a collagenase solution (Sigma, Oakville, ON, Canada). Upon removal from the digest the following day, the cells were rinsed in ammonium chloride (Sigma, ibid) to lyze any remaining red blood cells. Following this, the cells were plated out at a density of 4000 cells/cm^2 in α-MEM containing 5% fetal bovine serum (FBS) and antimicrobials: penicillin G 167 U/mL, gentamicin 50 μg/mL and amphotericin B 0.3 μg/mL (Sigma, ibid). The cells were passaged when they reached 75–80% confluence, which was approximately every 6–7 days. Additionally, aliquots of cells were frozen in 10% DMSO (Sigma, ibid) and shipped using a vapor liquid nitrogen shipper (Chart SC 4/3V) to the Karolinska Institute for independent analyzes.

Lymphocytes

White blood cells were extracted from heparinized blood taken from healthy donors. Cell separation was achieved by Ficoll–Paque™ PLUS density gradient (Amersham Biosciences, Pittsburgh, PA, US), in which the cells were spun for 35 min at 380 ***g***. The buffy coat was removed and counted using a ViCell-XR™ (Beckman Coulter, Mississauga, ON, Canada) with a protocol specific for lymphocytes, as determined by cell diameter and nucleus size. The cells were then plated out as per the requirements of the assay in RPMI-1640 media (Sigma, Oakville, ON, Canada) containing HEPES (25 mmol/L), L-glutamine (2 mmol/L), 10% FBS and antimicrobials (same constituents as HUCPVC).

Co-culture

Triplicates of 1×10^4 HUCPVC were plated in 96-well plates (Falcon, Mississauga, ON, Canada). Once the cells had attached (approximately 2 h), 10^5 peripheral blood lymphocytes (PBL) were added to each well. The plates were incubated at 37°C with 5% CO_2 air in RPMI-1640 media containing HEPES (25mmol/L), L-glutamine (2 mmol/L), 10% FBS and antimicrobials. The cells were allowed to incubate for 6 days, after which they were