Granulocyte colony-stimulating factor mobilized CFU-F can be found in the peripheral blood but have limited expansion potential

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Online Supplementary Material

Differentiation methods

Approximately two weeks after isolation, mobilized peripheral blood CFU-F were in sufficient number to differentiate and 50,000 cells per well were placed in a 6-well plate with the following media for osteogenic differentiation: α-MEM with 10% FCS, 10 mM B-glycerolphosphate, 0.2 mM ascorbic acid, and 10 μM dexamethasone.

For adipogenic differentiation the media contained DMEM (high-glucose) with 10% FCS, 0.5 mM isobutyl-methylxanthine, 200 μM indomethacin, 1 μM dexamethasone, and 10 μg/mL insulin. Media was changed every 2-3 days and the culture period was 21 days, after which cells were washed with PBS, fixed for 10 mins. in 10% buffered formalin at room temperature and stained with Alizarin Red (for osteogenic cells) or Oil Red-O (for adipogenic cells).

Isolation of mobilized peripheral blood CFU-F

Blood from G-CSF treated healthy volunteers (5 micrograms/kg/d for three days with cell harvest on day 4) was purchased from AllCells, LLC (Emeryville, CA, USA). Blood was collected in anticoagulant solution ACD-A.

Blood was diluted in RPMI (1:1 dilution) and peripheral blood mononuclear cells (PBMC) were isolated by ficoll density gradient centrifugation. The resulting PBMCs were washed twice in cold phosphate buffered saline (PBS) and then diluted in Iscov's media with 20% fetal calf serum (FCS) (Hyclone) and plated on plastic culture flasks at a density of approximately 250×10^6 per 300 cc flask.

Cells were left undisturbed for seven days without media changes and colonies of adherent cells were then noted on the flask. Culture flasks were washed with PBS and the adherent cells passaged by traditional methods using trypsin digestion. Cell numbers and population doublings were measured throughout the culture period.

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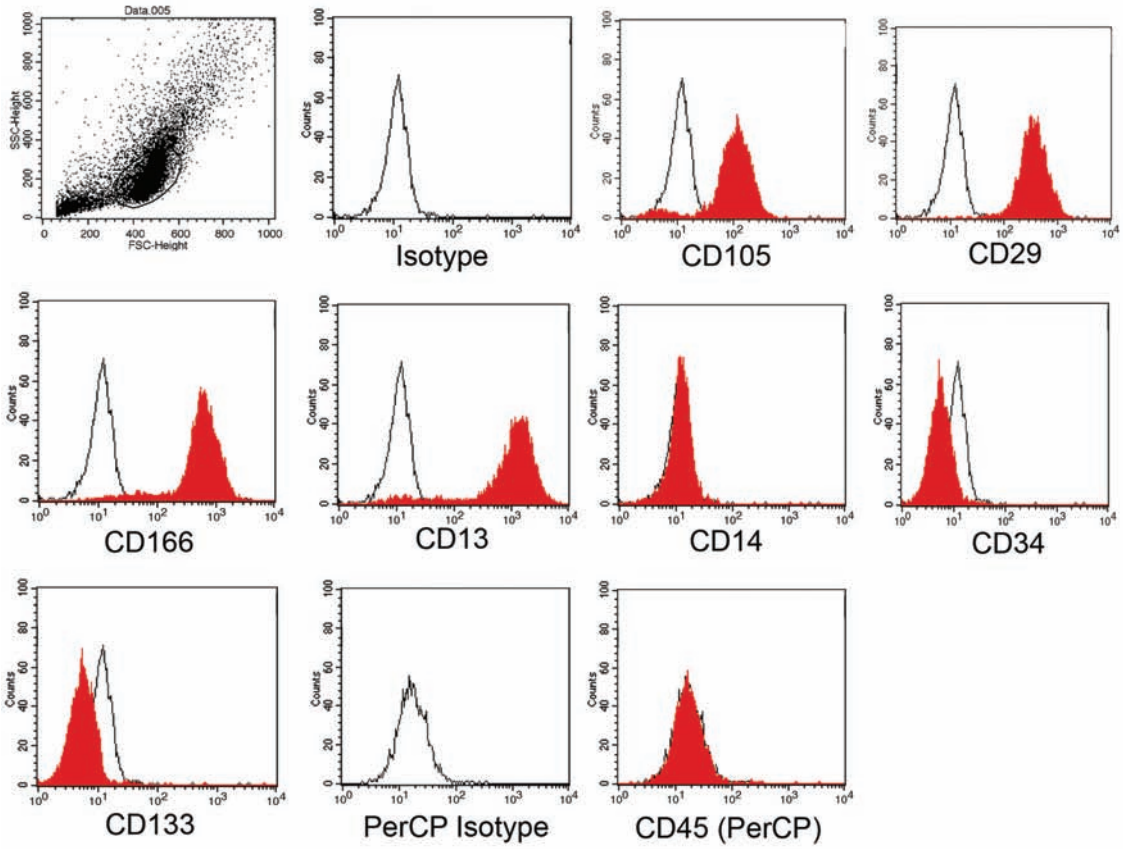
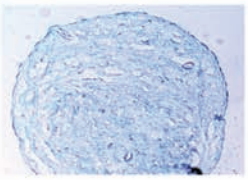
Supplemental Figure 1



Flow cytometry analysis.
Mobilized peripheral blood CFU-F at approximately 14-21 days in culture were subject to flow cytometry analysis of the following cell surface markers with antibodies to: CD105, CD166-phycoerythrin (R&D Systems, Minneapolis, MN), CD45-PerCP, CD34-phycoerythrin, CD13-phycoerythrin, CD29-phycoerythrin, CD14-phycoerythrin (Becton Dickinson, San Jose, CA), CD133-phycoerythrin (Miltenyi Biotec, Gladbach, Germany). Control mouse Ig with the appropriate conjugate was used in each analysis as control. Cells were harvested and incubated with the appropriate antibody in PBS containing 0.3% bovine serum albumin on ice for 30 minutes, then washed twice with PBS, and fixed in 2% paraformaldehyde until analysis on a FACSTAR flow cytometer.

Supplemental Figure 2



Chondrogenic differentiation of bone marrow mesenchymal stem cells. The method was as follows: 200,000 cells were pelleted in a 15 ml conical tube. Differentiation media contained high-glucose DMEM, 10 ng/ml recombinant human transforming growth factor beta-3 (rhTGF-B3), 100 nM dexamethasone, 6 µg/ml insulin, 100 µM ascorbic acid-2-phosphate, 1 mM sodium pyruvate, 6 µg/ml transferrin, 0.35 mM proline, and 1.25 mg/ml bovine serum albumin. Media was replaced every 2 days for a period of 21 days. At the end of the culture period the pellet (generally in the form a cell ball) was removed and flash frozen in a block of OCT at -80C, cryosectioned at 8 microns, transferred to slides and stained with toluidine blue.

The above section shows a typical chondrogenic differentiation from bone marrow mesenchymal stem cells. Cells from mobilized peripheral blood CFU-F would not form a cell ball at the end of the culture period and more often were a loose conglomeration of cells, therefore, could not be sectioned or stained. This may be evidence that these cells are not as multipotent as typical bone marrow mesenchymal stem cells, or perhaps the differentiation conditions required of them are different.