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Cytokine Profile of Human Adipose-Derived Stem Cells: Expression of Angiogenic, Hematopoietic, and Pro-Inflammatory Factors

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Adipose tissue serves as a source of adipokines and cytokines with both local and systemic actions in health and disease. In this study, we examine the hypothesis that multipotent human adipose-derived stem cells (ASCs), capable of differentiating along the adipocyte, chondrocyte, and osteoblast pathways, contribute to adipose tissue-derived cytokine secretion. Following exposure to basic fibroblast growth factor (bFGF) or epidermal growth factor (EGF), the ASCs significantly increase their secretion of hepatocyte growth factor (HGF), a cytokine implicated in hematopoiesis, vasculogenesis, and mammary epithelial duct formation. Ascorbic acid synergizes with these inductive factors, further increasing HGF levels. Following exposure to lipopolysaccharide, ASCs increase their secretion of both hematopoietic (granulocyte/monocyte, granulocyte, and macrophage colony stimulating factors, interleukin 7) and proinflammatory (interleukins 6, 8, and 11, tumor necrosis factor α) cytokines based on ELISA and RT-PCR. In co-cultures established with umbilical cord blood-derived $CD34^+$ cells, the ASCs support long-term hematopoiesis *in vitro*. Furthermore, in short-term 12-day co-cultures, the ASC maintain and expand the numbers of both myeloid and lymphoid progenitors. These observations are consistent with the functionality of the secreted cytokines and confirm recent reports by other laboratories concerning the hematopoietic supportive capability of ASCs. We conclude that the ASCs display cytokine secretory properties similar to those reported for bone marrow-derived mesenchymal stem cells (MSCs).
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Adipose tissue is a dynamic participant in endocrine physiology, serving as the source of secreted cytokines (reviewed in Kershaw and Flier, 2004; Trayhurn, 2005; Trayhurn and Beattie, 2001). The expression levels of adipose-derived cytokines are postulated to be risk factors for cardiovascular disease, diabetes, hypertension, and other components of the metabolic syndrome (Trayhurn, 2005). Adipose tissue contains multiple cell types, including endothelial cells and macrophages, which have been associated with cytokine production (Weisberg et al., 2003; Xu et al., 2003). There is mounting evidence that adipocytes and their progenitors can be a major source of cytokines in addition to adipokines (Trayhurn and Beattie, 2001; Rehman et al., 2003, 2004; Harkins et al., 2004; Kershaw and Flier, 2004; Nakagami et al., 2005; Trayhurn, 2005; Nakagami et al., 2006; Wang et al., 2006). Adipose-derived stem cells (ASCs) release angiogenic factors, such as hepatocyte growth factor (HGF), and this is postulated to contribute to their regenerative capability in ischemic injury models

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(Nakagami et al., 2005, 2006; Rehman et al., 2003, 2004; Wang et al., 2006). Indeed, circulating levels of HGF are elevated in obese patients (Rehman et al., 2003). Likewise, obesity is associated with elevated levels of pro-inflammatory cytokines, such as interleukin 6 (IL-6), interleukin 8 (IL-8), and tumor necrosis factor α (TNFα) (Bruun et al., 2001; Di Gregorio et al., 2005; Hotamisligil et al., 1995; Kern et al., 1995). Furthermore, adipose tissue expresses the hematopoietic growth factor, macrophage colony stimulating factor (M-CSF), and its expression can lead to adipose tissue volume expansion (Levine et al., 1998).

The plastic adherent cell stromal population expanded from collagenase digests of adipose tissue have been termed "Adipose-derived Stem Cells" (ASCs) based on a consensus reached by the Second Annual Meeting of the International Fat Applied Technology Society (Pittsburgh, PA, 2004). The ASCs display differentiation capacity along multiple lineage pathways at the clonal level (Zuk et al. 2003; Guilak et al. 2006) and have potential utility in regenerative medical and tissue engineering applications (reviewed in (Gimble and Guilak, 2003)). The differentiation potential of ASCs resemble those of bone marrow-derived mesenchymal stem cells or stromal cells (MSCs) (reviewed in (Caplan, 2005)). The similarities between these adult stem cells extend to the biochemical level. The immunophenotype of human ASCs and MSCs includes multiple surface proteins involved in hematopoietic cell adhesion, including CD9, CD29, and CD44 (Gronthos et al., 2001; Mitchell et al., 2006; Pittenger et al., 1999). Additional parallels may exist with respect to cytokine production, since bone marrow stromal cells or MSCs are known to express pro-inflammatory cytokines including IL-6, as well as M-CSF and related hematopoietic colony stimulating factors (CSFs) (Gimble et al., 1989, 1991; Majumdar et al., 1998, 2000). Cytokine expression levels in MSCs can be induced by exogenous factors, such as lipopolysaccharide (endotoxin) (Gimble et al., 1989). In light of the growing appreciation of adipose tissue as an endocrine organ, we set out to characterize the cytokine profile of primary cultures of undifferentiated and adipocyte differentiated human ASCs.

Materials and Methods

Isolation and culture of human adipose-derived stem cells

All protocols were reviewed and approved by the Western Institutional Review Board (Olympia, WA) or the Pennington Biomedical Research Center Institutional Research Boards (IRB) prior to the study. Liposuction aspirates from subcutaneous adipose tissue sites (abdomen, flank, thighs) were obtained from female subjects undergoing elective plastic surgical procedures. Tissues were washed three–four times with phosphate buffered saline and suspended in an equal volume of PBS supplemented with 1% bovine serum albumin and 0.1% collagenase type I (Worthington Biochemical Corporation, Lakewood, NJ) prewarmed to 37°C. The tissue was placed in a shaking water bath at 37°C with continuous agitation for 60 min and centrifuged for 5 min at 300–500 × g at room temperature (Aust et al., 2004; Dubois et al., 2005; Halvorsen et al., 2001; Sen et al., 2001). The supernatant, containing mature adipocytes, was aspirated. The pellet was identified as the stromal vascular fraction (SVF). The SVF were suspended and plated immediately in T225 flasks in Stromal Medium (DMEM/F 12 Ham's, 10% fetal bovine serum (Hyclone, Logan, UT), 100 U penicillin/100 μg streptomycin/0.25 μg Fungizone) at a density of 0.156 ml of tissue digest/cm^2 of surface area for expansion and culture. This initial passage of the primary cell culture was referred to as "Passage 0" (P0) for the adherent adipose-derived stem cell (ASC) population. Following the first 48 h of incubation at 37°C at 5% CO_2, the cultures were washed with PBS and maintained in stromal media until they achieved 75–90% confluence (approximately 6 days in culture). The cells were passaged by trypsin (0.05%) digestion and plated at a density of 5,000 cells/cm^2 ("Passage 1"). Cell viability and numbers at the time of passage were determined by trypan blue exclusion and hemacytometer cell counts. Cells were passaged at a density of 75–90% after approximately 6 days in culture and maintained in culture though Passage 2. Our published studies have demonstrated that the ASCs at P2 show an immunophenotype of >90% positivity for CD29, CD44, CD73, and CD90 and <5% positivity for CD45 and HLA-DR (McIntosh et al., 2006; Mitchell et al., 2006).

Adipogenic differentiation of human adipose-derived stem cells

Undifferentiated ASC (Passage 2) were seeded onto 12 well plates at a density of 5000 cells/cm^2. Cultures were maintained in stromal media until 75–90% confluency was obtained. The ASC were induced to undergo adipogenesis by replacing the stromal media with adipocyte induction medium composed of DMEM/F-12 with 3% FBS, 33 μM biotin, 17 μM pantothenate, 1 μM bovine insulin, 1 μM dexamethasone, 0.5 mM isobutylmethylxanthine (IBMX), 5 μM rosiglitazone, and 100 U penicillin/100 μg streptomycin/0.25 μg Fungizone. After 3 days, media was changed to adipocyte maintenance medium that was identical to induction media except for the deletion of both IBMX and rosiglitazone. Cells were maintained in culture for up to 9 days, with 90% of the maintenance media replaced every 3 days. Adipocyte differentiation was determined based on the morphological expression of lipid vacuoles.

Endotoxin (lipopolysaccharide) exposure

Undifferentiated ASCs were trypsin digested and seeded onto six well plates at a density of 3×10^4 cells /cm^2 in Stromal Medium, and fed with fresh medium after 24 h. Beginning on the fourth day in culture, the wells containing the ASCs were treated for varying lengths of time with endotoxin induction medium, consisting of Dulbecco's modified Eagles medium (High Glucose), 2% fetal calf serum, 100 U/ml penicillin, 100 μg/ml streptomycin, and 100 ng/ml lipopolysaccharide (*E. Coli* 0111:B4). On the fifth day, the individual wells were harvested simultaneously after 0, 1, 2, 4, 8, or 24 h of endotoxin exposure.

Cytokine and ascorbate exposure

Undifferentiated or adipocyte differentiated ASCs were induced with human epidermal growth factor (EGF) or human basic fibroblast growth factor (bFGF) at concentrations of 0, 0.1, 1, or 10 ng/ml in the absence or presence of ascorbic acid 2-phosphate (1 mM) for periods of 72 h. The conditioned medium was collected and frozen at −20°C for ELISA assay of hepatocyte growth factor (HGF) expression. Representative wells were harvested by trypsin digestion and cell numbers determined by trypan blue exclusion and hematocytometer counts. The remaining wells were harvested for total RNA using TriReagent according to the manufacturer's instructions (Molecular Research Center).

Enzyme linked immunosorbent assays

The ELISA for HGF was performed using a commercially available kit from R&D Systems (Minneapolis, MN) according to the manufacturer's instructions. All other cytokine assays were performed at the University of Maryland Cytokine Core Laboratory by two antibody ELISAs using biotin–streptavidin–peroxidase detection. Initial assays were performed using undiluted conditioned medium. For samples that exceeded the linear range of the assay, further assays were performed using serial five-fold dilutions.

RNA isolation and polymerase chain reaction

Total RNA was purified from tissues using TriReagent (Molecular Research Center) according to the manufacturer's specifications. Approximately 2 μg of total RNA was reverse transcribed using Moloney Murine Leukemia Virus Reverse Transcriptase (MMLV-RT; Promega), with Oligo dT at 42°C for 1 h in a 20μl reaction, or using the GeneAmp RNA PCR Kit (Perkin Elmer, Branchburg, NJ). Primers for genes of interest (listed in Table 1) were identified using Primer Express software (Applied Biosystems) or based on previously published sequence reports in the literature. Semiquantitative polymerase chain reactions were performed for 25 cycles (IL-6, IL-8, LIF, M-CSF, Actin) and 30 cycles (GM-CSF, G-CSF, Flt3-Ligand), respectively. Quantitative RT-PCR was performed on diluted cDNA samples with SYBR® Green PCR Master Mix (Applied Biosystems) using the 7900 Real Time PCR system (Applied Biosystems) under universal cycling conditions (95°C for 10 min; 40 cycles of 95°C for 15 s; then 60°C for 1 min). All results were normalized relative to a *Cyclophilin B* expression control. The identity of the PCR products was confirmed by automated sequencing, performed at the Genomic Core Facilities of the Oklahoma Medical Research Foundation (Oklahoma City, OK) and the Pennington Biomedical Research Center (Baton Rouge, LA).

TABLE 1. ASC donor demographics: age, gender, BMI

Donor	Gender	Age (Year)	Body Mass Index
L100799H1	F	37	34.64
L010400	F	39	25.88
L020800	F	55	23.56
L022900d	F	43	35.41
L031000A	F	23	22.55
L032400	F	41	20.29
L032600	F	23	24.72
L051900	F	35	23.87
L070700B	F	32	23.34
L080100	F	37	21.77
L082500A	F	27	21.36
L060113C	F	42	26.98
L060113W	F	44	21.32
L060117	F	24	22.88
L060302	F	47	30.07
L060412B	F	34	24.84
L060503	F	34	25.77
L060504	F	63	27.15
Mean ± S.D.		37.8 ± 10.6	25.4 ± 4.8

Hematopoietic Co-cultures

Hematopoietic co-cultures were established as a functional read-out for ASC, which were isolated and cultured as described above. The ASC were compared with bone marrow-derived stroma (MdS) that had been established from total mononuclear cells from human bone marrow. MdS were cultured in a standard Dexter's-style medium comprised of MEMα medium (Invitrogen/Gibco, Carlsbad, CA) supplemented with 12.5% fetal calf serum, 12.5% horse serum (Hyclone, Logan, UT), 55 μM 2-mercaptoethanol and 100 nM hydrocortisone (hemisuccinate salt; Sigma–Aldrich, St. Louis, MO). MdS were carried at least four passages before being used in culture assays. The developmental potentials for MdS cells were not defined; however, cells prepared in similar fashion have previously been demonstrated to support hematopoiesis (Storms et al., 2000). For all co-culture studies, monolayers of ASC or MdS were established in 24-well dishes ($4–6 \times 10^4$ cells per well) 48–72 h in advance. At confluence the monolayers were irradiated (10 Gy). The stroma feeder layers were derived from at least four independent donors.

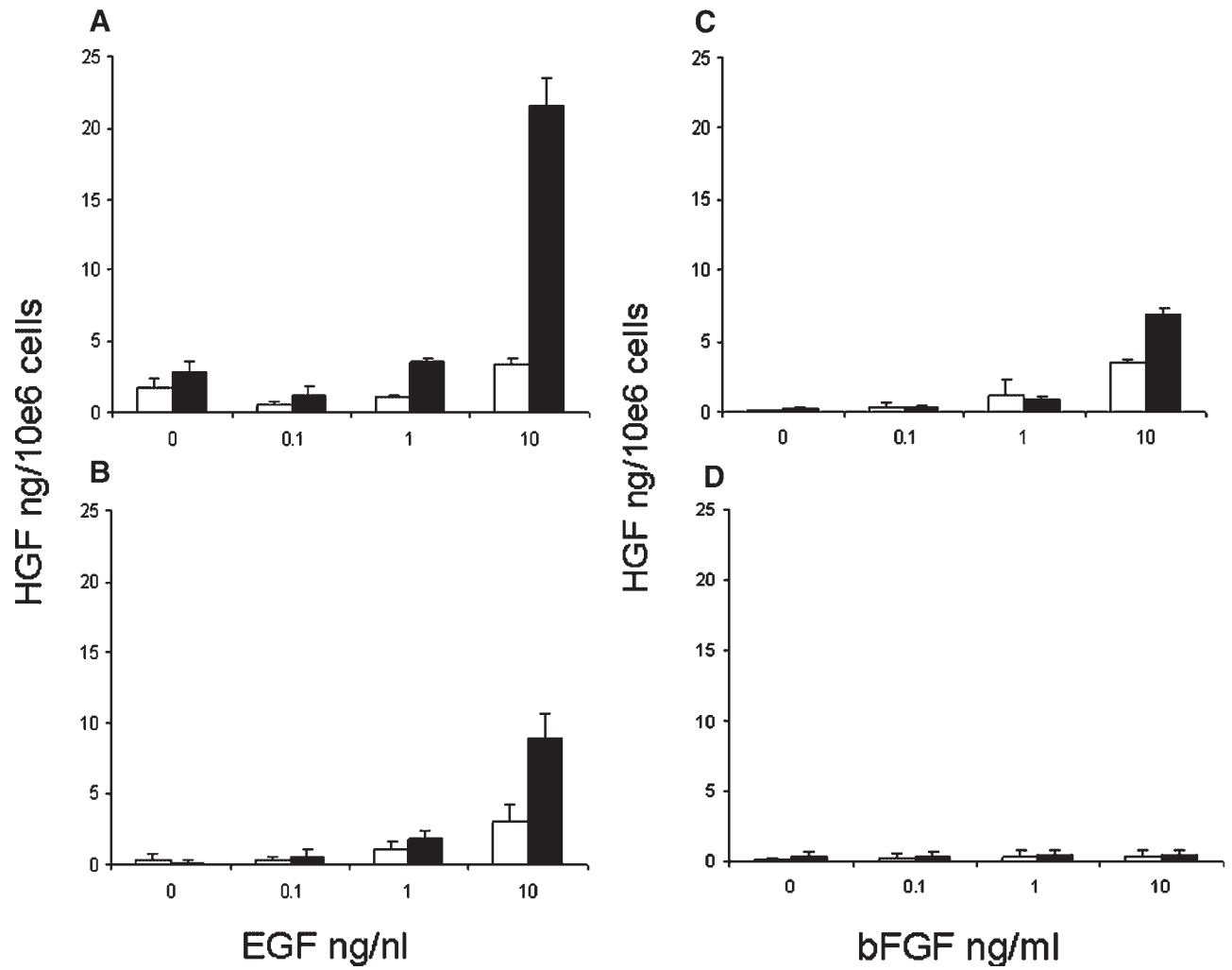

Fig. 1. Hepatocyte growth factor (HGF) Secretion. The secretion of HGF was determined by ELISA on conditioned medium from undifferentiated (A and C) and adipocyte-differentiated (B and D) ASCs following exposure to epidermal growth factor (EGF) (A and B) or basic fibroblast growth factor (bFGF) (C and D) in the absence (white bars) or presence (solid bars) of varying concentrations of 2-sodium ascorbic acid. The values represent the mean (ng/10^6 cells) ± S.D. of $n = 3$ ASC donors.

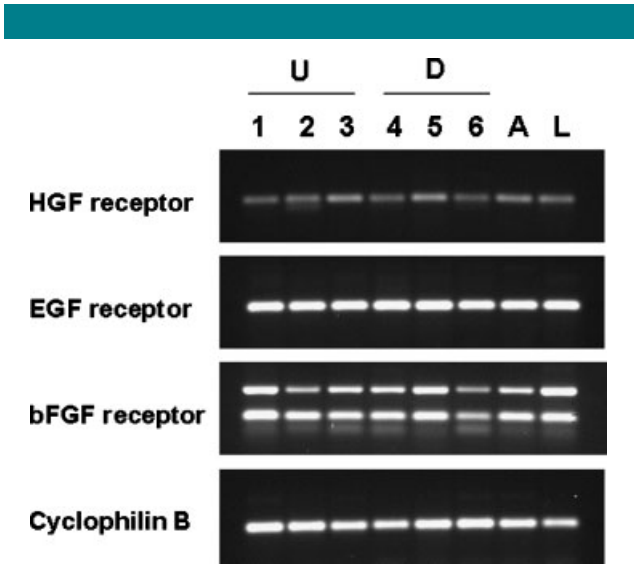

Fig. 2. Detection of mRNA encoding growth factor receptors. The total RNA isolated from the undifferentiated (U) and adipocyte differentiated (D) ASCs of three donors were used in semi-quantitative RT-PCR reactions using primers for the HGF receptor (c-met), the basic FGF receptor, and the EGF receptor. The level of cyclophilin B served as a control for equal loading across the lanes. Total RNA isolated from human adipose tissue (A) and human liver (L) served as positive controls.

All co-cultures were established using hematopoietic progenitor cells isolated from human umbilical cord blood (UCB) that were provided by the Carolina Cord Blood Bank at Duke University, per protocols reviewed and approved by the Duke University Institutional Review Board. For each study, "n" refers to the number of individual UCB that were prepared and assayed in co-culture. Initially, the lineage-committed UCB cells were depleted using a commercially available kit for immunomagnetic enrichment (StemSep enrichments using the progenitor cocktail; StemCell Technologies, Vancouver, BC). Subsequently, $CD34^+$ or $CD34^+$ $CD38^{neg}$ hematopoietic progenitor cells were purified by fluorescence-activated cell sorting on a FACStar Plus cell sorter (BD Biosciences, San Jose, CA). All fluorescent antibodies were purchased from either BD Biosciences or BD Pharmingen.

All long-term cultures (LTC) were established and maintained on cell monolayers in Dexter's medium in 24-well dishes. Initially, 500 or 2000 $CD34^+$ Lin^{neg} cells were cultured on ASC for either 3 or 5 weeks, respectively. Subsequent studies compared ASC and MdS for their capacity to support $CD34^+$ $CD38^{neg}$ Lin^{neg} cells in 5-week long-term cultures. At the end of each long-term culture, the cells were harvested using Trypsin-EDTA (Invitrogen/Gibco) and their content of clonogenic myeloid progenitors were monitored in MethoCult H4434 (StemCell Technologies).

Short-term hematopoietic co-cultures were established on ASC or MdS in DMEM/F12 medium (Invitrogen/Gibco) supplemented with 10% FCS, 55 μM 2-mercaptoethanol, 100 nM $ZnSO_4$, 100 nM $CuSO_4$ and 100 nM SeO_4. For these assays, 10,000 $CD34^+$ Lin^{neg} UCB were cultured on irradiated stroma (10 Gy) for 12 days. To monitor the influences of the stroma themselves, these cultures were not supplemented with exogenous cytokines or hydrocortisone. At 3-day intervals, the medium in these cultures was replenished by replacing half the culture volume. After 12 days, the cultures were harvested using Trypsin-EDTA and their total content of nucleated hematopoeitic ($CD45^+$) cells were enumerated by flow cytometry. At that time, the $CD45^+$ cells were also characterized for their relative percentages of $CD34^+$ and $CD7^+$ cells. Fluorescent 6-μm beads (Polysciences, Inc., Warrington, PA) were used to establish the absolute percentage of the culture that was acquired in each data file. All fluorescent antibodies were purchased from either BD Biosciences or BD Pharmingen, except antibodies directed against CD7 (Coulter-Immunotech, Miami, FL). At harvest, a small percentage of the culture was held in reserve to monitor clonogenic myeloid progenitors (CFC) using a commercially available medium as described above (MethoCult H4434, StemCell Technologies).

Results

Hepatocyte growth factor

A variety of agents and conditions have been reported to regulate the expression of angiogenic factors in fibroblasts and ASCs, including exposure to growth factors and ascorbate (Rehman et al., 2004; Wu et al., 1998). Treatment of undifferentiated ASCs with either bFGF or EGF led to increased levels of HGF release (Fig. 1A and B). At 10 ng/ml of either growth factor, HGF levels exceeded 3000 ng/10^6 cells, reflecting a 2–25-fold increase relative to baseline levels. The presence of ascorbic acid 2-phosphate further increased the inductive effects of bFGF and EGF on undifferentiated ASC release of HGF. At 10 ng/ml of either bFGF or EGF, HGF levels were increased an additional 2.0- and 6.3-fold, respectively. The baseline expression of HGF by the adipocyte-differentiated ASCs in the presence of EGF was relatively unchanged in comparison to the undifferentiated state in the absence of ascorbic acid (Fig. 1C). However, the overall induction by ascorbic acid was reduced by nearly 60% at 10 ng/ml of EGF. In contrast, bFGF failed to induce HGF expression in the adipocyte-differentiated ASCs (Fig. 1D). Subsequent analysis of total RNA by semi-quantitative RT-PCR demonstrated that both undifferentiated and adipoctye differentiated ASCs expressed the bFGF and EGF receptors, as well as that of HGF (c-met) (Fig. 2).

Proinflammatory and hematopoietic cytokines

Published studies have demonstrated that lipopolysaccharide (LPS) is a potent agonist for bone marrow stromal cell/MSC cytokine induction (Gimble et al., 1989). Since recent studies have documented that human ASCs express the multiple isoforms of the toll-like receptors responsible for LPS signal transduction (Cho et al., 2006), we exposed human ASCs to LPS and monitored the temporal cytokine expression profile (Fig. 3). The secreted levels of both IL-6 and IL-8 increased in a temporal-dependent manner following exposure to LPS, reaching maximal mean levels of 7845 and 6506 pg/ml conditioned medium, respectively, after 24 h of LPS exposure (Fig. 3A). The hematopoietic cytokines, M-CSF and GM-CSF, displayed inductive similar patterns, reaching maximal mean levels of 976 and 52 pg/ml, respectively, at 24 h (Fig. 3B and C). In contrast, TNFα reached its peak mean level of 112 pg/ml following 8 h of LPS exposure before declining at 24 h (Fig. 3C). While the mean levels of the B-cell inductive factor IL-7 and the pro-inflammatory cytokine IL-11 were low, they displayed a significant induction by ELISA, reaching maximal mean levels 24 h after LSP exposure of 3.4 and 12.7 pg/ml, respectively (Fig. 3D). Consistent with the ELISAs, the steady state levels of mRNAs for representative cytokines were elevated within 4 h following LPS exposure based on RT-PCR (Fig. 3E). Neither IL-1α, IL1-β, nor IL-12 protein was detected in the conditioned medium from undifferentiated ASCs ($n = 6$–8 subjects) by ELISA at any time following LPS exposure (data not shown).

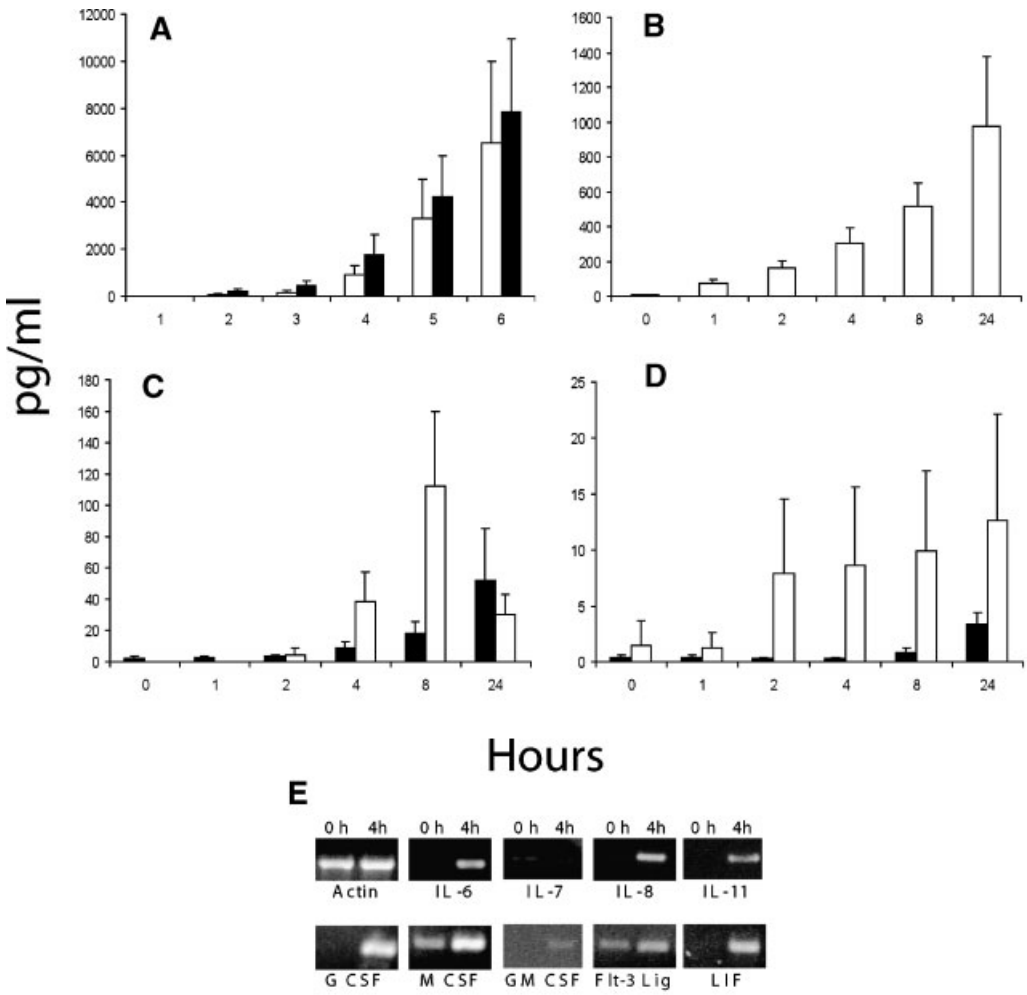

Fig. 3. Proinflammatory and hematopoietic cytokine Secretion. The conditioned medium from undifferentiated ASCs was assayed for secretion of selected cytokines at varying times following exposure to lipopolysaccharide (100 ng/ml) for periods of 0–24 h; (A) IL-6 (solid bar) and IL-8 (clear bar); (B) M-CSF; (C) GM-CSF (clear bar) and TNF (solid bar); (D) IL-7 (clear bar) and IL-11 (solid bar). The values represent the mean (pg/ml) ± S.E.M. of $n = 6$–8 ASC donors. (E) The mRNA levels of selected cytokines in ASCs from a representative donor were assayed by PCR analysis following exposure to lipopolysaccharide (100 ng/ml) for 0 or 4 h.

Functional analysis of hematopoietic support

One of the most fundamental properties for bone marrow-derived mesenchyme is a capacity to provide long-term hematopoietic support. The data regarding cytokine expression by ASC suggested that these cells might provide a similar function. To assay this potential, purified $CD34^+$ Lin^{neg} cells were used to initiate long-term culture assays on ASC. After either 3 or 5 weeks, the cultures were harvested to assay whether clonogenic myeloid cells (CFC) had been maintained (Fig. 4, part A). Robust hematopoiesis was maintained in 3-week cultures on ASC; however, by 5 weeks many fewer clonogenic progenitors had been maintained.

The preliminary LTC assays suggested that ASC were capable of supporting hematopoiesis in vitro. Therefore, LTC assays were subsequently established to directly compare ASC with marrow-derived stroma (MdS). These cultures were established with between 25 and 200 purified $CD34^+$ $CD38^{neg}$ Lin^{neg} cells, a cell population that is highly enriched with early hematopoietic progenitors. After 5 weeks, the cultures were harvested to monitor their content of myeloid CFC (Fig. 4, panel B). All of the cultures established on MdS successfully supported CFC. From every 100 $CD34^+$ $CD38^{neg}$ Lin^{neg} cells used to initiate the cultures, the MdS gave rise to 64.1 CFC (±11, SEM). In parallel cultures, ASC supported 24.7 CFC (±9, S.E.M.; $P = 0.009$). We also noted that 50% of the cultures established on ASC failed to support hematopoiesis when 100 $CD34^+$ $CD38^{neg}$ Lin^{neg} cells or fewer were used to initiate the cultures. However, nearly all ASC cultures established with 200 $CD34^+$ $CD38^{neg}$ Lin^{neg} cells were successful. In total, these data suggest that the marrow-derived stroma provided more efficient long-term support for primitive progenitors. However, while ASC were less efficient then MdS, these data did suggest that ASC exhibit some true capacity to fulfill this function.

To monitor the influences of the stroma themselves, short-term co-cultures were established on both the ASC and MdS feeder layers. Parallel cultures were maintained 12 days without exogenous cytokine support. Both ASC and MdS supported comparable levels of total hematopoietic cell expansion, 3.8 ± 1.1-fold and 3.4 ± 1.3-fold, respectively (Fig. 5, part E) Similarly, after 12 days the total expansion of clonogenic

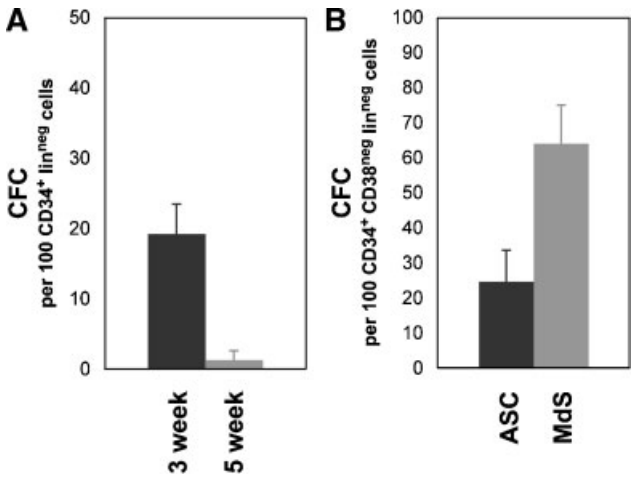

Fig. 4. Long-term hematopoietic co-cultures. A: Five hundred or 2000 $CD34^+$ Lin^{neg} hematopoietic progenitors cells were cultured on irradiated ASC feeder layers for either 3 or 5 weeks, respectively. When harvested, the cultures were monitored for clonogenic myeloid progenitors (CFC) in colony forming assays performed in methylcellulose. Bar graphs represent the mean ± S.E.M. ($n = 8$). B: Twenty-five to 200 $CD34^+$ $CD38^{neg}$ Lin^{neg} cells were cultured on irradiated ASC or MdS feeder layers for 5 weeks. When harvested, these cultures were compared for their total content of clonogenic myeloid progenitors (CFC) in standard colony forming assays. Bar graphs represent the mean ± S.E.M. ($n = 8$).

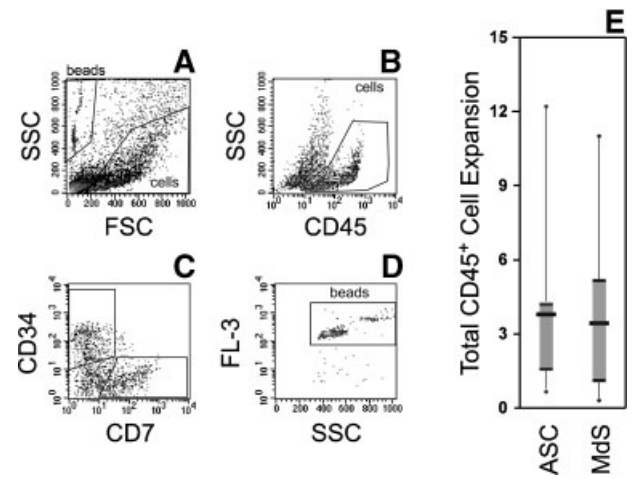

Fig. 5. Short-term hematopoietic co-cultures. Ten thousand $CD34^+$ cells were cultured on ASC or MdS in the absence of exogenous cytokines ($n = 8$). After 12 days, the cultures were harvested and fluorescent beads were added to each sample. A: Using flow cytometry, the hematopoietic cells and fluorescent beads were discriminated based on their size (as labeled). B: Nucleated hematopoietic cells were accurately identified based on their expression of CD45. C: A representative flow dot plot documents typical $CD34^+$ or $CD7^+$ hematopoietic cell phenotypes derived from these cultures. D: Beads initially identified based on their size (see part A) were enumerated based on their fluorescence. The beads were used to calculate the absolute percentage of the culture represented in each data file. E: The total $CD45^+$ hematopoietic cell expansion was determined for ASC and MdS feeder layers. For each stroma, these data are represented to show the mean (heavy bar), the range from the 25th to 75th quartile (shaded box) and the farthest outlying data points.

myeloid progenitors was not significantly different between cultures established on ASC or MdS ($P = 0.366$) (Table 3). Short-term cultures established on MdS did contain significantly higher percentages of $CD34^+$ cells. In contrast, the percentage of $CD7^+$ progeny was significantly greater on ASC. However, the significance of both of these observations was diminished when we examined the total output of $CD34^+$ or $CD7^+$ cells on either stroma background. (Table 3).

Discussion

Adipose tissue is an abundant and accessible source of adult/somatic stem cells for tissue engineering and regenerative medicine (Gimble and Guilak, 2003). The ASCs are multipotent and differentiate along selected lineage pathways in response to specific growth factors and environmental cues (Gimble and Guilak, 2003). While ASCs have potential utility solely as a function of their differentiation capacity, it has been postulated that they can also promote tissue recovery through the delivery and localized secretion of cytokines. Recent in vivo studies in rodent models support this latter hypothesis. Intravenous infusion of ASCs improved recovery of hind limb function in mice and rats following an ischemic vascular injury (Miranville et al., 2004; Planat-Benard et al., 2004; Rehman et al., 2004). The therapeutic effect of ASCs in ischemic models has been ascribed to their secretion of angiogenic cytokines, such as HGF and VEGF (Miranville et al., 2004; Planat-Benard et al., 2004; Rehman et al., 2004). Human ASCs have been found to secrete both factors constitutively (Rehman et al., 2004). *In vitro* studies have implicated adipocyte-derived HGF in both physiological and pathological processes. Secretion of HGF by murine 3T3-L1 adipocytes has been reported to promote tubule formation by vascular endothelial cells and this action was found to be

TABLE 2. Human gene PCR primer sets

Gene	Forward	Reverse	Size
B-Actin	AGCCATGTACGTTGCTA	AGTCCGCCTAGAAGCA	800 bp
EGF-R	GCCTTGACTGAGGACAGCATAGA	GCCAGCGGGCCTTTTG	83 bp
bFGF-R	CAACGTTCAAGCAGTTGGTAGAA	TGGCTGAGGTCCAAGTATTCCT	78 bp
Cyclophilin B	GGAGATGGCACAGGAGGAAA	CGTAGTGCTTCAGTTTGAAGTTCTCA	72 bp
Flt-3 Ligand	TGGAGCCCAACAACCTATCTC	GGGCTGAAAGGCACATTTGGT	333 bp
G-CSF	AGCTTCCTGCTCAAGTGC	TTCTTCCATCTGCTGCCAGATGGT	346 bp
GM-CSF	GTCTCCTGAACCTGAGTAGAGACA	AAGGGGATGACAAGCAGAAAGTCC	286 bp
HGF-R	TTATCCTGACGTAAACACCTTTGATATAAC	CTGGGCAGTATTCGGGTTTGA	79 bp
IL-6	GTAGCCGCCCCACACAGACAGCC	GCCATCTTTGGAAGGTTC	174 bp
IL-7	ATGTTCCATGTTTCTTTTAGGTATAC	TGCATTTCTCAAATGCCCTAATCCG	681 bp
IL-8	TCTGCAGCTCTGTGTGAAGGT	TGAATTCTCAGCCCTCTTCAA	252 bp
LIF	AACAACCTCATGAACCAGATCAGGAGC	ATCCTTACCCGAGGTGTCAGGGCCGTAGG	405 bp
M-CSF	TTGGGAGTGGACACCTGCAGTCT	CCTTGGTGAAGCAGCTCTTCAGCC	248 bp

TABLE 3. Hematopoietic expansion on human ASC and MdS feeder layers

	CFU expansion	Percent, $CD34^+$	Output, $CD34^+$	Percent, $CD7^+$	Output, $CD7^+$
ASC	0.9 ± 0.3-fold	17.4 ± 5.1%	4422 ± 816	28.6 ± 5.6%	5322 ± 1323
MdS	1.07 ± 0.3-fold	28.7 ± 6.5%	8730 ± 3195	19.5 ± 4.2%	4161 ± 1763
n, UCB	8	8	8	6	6
P value	0.366	0.002	0.042	0.003	0.107

$CD34^+$ Lin^{neg} cells were cultured on ASC or marrow-derived stroma (MdS) without exogenous cytokine support. After 12 days we compared the relative yield of clonogenic myeloid progenitors (CFU expansion) as well as the relative percentages of $CD34^+$ or $CD7^+$ cells. In addition, the cultures were compared for their relative output of $CD34^+$ or $CD7^+$ cells per 10,000 cells that were used to initiate the culture. All values given are the average ± SEM.

TABLE 4. Reported Cytokine Expression Profile of Human ASCs and MSCs

Cytokine	ASC	MSC
Angiogenic		
HGF	+ (E) Current, (Rehman et al., 2004)	+ (R) (Chen et al., 2002; Matsuda-Hashii et al., 2004; Takai et al., 1997; Weimar et al., 1998)
VEGF	+ (E) (Rehman et al., 2004)	+ (E) (Chen et al., 2002; Kinnaird et al., 2004)
Hematopoietic		
Flt-3 Ligand	+ (R) Current	+ (R) (Majumdar et al., 1998; Majumdar et al., 2000)
G-CSF	+ (E,R) Current	+ (R) (Majumdar et al., 1998; Majumdar et al., 2000)
GM-CSF	+ (E,R) Current	+ (R) (Majumdar et al., 1998; Majumdar et al., 2000)
IL-7	+ (E) Current	
IL-12	− (E) Current	+ (R) (Majumdar et al., 1998; Majumdar et al., 2000)
M-CSF	+ (E,R) Current	+ (R) (Majumdar et al., 1998; Majumdar et al., 2000)
SCF	Not Done	+ (R) (Majumdar et al., 1998; Majumdar et al., 2000)
Proinflammatory		
IL-1α	− (E) Current	+ (R) (Majumdar et al., 1998; Majumdar et al., 2000)
IL-6	+ (E,R) Current	+ (R) (Majumdar et al., 1998; Majumdar et al., 2000; Rougier et al., 1998)
IL-8	+ (E,R) Current	+ (R) (Majumdar et al., 1998; Majumdar et al., 2000; Rougier et al., 1998)
IL-11	+ (E) Current	+ (R) (Majumdar et al., 1998; Majumdar et al., 2000)
LIF	+ (R) Current	+ (R) (Majumdar et al., 1998; Majumdar et al., 2000)
TNFα	+ (E) Current	

+, Present; −, Absent; E, ELISA assay; R, RNA assay.

independent of the presence of VEGF (Saiki et al., 2006). Likewise, 3T3-L1 cell secretion of HGF enhanced the proliferation of mammary tumor epithelial cells (Rahimi et al., 1994). The current study has documented the constitutive and inducible secretion of HGF by ASCs *in vitro*. These properties are dependent on the differentiation status of the ASCs. Following adipocyte differentiation, the ASCs lost their responsiveness to bFGF, despite the continued expression of bFGF receptor mRNA levels. While the mechanism accounting for this change remains to be determined, adipogenesis may modulate the bFGF signal transduction pathway. The undifferentiated ASC secretion of HGF paralleled that reported for human foreskin fibroblasts (Wu et al., 1998). In both cell populations, bFGF and EGF induced HGF secretion; the addition of ascorbic acid increased this level by a factor of 2-fold or greater (Wu et al., 1998). Thus, it is possible to modulate the ASC production of HGF by an order of magnitude or more by supplementing the culture medium with growth factors and ascorbic acid. This may have benefits as a pre-conditioning regimen prior to the infusion of ASCs for the treatment of ischemic injuries.

Our co-culture experiments document the functionality of the ASC-derived cytokines and confirm previous reports of the ability of ASC to provide hematopoietic support (Corre et al., 2006). In the present study, the human ASC and MdS feeder layers supported a similar overall level of hematopoietic cell expansion. However, we observed that in the absence of exogenous cytokines ASCs supported CFUs and produced CD34 cells at levels ~2-fold less than that observed with marrow derived stroma (MdS). Nevertheless, we can conclude that hematopoietic progenitor cells in the presence of ASCs are maintained for periods of 12 days to 5 weeks and retain the ability to differentiate along the lymphoid and myeloid lineage pathways. These hematopoietic events depend on the coordinated action of M-CSF, G-CSF, GM-CSF, and IL-7, in addition to additional cytokines and cell surface molecules (Kincade et al., 1989). The cytokine profile of ASCs is similar to that reported for human bone marrow-derived MSCs as summarized in Table 4. Both cell types secrete angiogenic, pro-inflammatory, and hematopoietic-supportive cytokines following exposure to common inductive factors including LPS. Some discrepancies are noted; for example, while MSCs secreted IL-12, ASCs did not. Differences in culture conditions could account for such variation in expression profiles. Nevertheless, the study provides additional documentation of the shared characteristics of ASCs and human bone marrow-derived MSCs, consistent with previous analyses of their comparable surface immunophenotypes and differentiation potential (Gronthos et al., 2001; Mitchell et al., 2006; Pittenger et al., 1999). This suggests that ASCs, like MSCs (Koc et al., 1998; Koc and Lazarus, 2001), may have clinical utility for patients undergoing hematopoietic stem cell (HSC) transplantation following high dose chemotherapy. It may be possible to co-infuse ASCs with HSC to enhance and accelerate the recovery of normal blood cell production and restoration of immune competence.

The cytokine expression profile of ASCs has direct relevance to adipose tissue function and human disease. The angiogenic factors released by ASCs may stimulate the vasculogenesis and blood supply required for the expansion of adipose tissue depots during the development of obesity (Hausman and Richardson, 2004). Furthermore, obesity is characterized by elevated serum levels of pro-inflammatory and chemotactic cytokines (Bruun et al., 2001; Di Gregorio et al., 2005; Hotamisligil et al., 1995; Kern et al., 1995; Rehman et al., 2003). These same cytokines have been implicated as causative factors for the cardiovascular disease, diabetes, and hyptertension which, together with obesity, constitute "the metabolic syndrome" (Trayhurn, 2005). Elevated serum pro-inflammatory cytokine levels have been attributed, in part, to the increased number of resident macrophages within adipose tissue of obese individuals. The current findings suggests that

undifferentiated and/or adipocyte differentiated ASCs within intact adipose tissues also may contribute to the elevated proinflammatory cytokines levels found in obese individuals.

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