Fluorescent Activated Cell Sorting for the Characterization of Primary Rabbit Bone Marrow Fat Cell, Bone Marrow Stromal Cell, and Osteoblastic Cell Populations

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Introduction

As interest in the use of mesenchymal stem cell (MSC) based therapies increases, it will become important to fully characterize the cell fractions used. Several investigations have demonstrated the existence of MSCs in bone marrow stromal cells isolated from murine[1] and human.[2] Rabbits are a commonly used model for the evaluation of orthopaedic devices, but to date, bone marrow and osteogenic cell fractions from rabbit have not been characterized. We hypothesized that following monoclonal antibody labeling, fluorescent activated cell sorting (FACS) analysis could be used to characterize cell populations from rabbit bone and bone marrow stroma. Monoclonal antibodies (MAbs) for CD29, CD44, CD45, and cytokeratin were used to characterize osteoblastic, bone marrow-derived fat, and bone marrow-derived stromal cell (BMSC) fractions. Results indicated the three cell fractions were similar in composition and are a potential source of MSCs for use in the evaluation of orthopaedic cell based therapies.

Materials and Methods

Cell Isolation - Osteoblastic cells were harvested from the long bones of 1.0 kg New Zealand White rabbits following a previously published protocol.[3] The bone marrow was suspended in DMEM supplemented with 2% antibiotics, passed 3X through 16- and 21-gauge needles to yield single cell suspensions, and centrifuged. The resultant top layer of bone marrow fat was plated to T-75 flasks and the bottom layer of BMSCs underwent a red blood cell lysis procedure prior to plating. Adherent cells up to passage 7 were used for FACS analysis.

FACS analysis of cultured cells - The following MAbs were selected to characterize the primary rabbit cell fractions: anti-rabbit CD44-PE (Research Diagnostics, Inc.), which is specific for the hyaluronic acid receptor; anti-rabbit CD45-FITC, which is a hematopoietic marker; anti-human CD29-APC (BD Pharmingen), which is a marker for the β1 integrin; and anti-human cytokeratin (Miltenyi Biotec MACS), which identifies fibroblastoid cell types. The optimal concentration and volumes of MAbs used were determined through MAb titering and matched for the fluorescent conjugated marker and isotype control (unconjugated antibody).

Cultured cell fractions were trypsinized, washed, blocked with mouse IgG (Lampire Biological Laboratories), and stained with the appropriate MAb. Final cell suspensions containing 1 x 10^6 cells in 500µl of PBS supplemented with 2% heat-inactivated FBS were analyzed on a FACS Calibur machine (Becton Dickinson). Scatter gates were set to exclude debris with very low forward scatter or very high side scatter, and 10,000 or 15,000 gated events were collected for each sample. Data analysis was completed using Flowjo 6.3.3 software.

Results

Table I: Quantitative FACS analysis of rabbit bone marrow fat cell, bone marrow stromal cell, and osteoblastic cell fractions following labeling with the isotype control or MAb. Results indicated the cell populations contained cells that expressed the cell surface antigen CD44, a MSC marker, and intracellular cytokeratin, a fibroblastoid cell marker. The low expression of the CD45 indicated a small hematopoietic progenitor population. The lack of anti-human CD29 expression indicated a potential inability of this MAb to cross-react.

Discussion/Conclusion

Primary rabbit bone marrow fat, bone marrow stromal, and osteoblastic cell populations contained cells positive for the mesenchymal stem cell marker CD44, as well as the fibroblastoid cell marker cytokeratin. These findings indicate a multipotential cell population likely exists in these cell populations. These findings are consistent with results obtained using murine[1] and human[2] bone marrow aspirates.

A CD-45 MAb was used to determine whether a hematopoietic progenitor population co-existed with the progenitor populations. The low percentage of CD-45 positive cells indicated hematopoietic leukocytes were not common in the adhered osteoblastic and bone marrow stromal cell populations. Leukocytes were likely lysed during the cell lysis procedure or removed during routine cell feeding. These results agree with the findings of Deschaseaux et al.[4] in which cultured human MSCs are CD-45 negative.

A low percentage of CD-29 positive cells indicate that either a low percentage of cells expressed the β-1 integrin or, more likely, the anti-human CD-29 MAb did not exhibit cross reactivity with cells derived from rabbit.

Further investigations are required to determine whether the CD-44 positive primary rabbit cells are self-renewing, multipotent cells capable of differentiating down osteogenic, chondrogenic, and adipogenic pathways under appropriate culture conditions.

Table I

FACS ANALYSIS OF RABBIT BONE MARROW FAT CELL FRACTION			
	Number of cells gated	Negative	Positive
CD44 isotype control	12,417 (81%)	12,030 (97%)	387 (3%)
CD44-PE MAb	12,045 (78%)	8,922 (52%)	3,123 (26%)
CD29 isotype control	9,854 (89.9%)	9,611 (97.5%)	243 (2.47%)
CD29-APC MAb	9,878 (90.8%)	9,576 (96.6%)	302 (3.06%)
cytokeratin isotype control	9,150 (92%)	8,883 (97%)	267 (3%)
cytokeratin-FITC MAb	9,153 (92%)	2,042 (22%)	7,111 (78%)
CD45 isotype control	10,314 (85.2%)	10,029 (97.2%)	285 (2.76%)
CD45-FITC	10,347 (84.9%)	9,761 (94.3%)	586 (5.66%)

FACS ANALYSIS OF RABBIT BONE MARROW STROMAL CELL FRACTION			
	Number of cells gated	Negative	Positive
CD44 isotype control	13,041 (96.4%)	12,653 (97%)	388 (2.98%)
CD44-PE MAb	12,791 (95.4%)	7,966 (62.3%)	4,825 (37.7%)
CD29 isotype control	9,992 (90.5%)	9,713 (97.2%)	279 (2.79%)
CD29-APC MAb	10,021 (91.1%)	9,716 (97%)	305 (3.04%)
cytokeratin isotype control	10,267 (95.8%)	9,964 (97%)	303 (2.95%)
cytokeratin-FITC MAb	10,284 (98.3%)	7,936 (77.2%)	2,348 (22.8%)
CD45 isotype control	10,417 (81.5%)	10,097 (96.6%)	320 (3.07%)
CD45-FITC	10,353 (84.4%)	9,797 (94.6%)	556 (5.37%)

FACS ANALYSIS OF RABBIT OSTEOBLASTIC CELL FRACTION			
	Number of cells gated	Negative	Positive
CD44 isotype control	8,937 (89.4%)	8,649 (96.8%)	288 (3.22%)
CD44-PE MAb	8,868 (88.7%)	795 (9.0%)	8,073 (91.0%)
CD29 isotype control	8,534 (85.3%)	8,271 (96.9%)	263 (3.08%)
CD29-APC MAb	8,812 (88.1%)	8,489 (96.3%)	323 (3.67%)
cytokeratin isotype control	9,909 (99.1%)	9,629 (97.2%)	280 (2.83%)
cytokeratin-FITC MAb	9,961 (99.6%)	3,157 (31.7%)	6,804 (68.3%)
CD45 isotype control	9,856 (96.9%)	9,551 (96.9%)	305 (3.09%)
CD45-FITC	9,896 (99.0%)	9,492 (95.9%)	404 (4.08%)

References

[1] Baddoo M, et al. J Cell Biochem. 89: 1235-1249 (2003). **[2]** Gronthos S et al. J Cell Sci 116: 1827-1835 (2003). **[3]** Kofron MD, et al. J Orthop Res. 21: 1005-1010 (2003). **[4]** Deschaseaux et al. Br J Haematology 122: 506-517 (2003).