Human primary fibroblasts perform similarly to MSCs in assays used to evaluate MSC safety and potency

Barbara A. Christy[1,2] *Maryanne C. Herzig,*[1] *Christopher Delavan,*[1] *Carolina Cantu,*[1] *Christi Salgado,*[1] *James A. Bynum,*[1,3,4] *and Andrew P. Cap*[1,5]

BACKGROUND: Cellular therapeutic agents may benefit trauma patients by modulating the immune response to injury, and by reducing inflammation and vascular leakage. Administration of allogeneic mesenchymal stromal cells (MSCs) shows some benefit in preclinical and clinical trials, but less testing has been performed with other cell types. Human primary fibroblasts (FBs) were compared to MSCs in assays designed to evaluate MSCs to determine if these assays actually evaluate properties unique to MSCs or whether related cell types perform similarly.

STUDY DESIGN AND METHODS: MSC-related surface marker expression, tissue factor, and human leukocyte antigen–D related were evaluated by flow cytometry, and in vitro adipogenic and osteogenic differentiation potential were determined. Procoagulant activity was determined by thromboelastography. Two potency assays correlated with immunomodulation potential were utilized: the mixed lymphocyte reaction and indoleamine 2,3-dioxygenase enzyme activity assays.

RESULTS: Human primary FBs performed similarly to MSCs in assays designed to evaluate MSC characteristics and potency. Although similar for MSC-positive cell surface marker expression, FBs did not show robust adipose differentiation and expressed some level of markers not expected on MSCs.

CONCLUSIONS: Human primary FBs are very similar to human MSCs, at least in assays currently used to evaluate MSC potency. Preclinical and clinical testing are required to determine if FBs show similar activity to MSCs in vivo. If FBs show inferior activity in vivo, development of new MSC-specific potency assays will be necessary to evaluate properties relevant to their unique clinical benefits.

Cellular therapeutics have the potential to benefit both military and civilian trauma patients. Based on their previously described immune modulatory and anti-inflammatory properties, stem or progenitor cells may be good therapeutic agents in the acute

ABBREVIATIONS: AD-MSCs = adipose-derived mesenchymal stromal cells; ATP = adenosine triphosphate; BM-MSCs = bone marrow–derived mesenchymal stromal cells; EC_{50} = half maximal effective concentration; FBs = fibroblasts; HLA-DR = human leukocyte antigen–D related; HLFs = human primary lung fibroblasts; IDO = indoleamine 2,3-dioxygenase; IFNγ = interferon-gamma; MLR = mixed lymphocyte reaction; MSCs = mesenchymal stromal cells; NHDFs = normal human dermal fibroblasts; PBMCs = peripheral blood mononuclear cells; PPP = platelet poor plasma; TEG = thromboelastography; TF = tissue factor; TNFα = tumor necrosis factor-alpha.

From the [1]Coagulation & Blood Research, US Army Institute of Surgical Research, JBSA Fort Sam Houston, Texas; [2]Department of Molecular Medicine, UT Health San Antonio, San Antonio, Texas; [3]Institute of Biomedical Studies, Baylor University, Waco, Texas; [4]Joint Interdisciplinary Biomedical Engineering Program, UT San Antonio & UT Health San Antonio, San Antonio, Texas; and the [5]Department of Surgery, UT Health San Antonio, San Antonio, Texas.

Address reprint requests to: Barbara A. Christy, PhD, Coagulation & Blood Research, US Army Institute of Surgical Research, 3650 Chambers Pass, Bldg. 3610, JBSA Fort Sam Houston, TX 78234-4504; e-mail: barbara.christy3.ctr@mail.mil.

The studies presented here were funded by the US Army Medical Research and Materiel Command Combat Casualty Care Research Program.

The opinions or assertions contained herein are the private views of the authors and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense.

Received for publication November 27, 2018; revision received January 18, 2019, and accepted January 18, 2019.

doi:10.1111/trf.15187

TRANSFUSION 2019;59;1593–1600

phase after injury.[1,2] Sequelae of the initial injury include inflammation, ischemia, and edema; modulation of the immune response to injury may mitigate further tissue damage. Because cell therapy agents also have angiogenic and wound-healing properties, benefits may extend beyond the acute injury. Mesenchymal stromal cells (MSCs) are multipotent progenitor cells with stem cell–like properties. They can be derived from many different sources, but are most commonly obtained from bone marrow and adipose or birth-associated tissues (umbilical cord, cord blood, placenta, or amnion). MSCs are primary cells that have not been genetically modified, minimizing the risk of long-term retention and tumorigenesis. Trauma patients in particular may have only a temporary need, and long-term retention may be unnecessary. MSCs are thought to be immune privileged compared with most other human cells, suggesting that they will be safer for human allogeneic administration. However, there are challenges. Because they have a finite life span, new MSC isolates must be continually derived and will require evaluation to determine safety and potency. All MSCs are not equivalent; they are sensitive to donor factors and to isolation and handling conditions.[3–8] Every individual MSC likely has unique properties affecting potency and efficacy. One challenge with bone marrow–derived MSCs is that massive expansion from a relatively small sample is necessary to obtain cells for clinical use, which may reduce effectiveness.

Although MSCs are promising cell therapy candidates, other human primary cells may also deliver similar benefits. Fibroblasts (FBs) in particular share many characteristics with MSCs and may share at least some functional capabilities. FBs are easy to expand and maintain in culture and can be obtained noninvasively in large numbers from discarded medical waste or from organ donors.[9] In this study, we analyzed several human primary FB populations to determine if they perform similarly to human MSCs in assays commonly used to gauge MSC potency.

METHODS

Cells and cell culture

WI38 human lung FBs (CCL-75, Lot 63913711, passage 18, 32 population doublings, from 3 months' gestation female fetus) and normal human primary lung fibroblasts (HLFs; PCS-201-013, Lot 63710262, passage 2, from 14-year-old female) were obtained from American Type Culture Collection. Normal human dermal fibroblasts (NHDFs; Lot 0000481450, passage 1, 42-year-old female) were obtained from Lonza, Inc. MSCs were obtained from RoosterBio, Inc. Cells were cryopreserved at an early passage number. For comparison within assays, cells from different sources were adapted for growth in a common medium (hMSC High Performance media, RoosterBio).

In vitro differentiation (adipose and bone)

Adipogenesis was induced with mesenchymal stem cell adipogenic differentiation medium 2 (PromoCell GMBH), and differentiation was assayed using an assay reagent (AdipoRed, Lonza, Inc.) to quantify fat droplets on a fluorescence plate reader (Spectramax M5, Molecular Devices; excitation 485 nM, 530 cutoff, emission 590 nM). Osteoblast differentiation was induced with osteogenesis differentiation medium (OsteoMAX-XF, EMD Millipore). Mineralized hydroxyapatite nodules were detected with an assay reagent (Osteoimage, Lonza, Inc.). Relative fluorescence was determined (excitation 485 nM, 515 cutoff, emission 538 nM). Fluorescent images were captured on Days 7 and 14.

Flow cytometry

Cell surface expression of tissue factor (TF) was determined by flow cytometry using anti-CD142 (BD Biosciences); expression of positive and negative MSC markers was determined using a flow cytometer (Human MSC Analysis Kit, BD Stemflow). Human leukocyte antigen–D related (HLA-DR) surface expression was determined separately using anti-HLA-DR (BD Biosciences). Nonviable cells were excluded using 7-aminoactinomycin D (BD Pharmingen).

Thromboelastography

Thromboelastography (TEG) was performed using a coagulation analyzer (Haemoscope TEG 5000, Haemonetics Corporation). FBs and MSCs were detached and washed three times in Dulbecco's phosphate-buffered saline before suspension in Dulbecco's phosphate-buffered saline and addition to pooled platelet-poor plasma (PPP). Total sample volumes were 340 μL; final cell concentration was 2×10^4 cells/mL. Control samples received an equivalent volume of Dulbecco's phosphate-buffered saline alone. Values obtained for R time (time to initial fibrin clot formation) were an average of duplicate reactions.

Preparation of pooled PPP

Blood was obtained by the USAISR Research Blood Bank under an approved standard operating procedure. Whole blood was collected from 10 healthy deidentified donors into 4.5-mL blood collection tubes (Vacutainer tubes, BD) with sodium citrate (10 tubes per donor) and processed to separate plasma by centrifugation at 3,000× *g* (10 minutes at 4°C). A second centrifugation at 3000× *g* removed residual platelets. PPP from all donors was pooled to minimize donor-to-donor differences and enhance reproducibility and stored frozen at –80°C.

Mixed lymphocyte reaction assay

A modified mixed lymphocyte reaction (MLR) assay was used to determine the ability of FBs to suppress mitogen-stimulated T-lymphocyte proliferation. Peripheral blood mononuclear cells (PBMCs) were separated from whole blood using a

sterile density medium (Ficoll-Paque Premium, GE Healthcare). PBMCs from 10 donors were pooled to minimize donor variation, cryopreserved, and stored in liquid nitrogen. A constant number of pooled PBMCs were incubated in the presence or absence of the mitogen phytohaemagglutinin A (5 μg/mL) with increasing numbers of FBs cultured (in triplicate) in 96-well plates to evaluate different ratios of FBs to PBMCs. Controls had PBMCs alone; FB-to-PBMC ratios ranged from 1 FB:2.5 PBMC to 1 FB:200 PBMC at plating. Because most of the PBMCs (largely T lymphocytes) are nonadherent, they can be quantified separately from the adherent MSCs. Relative numbers of nonadherent PBMCs after 72-hour coculture were estimated using a luminescent adenosine triphosphate (ATP) assay (Cell Titre Glo 2.0, Promega Corporation); in parallel, the number of adherent FBs or MSCs was estimated from each well by ATP assay. The ATP assay gives an excellent signal-to-noise ratio at 72 hours, is rapid, and allows for high throughput using relatively low cell numbers. A relative measure of immunosuppression efficiency (half maximal effective concentration [EC_{50}]) was calculated for each FB or MSC. The EC_{50} is defined as the amount of (adherent) ATP per well needed to achieve 50% suppression of the PBMC number.

Assay of indoleamine 2,3-dioxygenase enzymatic activity

Indoleamine 2,3-dioxygenase (IDO) activity was determined by colorimetric assay of the product kynurenine secreted into culture medium with or without treatment with interferon-gamma (IFNγ) and tumor necrosis factor-alpha (TNFα) for 24 hours. Relative kynurenine concentrations were determined by comparison with a standard curve generated using purified kynurenine (Millipore-Sigma).

RESULTS

Human primary FBs express stem cell surface markers and can differentiate in vitro

Three human primary FBs (WI38 human fetal lung FBs, HLFs, NHDFs) were tested using a commercial panel of flow cytometry markers designed to establish minimal MSC stem cell identity. As shown in Table 1, all three FBs (similar to MSCs) show expression of the stem cell surface markers CD90, CD73, CD105, and CD44. In the flow cytometry kit, negative markers are a mixture labeled with the same fluorophore and include CD45, CD34, CD11b, CD19, and HLA-DR; the FBs show a higher percentage of cells expressing these negative markers than expected for MSCs.

Each FB population was assessed for its ability to differentiate in vitro and undergo osteogenesis and adipogenesis, and compared to two bone marrow–derived mesenchymal stromal cells (BM-MSCs). As shown in Fig. 1A and Fig. 1C (left), HLFs and NHDFs have the capacity to undergo osteogenic differentiation, comparable to BM-MSCs at 2 weeks. As shown in Fig. 1B and Fig. 1C (right), all three FBs showed only a low level of adipocyte differentiation. However, variability among different BM-MSCs was also seen (BM-3, BM-4). One FB population (WI38) did not demonstrate either osteogenic or adipogenic differentiation, indicating a low differentiation potential relative to other FBs or MSCs tested.

Human primary FBs express cell surface tissue factor and exhibit procoagulant activity

We previously showed that human adipose-derived MSCs (AD-MSCs) express high levels of cell surface TF (CD142), and are highly procoagulant when mixed with whole blood or pooled human PPP. BM-MSCs are less procoagulant and express lower and more variable levels of TF.[5] It was subsequently shown that human MSCs derived from amniotic fluid also express high levels of TF, while umbilical cord MSCs and other cellular therapeutics express lower TF levels, similar to BM-MSCs.[6] FBs were similarly examined for TF expression and procoagulant activity. FBs were subjected to flow cytometry to determine expression of cell surface TF (CD142). As shown in Fig. 2A, the percentage of WI38 and NHDF cells expressing cell surface TF is high (>90%), comparable to the percentages seen in AD-MSCs, which range from 75% to 99% CD142+. The percentage of HLF cells expressing cell surface TF is lower (56%) but still higher than the range generally seen in BM-MSCs (2.5%–35% CD142+). To determine if the high levels of TF-positive cells in these populations predicts procoagulant activity, washed cells were mixed with pooled human PPP and clot initiation was followed by TEG. As shown in Fig. 2B, all FBs tested were procoagulant, significantly reducing R time (time to clot initiation). WI38 cells (98% CD142+) have more procoagulant activity than the other FBs tested, although the difference only reaches significance compared to HLF cells (56% CD142+).

TABLE 1. Analysis of stem cell identity markers on human fibroblast cell surface by flow cytometry

	Positive stem cell markers				
Cells	CD90 + (%)	CD73 + (%)	CD105 + (%)	CD44 + (%)	Negative markers
WI38	**97.9**	**99.6**	**98.3**	**99.5**	**10.6**
HLF	**91.9**	**100**	**99.2**	**99.9**	**19.1**
NHDF	**99.1**	**99.8**	**98.4**	**99.7**	**24.6**

HLF = human primary lung fibroblast; NHDF = normal human dermal fibroblast.

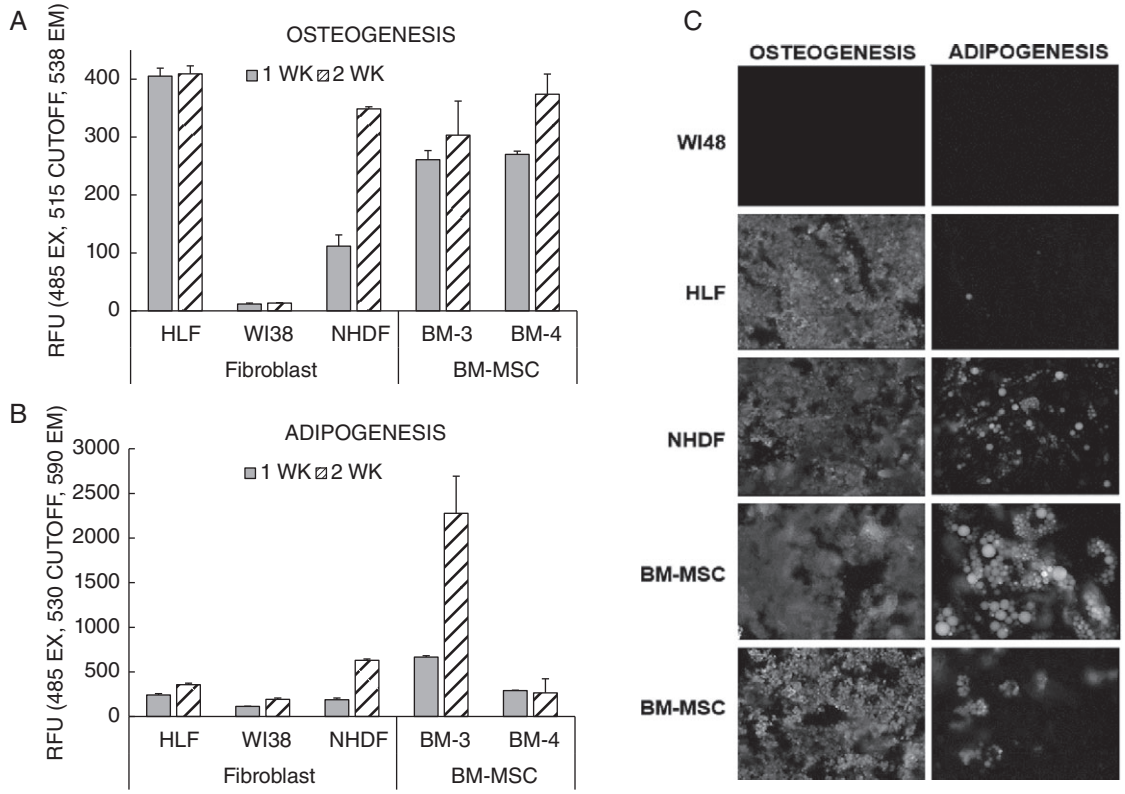

Fig. 1. Human primary FBs, like MSCs, can differentiate in vitro into bone or adipose under the appropriate conditions, although the differentiation potential is variable. Three human FB populations (HLF, WI38, and NHDF) were switched from growth medium to osteoblast or adipose differentiation media and assayed at 7 or 14 days. Two BM-MSCs were treated similarly in the same experiment. (A) Osteogenesis was measured at 1 week (solid bars) or 2 weeks (striped bars) by fluorescent detection of hydroxyapatite nodules. (B) Adipogenesis was measured at 1 week (solid bars) or 2 weeks (striped bars) using AdipoRed to detect fat droplets. (C) Fluorescent images of FB and BM-MSC differentiation at 2 weeks. RFU = relative fluorescence units.

HLA-DR expression is up regulated in both FBs and MSCs by treatment with inflammatory agents

Because HLA-DR is one of the negative markers included in a cocktail from the human MSC analysis kit labeled with the same fluorophore (Table 1), we were unable to determine whether the FBs with some negative marker expression were expressing HLA-DR or another marker. Expression of cell surface HLA-DR might suggest that the tested FBs are less immune privileged than MSCs, which are defined by their lack of HLA-DR. Therefore, HLA-DR surface expression was tested separately by flow cytometry. Some expression of HLA-DR is seen in both HLF and NHDF without treatment (Fig. 3); however, the representative BM-MSCs tested here also show a low level of HLA-DR–positive cells. Upon stimulation of the cells for 24 hours with IFNγ and TNFα, the number of HLA-DR–positive cells increases in both FBs and MSCs, with the maximal increase in the stimulated BM-MSCs.

Human primary FBs respond to inflammatory signaling by up regulating IDO enzyme activity

Assay of IDO enzyme activity is commonly used to evaluate immunomodulation potential of human MSCs.[4] It is believed that elevated IDO depletes tryptophan, raising a metabolic blockade to lymphocyte proliferation.[10] Assay of IDO activity is an indirect measure to determine the potential for human MSCs to respond to inflammatory conditions by up regulating IDO activity, mainly by increasing the amount of IDO mRNA and protein. Enzymatic activity is measured by assaying the enzyme's product kynurenine secreted into culture medium. As shown in Fig. 4, all FB populations tested respond to IFNγ and TNFα by secreting detectable amounts of kynurenine into the culture medium. In the absence of stimulation, levels of kynurenine are low. The levels of kynurenine produced by stimulated FBs is comparable to the average levels seen with AD-MSCs (n = 3) and BM-MSCs (n = 6). Similar to MSCs, IDO mRNA was also induced in WI38 and HLFs by IFNγ + TNFα treatment (not shown).

Human primary FBs inhibit lymphocyte proliferation in an MLR-type assay

The MLR is the most common functional assay used to evaluate immunomodulation potential of MSCs.[3,11] It measures the ability of MSCs to suppress proliferation and survival of lymphocytes when cocultured directly or indirectly with

A

Cells	% CD142+
WI38	98%
HLF	56%
NHDF	94%
BM-MSCs	2.5-35%
AD-MSCs	75-99%

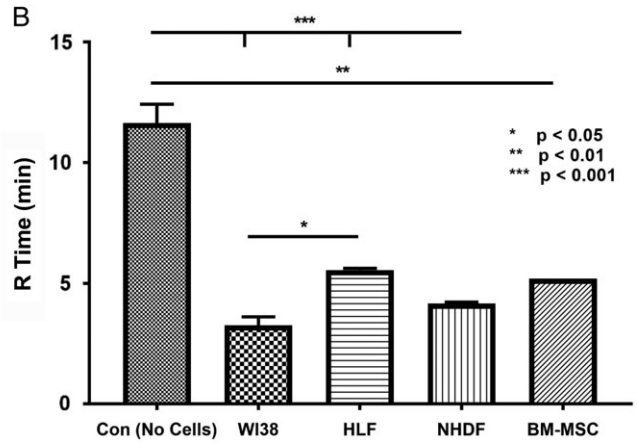

Fig. 2. Human primary FBs express cell surface TF and are procoagulant. (A) The percentage of cells expressing cell surface TF (CD142) was determined by flow cytometry. Three human FB populations were assayed. For comparison, the range of CD142+ percentages are shown for BM-MSCs (n = 6) and AD-MSCs (n = 3). (B) Procoagulant activity of the three FBs was determined by TEG when washed cells were incubated with pooled human plasma. One representative BM-MSC is shown for comparison. R time represents the time to clot initiation, and a reduced R time in the presence of added FBs or MSCs compared with the control (vehicle alone) indicates procoagulant activity. * p < 0.05; ** p < 0.01; *** p < 0.001.

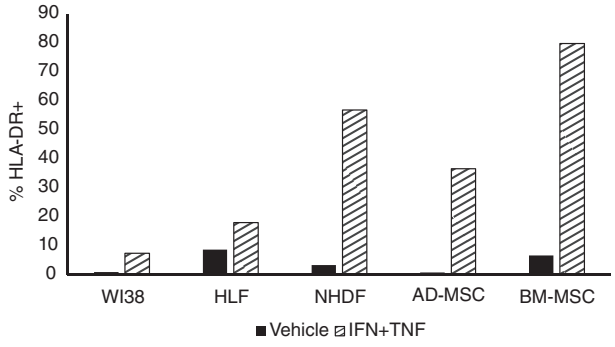

Fig. 3. HLA-DR expression is induced by inflammatory signaling in both FBs and MSCs. FBs from three different sources (WI38, HLF, and NHDF) were analyzed for HLA-DR expression by flow cytometry following 24-hour treatment with vehicle alone (solid bars) or 10 ng/mL IFNγ +10 ng/mL TNFα (striped bars). For comparison, one AD-MSC and one BM-MSC were treated and analyzed similarly.

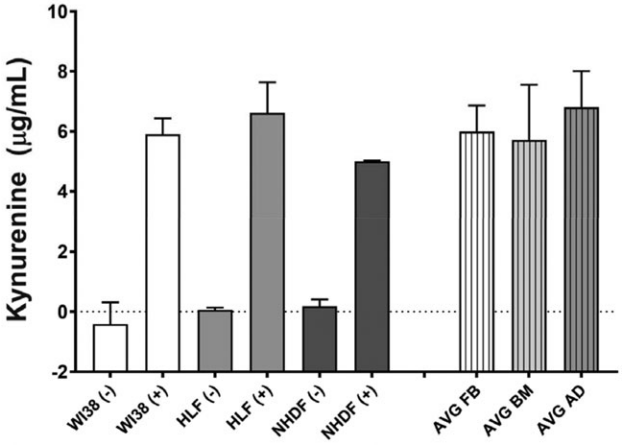

Fig. 4. IDO enzymatic activity is induced by inflammatory signaling in FBs. FBs from three different sources (WI38, HLF, and NHDF) were treated for 24 hours with (+) or without (−) 10 ng/mL IFNγ + 10 ng/mL TNFα in low-serum assay medium. Conditioned medium was harvested and the product of IDO enzyme activity (kynurenine) was assayed and quantitated relative to a standard curve generated with purified kynurenine. For comparison, the average values for FBs (n = 3), BM-MSCs (n = 6) and AD-MSCs (n = 3) are shown. Control wells were treated with vehicle alone and contained almost no detectable kynurenine.

human PBMCs or purified lymphocytes. We tested each FB population in a modified MLR assay in which increasing numbers of FB cells were cocultured directly with a constant number of phytohaemagglutinin A–stimulated PBMCs. Relative cell numbers for both the adherent FBs or MSCs and the nonadherent PBMCs were determined by cellular ATP assay. An EC_{50} (ng/well ATP at which 50% PBMC cell number suppression is achieved) was calculated for each FB and compared with EC_{50} values obtained for BM-MSCs and AD-MSCs assayed in a similar manner. As shown in Table 2, all three FB types show suppression activity, with NHDF cells being the most active (lowest EC_{50} value). For comparison, the range of values obtained in similar testing of MSCs is shown; the range is especially wide for BM-MSCs but the FB values fall within this range.

DISCUSSION

Beyond the initial tissue damage occurring directly from traumatic injury, cellular and systemic responses such as

TABLE 2. Human primary fibroblasts show immunomodulation activity in a mixed lymphocyte reaction assay

Cells	MLR EC_{50}	SEM
W138	21.68	1.59
HLF	81.44	1.19
NHDF	11.16	2.31
BM-MSCs	27.24–478.9	0–1.42
AD-MSCs	6.82–41.42	1.91–1.95

A lower EC_{50} value signifies more potent immunomodulation activity in this assay. For comparison, the range of EC_{50} values calculated for BM-MSCs (n = 6) and AD-MSCs (n = 3) is included.
AD-MSCs = adipose-derived mesenchymal stromal cells; BM-MSCs = bone marrow–derived mesenchymal stromal cells; EC_{50} = average amount of FB or MSC ATP (ng/well) sufficient to produce a 50% suppression of lymphocyte proliferation; HLF = human primary lung fibroblast; MLR = mixed lymphocyte reaction; NHDF = normal human dermal fibroblast; SEM = standard error of the mean.

inflammation, vascular leakage, and immune responses often cause secondary tissue damage. Because cell therapies have the potential to mitigate all of these processes, they might interrupt the progression of systemic alterations that leads to secondary damage and more profound consequences to the patient. Thus, cellular therapies may be of value for treating both military and civilian injuries.

Human MSCs or other progenitor cells are currently being tested in multiple clinical trials for a variety of indications.[12–14] For acute trauma in otherwise healthy individuals, the need is temporary, and persistence of administered cells may not be required. MSCs are primary cells derived from adult tissue without genetic modification, reducing the risk of mutation or tumorigenesis. Although MSCs may not be true "stem" cells, they do have stem cell–like characteristics. While MSCs are good candidates to provide clinical benefits, other human primary cells may also be of value. In this study, we tested several commercial FB cell populations using assays commonly used to define cells as MSCs and to evaluate their functional potential.

No exclusive cell surface marker exists that defines cells specifically as either MSCs or FBs, but a minimal set of characteristics to define human MSCs has been established.[15] These include the ability to adhere and grow on tissue culture plastic, a property that allows separation of MSCs from many other cell types in tissue (but is shared with FBs). Other criteria include expression of cell surface markers CD90, CD73, CD105, and CD44, although expression is not specific to MSCs. A lack of expression of several "negative" markers that are characteristic of non–stem cell lineages is also required (CD45–, CD34–, CD11b–, CD19–, HLA-DR–). Finally, MSCs must be multipotent and possess the ability to differentiate into multiple cell types under appropriate conditions in vitro. FB cells are mesenchymal derivatives and share many characteristics with MSCs; therefore, they are also expected to share some functional capabilities. Three FB populations tested all expressed MSC surface markers (CD90, CD73, CD105, and CD44), similar to a previous report.[16] Ten percent to 25% of the FBs tested here were expressing one or more "negative" markers characteristic of non–stem cell lineages (a cocktail of CD45, CD34, CD11b, CD19, and HLA-DR). This would disqualify these FB populations from being called MSCs based on the minimal criteria and could suggest heterogeneity within the populations. Although Alt et al.[16] did not find expression of negative markers, their negative marker set included only CD14, CD45, and CD31. Thus, the low-level expression of negative markers seen in our testing could be due to a subset of cells positive for CD34, CD11b, CD19, or HLA-DR. A separate study[17] showed comparable results, indicating similar expression of MSC-defining cell surface antigens between FBs and MSCs. Overall, human MSCs and FBs are similar in regards to their expression of MSC surface markers.

Another criterion used to define MSCs is the ability to differentiate into multiple cell types under appropriate conditions, including bone, adipose, and cartilage.[15] Previous reports generated conflicting results when FB cell populations were differentiated in culture.[16,17] Although some reports conclude that FBs are *not* capable of in vitro differentiation, several studies indicate that they behave similarly to MSCs when differentiated under the same conditions.[17] In the current study, we used staining methods that allowed us to quantitatively compare cell populations in addition to visual detection. FBs varied in their differentiation potential, although there were also differences between two representative BM-MSCs tested at the same time. We conclude that differentiation potential varies between both FB and MSC populations. FBs, similar to MSCs, are heterogeneous and differ depending on the particular tissue or body site from which they were derived. FB populations vary in their developmental origin even among different populations of skin FBs.[18] Although Alt et al.[16] also observed differentiation of early passage primary FB populations but not the well-established WI38 cells, they concluded that the differentiation observed could be the result of contaminating stem cells in the primary FB population. Because we observed differentiation at a similar level to BM-MSCs in some cases (Fig. 1) and both are likely to be heterogeneous populations, we believe that some ability to differentiate is retained in some FB populations. Although the inability of WI38 to differentiate could be attributed to being a more pure FB population as suggested by Alt et al.,[16] it is more likely that there was a loss of differentiation potential in cells that were derived in the early 1960s,[19,20] which we obtained at passage 18, as opposed to the much earlier passage numbers for the other FBs (passages 1–2) and the BM-MSCs (passages 4–5) tested. Additionally, WI38 cells were derived from fetal tissue, which could affect their properties but would not be expected to limit their differentiation potential. We and others previously showed that human MSCs are procoagulant when mixed with human blood or plasma,[5,6] a

property that has safety implications. In those studies, AD-MSCs and amniotic fluid-derived MSCs were highly procoagulant and expressed correspondingly high levels of cell surface TF. BM-MSCs and other cellular therapeutics were still procoagulant, but demonstrated lower TF expression and procoagulant activity, suggesting that safety could be improved by selecting MSCs with lower TF and procoagulant potential.[5,6] Although recent clinical trials have concluded that administration of MSCs is safe at the levels tested, there have been thrombotic complications described both in patients and in animal testing.[21–23] It is important to approach human administration with an abundance of caution, especially when considering increasing MSC dose to increase efficacy. The human FBs evaluated here expressed higher levels of TF than BM-MSCs. Two of the three expressed TF at a similar level to that seen in AD-MSCs, while one had a percentage of TF-positive cells intermediate between BM-MSCs and AD-MSCs (Fig. 2). When mixed with pooled human PPP and subjected to TEG analysis, FBs were indeed procoagulant (Fig. 2). The two FBs with the highest percentage of TF-positive cells (WI38 and NHDF) reduced R time the most, but a significant difference between FBs and BM-MSCs was not seen.

Because testing of stem cell–negative markers could not distinguish whether the FB cells expressed HLA-DR in a higher percentage of the population than MSCs (which could be a safety concern when injecting allogeneic MSCs into patients), we tested HLA-DR expression separately before and after treatment with IFNγ + TNFα to mimic inflammatory signaling. As shown in Fig. 3, the percentage of HLA-DR–positive cells did increase in FBs following treatment. However, AD-MSC or BM-MSC cells show similar results under these conditions. We conclude that human MSCs are not actually immune privileged compared to primary human FBs. This could have implications for patient safety and efficacy but suggests that allogeneic administration of human primary FBs could be considered for clinical use.

A critical characteristic for allogeneic cell therapy is likely to be immunomodulation potential. When several FBs were tested in two common MSC potency assays, the MLR and IDO activity assays, FBs performed similarly to MSCs. Differences in in vitro potency were observed among FBs, but they fall within the range of potencies observed with different MSCs in the same assays. For the MLR assay, the FBs tested fall within the lower range of EC_{50} values obtained for MSCs (all FBs more active than three of six BM-MSCs). FBs and MSCs similarly respond to inflammatory signaling by upregulating IDO activity, an activity thought to correlate with immunomodulation in human MSCs.[4] We conclude that activity in these commonly used potency assays is not specific to MSCs. This suggests that FBs could be good candidates for preclinical and clinical testing.

Do MSCs have properties or activities that make them ideal candidates for cell therapy agents relative to other cell types? From this study and others,[9,16,17] it appears that human primary MSCs and FBs have very similar phenotypic and functional properties. Both FBs and MSCs demonstrate differences in potency that are likely based on different sites of origin within the body, individual donor differences, or different cell isolation and handling methods. Although some FBs within tissue may be terminally differentiated, less mature FBs would be expected to expand in culture (similar to MSCs). Perhaps as suggested by Ichim et al.,[9] FBs could be an alternative to MSCs for clinical use because they could be easily harvested in large numbers from medical waste or organ donors. Starting with large numbers would obviate the need for massive expansion of cells derived from small tissue or bone marrow samples that may lead to loss or reduction of cell function. Alternatively, it may be that MSCs do have special properties that make them more potent cell therapy agents relative to primary FBs or other cell types, but the assays routinely used for MSC evaluation do not measure these special properties. In that case, it will be important to develop additional functional assays that measure clinically relevant properties. Because potency assays are performed in vitro, preclinical testing in animals will be required to determine how well the they predict in vivo efficacy. However, human primary FB cells may provide an alternative to MSC use, based on their ease of isolation in large numbers, cost effectiveness, and similar characteristics, and should be investigated further.

ACKNOWLEDGMENTS

The authors thank the USAISR Research Blood Bank and the de-identified volunteer blood donors who provided blood for preparation of plasma and PBMCs. Support was provided by the US Army Medical Research Command (MRMC).

CONFLICT OF INTEREST

The authors have disclosed no conflicts of interest.

REFERENCES

1. Pati S, Pilia M, Grimsley JM, et al. Cellular therapies in trauma and critical care medicine: forging new frontiers. Shock 2015; 44:505-23.
2. Matthay MA, Pati S, Lee J-W. Concise review: mesenchymal stem (stromal) cells: biology and preclinical evidence for therapeutic potential for organ dysfunction following trauma or sepsis. Stem Cells 2017;35:316-24.
3. Ketterl N, Brachtl G, Schuh C, et al. A robust potency assay highlights significant donor variation of human mesenchymal stem/progenitor cell immune modulatory capacity and extended radio-resistance. Stem Cell Res Ther 2015;6:236.
4. Francois M, Romieu-Mourez R, Li M, et al. Human MSC suppression correlates with cytokine induction of indoleamine

2,3-dioxygenase and bystander M2 macrophage differentiation. Mol Ther 2012;20:187-95.
5. Christy BA, Herzig MC, Montgomery RK, et al. Pro-coagulant activity of human mesenchymal stem cells. J Trauma Acute Care Surg 2017;83:S164-9.
6. George MJ, Prabhakara K, Toledano-Furman NE, Wang Y-W, Gill BS, Wade CE, Olson SD, Cox CS Jr. Clinical cellular therapeutics accelerate clot formation. Stem Cells Transl Med 2018; 7:731-9.
7. Mattar P, Bieback K. Comparing the immunomodulatory properties of bone marrow, adipose tissue, and birth-associated tissue mesenchymal stem cells. Front Immunol 2015;6:560.
8. Talwadekar MD, Kale VP, Limaye LS. Placenta-derived mesenchymal stem cells possess better immunoregulatory properties compared to their cord-derived counterparts-a paired sample study. Sci Rep 2015;5:15784.
9. Ichim TE, O'Heeron P, Kesari S. Fibroblasts as a practical alternative to mesenchymal stem cells. J Transl Med 2018;16:212.
10. Hoogduijn MJ. Indoleamine 2,3-dioxygenase does it. Transplantation 2015;99:1751-2.
11. Bloom DD, Centanni JM, Bhatia N, et al. A reproducible immunopotency assay to measure mesenchymal stromal cell-mediated T-cell suppression. Cytotherapy 2015;17:140-51.
12. Galipeau J, Sensebe L. Mesenchymal stromal cells: clinical challenges and therapeutic opportunities. Cell Stem Cell 2018; 22:824-33.
13. Cox CS Jr. Cellular therapy for traumatic neurological injury. Pediatr Res 2018;83:325-32.
14. Ward MR, Abadeh A, Connelly KA. Concise review: rational use of mesenchymal stem cells in the treatment of ischemic heart disease. Stem Cells Transl Med 2018;7:543-50.
15. Dominici M, Le Blanc K, Mueller I, et al. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy 2006;8:315-7.
16. Alt E, Yan Y, Gehmert S, et al. Fibroblasts share mesenchymal phenotypes with stem cells, but lack their differentiation and colony-formation potential. Biol Cell 2011;103:197-208.
17. Denu RA, Nemcek S, Bloom DD, et al. Fibroblasts and mesenchymal stromal/stem cells are phenotypically indistinguishable. Acta Haematol 2016;136:85-97.
18. Drikell R, Watt FM. Understanding fibroblast heterogeneity in the skin. Trends Cell Biol 2015;25:92-9.
19. Hayflick L, Moorhead PS. The serial cultivation of human diploid cell strains. Exp Cell Res 1961;25:585-621.
20. Hayflick L. The limited in vitro lifetime of human diploid cell strains. Exp Cell Res 1965;37:614-36.
21. Jung JW, Kwon M, Choi JC, et al. Familial occurrence of pulmonary embolism after intravenous, adipose tissue-derived stem cell therapy. Yonsei Med J 2013;54:1293-6.
22. Cyranoski D. Korean deaths spark inquiry. Nature 2010; 468:485.
23. Gleeson BM, Martin K, Ali MT, et al. Bone marrow-derived mesenchymal stem cells have innate procoagulant activity and cause microvascular obstruction following intracoronary delivery: amelioration by antithrombin therapy. Stem Cells 2015;33: 2726-37.