Analysis of the hematopoietic potential of muscle-derived cells in mice

Hartmut Geiger, Jarrod M. True, Barry Grimes, Elizabeth J. Carroll, Roger A. Fleischman, and Gary Van Zant

Introduction

An emerging theme in stem cell biology is the flexibility or “plasticity” retained by adult stem cells such as hematopoietic and neural stem cells to express differentiation programs appropriate to specific tissue microenvironments.1-3 Plasticity was also ascribed to skeletal muscle stem cells, which were reported to differentiate into hematopoietic stem cells when transplanted into lethally irradiated animals.4-6 To investigate the developmental potential and plasticity of cells in skeletal muscle, we sorted cells derived from murine muscle tissue on the basis of expression of surface markers found on hematopoietic cells, endothelial cells, and muscle stem and progenitor cells. The potential of stem and progenitor cells was analyzed in vitro and in vivo to identify the hematopoietic repopulating activity found in muscle tissue of mice by antibody staining and cell sorting. We confirmed existence of a hematopoietic repopulating cell in muscle tissue, but the data strongly suggest that repopulation is due not to muscle stem cells but to hematopoietic cells present in muscle tissue. Unexpectedly, the blood-forming cells were enriched in muscle relative to their frequency in peripheral blood. (Blood. 2002;100:721-723)

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Study design

Mice

C57BL/6J mice were from The Jackson Laboratory (Bar Harbor, ME). DBA/2CR, C57BL/6CR, and B6.SJL (Ptprc a [Ly5.1]) mice were from Charles River Laboratories (Frederick, MD). For CAFC assays, muscle tissue was derived from DBA/2 mice; for transplantation experiments, C57BL/6 and C57BL/6 Ly5.1 mice were used.

Muscle cell preparation

Mice (5-8 weeks of age) were given a perfusion of 20 mL cold phosphate-buffered saline (PBS) at a flow rate of 4 mL/minute. Per fusate was pumped into the left ventricle of the heart and removed by means of the right atrium. The gastrocnemius, soleus, and plantaris muscles were excised and manipulated in further steps on ice in Iscoves modified Dulbecco medium (Life Technologies, Grand Island, NY), 15% fetal-calf serum (FCS; Hyclone Laboratories, Logan, UT), 0.5% chick-embryo extract (Sigma, St Louis, MO), 80 U/mL penicillin (Life Technologies), and 80 mg/mL streptomycin (Life Technologies). Muscle tissue was finely minced into pieces of about 1 mm³, washed, resuspended in collagenase and dispase (0.1 U/mL and 0.8 U/mL in PBS; Roche Molecular Biochemicals, Indianapolis, IN), and incubated for 1.5 to 2 hours at 37°C with gentle agitation. The tissue was then vigorously triturated and successively filtered through cell strainers with a pore size of 70 and 40 μm (BD Biosciences, San Jose, CA). Single cells were collected by centrifugation (400g at 4°C), resuspended, and counted. For flow cytometric analyses, cells were stained with antibodies in ice-cold Hanks medium containing 2% FCS, sorted, and either plated on culture trays or injected into mice.

CAFC assay

The CAFC assay was performed without modification as described previously for assessment of hematopoietic cells.7

Transplantation

Transplant-recipient female mice were given antibiotics in drinking water 1 week before irradiation. The mice received a dose of 9 Gy (11 Gy in 1 experiment) at least 4 hours before transplantation (11 Gy was split into 2 doses). In case of competitive transplantation, the competitor cells were of the same genotype as the recipient.

Flow cytometry

Cell suspensions were stained with antibodies according to standard procedures. The analyses were performed on either a FACS Vantage or FACSscan device (BD Biosciences). The following antibodies (all from BD Biosciences) were used: CD5 (53-7.3), B220 (RA3-6B2), Mac-1 (M1/70), CD8a (53-6.7), Gr-1 (RB6-8C5), TER-119, c-Kit (2B8), Sca-1 (E13-161.7), Thy1.2 (30-H12), CD34 (RAM34), and CD31 (Mec13.1).

Results and discussion

The expression patterns of hematopoietic epitopes on muscle-derived cells were analyzed by flow cytometry (Figure 1A). Although the mice were perfused with 10 times their blood volume to remove contaminating peripheral blood, a high percentage of muscle-derived cells expressed hematopoietic cell markers, including CD45 (29%), in agreement with the findings of McKinney-Freeman et al8; and B220 (26%),...
A high frequency of CAFCs in muscle cells mismatched mice, and in some cases, admixed with recipient-type according to regions used for the CAFC assays (Figure 1B) and repopulate the hematopoietic system of irradiated mice was reduced CAFC activity to almost 25% that in unstained cells. Cells in contrast to the results of Gussoni et al.4 The high percentage of cells positive for Thy-1.2 (53%), an epitope found mostly on T cells, is negative (–) or intermediate (int) for Sca-1, CD34, CD31, and CD45. (B) Flow cytometry regions that were applied for sorting using antibodies to Sca-1, CD34, CD31, and CD45. (C) CAFC frequency in sorted cell populations derived from muscle tissue and in WBCs. The CAFC assay permits determination of frequencies of cells with proliferation potential at limiting dilution and provides temporal data indicating the hierarchy of stem and progenitor cells because later-developing colonies arise from more primitive cells. CAFC frequencies/100 000 cells (± SEM) from 3 independent analyses are shown. Muscle tissue for these experiments was derived from DBA/2 mice. WBCs (1 x 10⁶) were, after lysis of erythrocytes, derived from approximately 45 μL of blood. Preliminary analyses showed a stable engraftment pattern from 3 months to 8 months after transplantation. Mice were given transplants of the same sorted cell populations derived from muscle tissue and from WBCs reported for the CAFC analysis, allowing a direct comparison of the in vivo and in vivo potential of the cells. The percentage of donor-derived cells in peripheral blood (± SEM) is shown. Data are based on at least 2 independent cell sorts and transplantations for each cell population. In each experiment, each sorted cell population was transplanted into at least 2 mice; c.c. indicates 2 x 10⁶ competitor cells.

Muscle-derived cells with the ability to repopulate the hematopoietic compartment were Sca-1–/int, CD34–, CD31–/int, and CD45– (Figure 1D). Thus, except for CD34, cells with in vitro and in vivo stem and progenitor cell activity showed the same pattern of epitope expression. Because staining with the CD31 antibody did not interfere with homing or engraftment of muscle-derived cells (data not shown), masking of functional protein domains by the antibody could not have accounted for the difference between the results of the 2 assays.

Taken together, the Thy-1 skewing characteristic of repopulation from peripheral blood progenitors and the surface phenotype of the repopulating cell indicate a peripheral hematopoietic stem cell as the repopulating unit. This conclusion is in agreement with observations by Kawada and Ogawa.12 We detected the same Thy-1 skewing when white blood cells (WBCs) were used as donor cells, in accordance with a previous study of mobilized peripheral blood cells.13

Muscle-derived cells showed long-term (> 8 months) multilineage repopulation and chimerism in secondary BM transplants, confirming the presence of stem cell activity (data not shown). Spleen chimerism reflected the percentage found in peripheral blood (Figure 1D), whereas donor chimerism in the thymus and BM was typically 10% to 20% of the percentage observed in the periphery (data not shown). Interestingly, even in mice showing multilineage reconstitution, most donor-derived cells in peripheral blood were Thy-1–, a feature also reported, but not emphasized, by Kawada and Ogawa.12

The ability of distinct muscle-derived cell populations to repopulate the hematopoietic system of irradiated mice was analyzed in a transplantation assay (Figure 1D). Cells were sorted according to regions used for the CAFC assays (Figure 1B) and subsequently transplanted into lethally irradiated Ly5.1-Ly5.2 mismatched mice, and in some cases, admixed with recipient-type BM competitor cells.5,11
As shown in Figure 1C and D, peripheral WBCs also contained CAFCs and repopulating activity. Transplantation of $2.5 \times 10^6$ muscle-derived cells, the number we obtained on average from a C57BL/6 mouse, resulted in a chimerism of 8% (Figure 1D). This is 16-fold higher than the chimerism obtained with $1.2 \times 10^5$ WBCs (0.5%), which correspond to 55 µL of blood. Indeed, to achieve the same percentage of chimerism observed after transplantation of muscle cells derived from a single mouse would require WBCs derived from 900 µL of blood. This suggests that these repopulating cells are not contaminating stem cells found in peripheral blood. Given that we obtained on average $1.8 \times 10^6$ muscle-derived cells from a DBA/2 mouse, the CAFC activity in muscle tissue dissected from one animal would be contained in 1.8 mL of blood (Figure 1C).

Thus, we found that skeletal muscle tissue is enriched for hematopoietic stem cells. Further analyses will identify the location of these cells in muscle and determine whether they are sequestered in the wall of the vasculature or within the muscle tissue itself.

Since this manuscript was submitted for publication, McKinney-Freeman et al. reported that muscle-derived hematopoietic stem cells are hematopoietic of origin, a finding supported by our analyses.

References

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