SCA-1$^+$ Cells with an Adipocyte Phenotype in Neonatal Mouse Skin

To The Editor:

There is considerable current interest in stem cells, not only in order to understand the processes of development, regeneration, and carcinogenesis but also because readily accessible tissues such as the skin may be an excellent resource of autologous stem cells to treat human disease (Baksh *et al*, 2004). Cells that mature into functionally different lineages have been described in the skin (Taylor *et al*, 2000; Albert *et al*, 2001; Potten and Booth, 2002; Jahoda *et al*, 2003; Trempus *et al*, 2003; Dyce *et al*, 2004). For example, skin-derived precursor cells have been described that can differentiate into neurons, muscle, and adipocytes (Toma *et al*, 2001). With the exception of keratinocyte and melanocyte stem cells, the origin and characteristics of skin stem cells are, however, not well defined.

Stem cell antigen-1 (Sca-1; Ly-6A/E) is a well-established marker for bone marrow-derived murine stem cell enrichment for both hematopoietic and mesenchymal stem cells (Patterson *et al*, 2000; Baddoo *et al*, 2003; Bonyadi *et al*, 2003) but has been little investigated in skin. We have identified in neonatal mouse skin a major population of Sca-1$^+$ cells that do not express c-kit or CD45 but that express adipocyte markers. A preliminary report of this study has been made.[1]

Sca-1 visualized by immunohistochemistry in neonatal skin showed positive staining cells in the lower dermis, in the sebaceous glands, and in the panniculus adiposus of the hypodermis (Fig 1*A*, *C*; for methods, see Supplementary data and Table S1). It was unexpected to find such a large population of Sca-1$^+$ cells in the skin, since in the bone marrow, less than 1% of cells are positive for Sca-1. Double staining with antibodies to the stem cell marker CD34 indicated the Sca-1$^+$ cells in the lower dermis and sebaceous glands were mostly negative for CD34, whereas Sca-1$^+$CD34$^+$ cells positive for both markers were readily identifiable in the panniculus adiposus (Fig 1*A*). Further, CD34$^+$ cells that did not express Sca-1 were found chiefly in the upper dermis (Fig 1*A*). Since adipocytes are a major component of the lower dermis and the panniculus adiposus, skin sections were stained with red oil that visualizes fat and also with an adipocyte-specific antibody to fatty-acid binding protein 4 (FABP4, Ap2) (Fruhbeck *et al*, 2001) (Fig 1*B*, *D*). Red oil staining, indicating the presence of fat, was readily observed in the lower dermis and in the sebaceous glands, corresponding to the localization of Sca-1$^+$CD34$^-$ cells (Fig 1*D*). Interestingly, the panniculus adiposus, the major source of Sca-1$^+$CD34$^+$ cells, did not stain with red oil, suggesting that these cells may be immature cells of the adipocyte lineage since, in development (Hausman *et al*, 1981), adipocyte precursors progress from the hypodermis to the dermis. Positive staining for FABP4 observed in the lower dermis and in the hypodermis, although not in sebocytes, supported this contention (Fig 1*B*). Double staining of frozen sections with antibodies to Sca-1 and to 6-α integrin revealed rare double-positive cells (Fig S5*A*).

To further characterize Sca-1$^+$ cells, single-cell suspensions were prepared from neonatal mouse skin (for methods, see Supplementary data and Table S1) and examined by FACS analysis for surface expression of the stem cell markers Sca-1, CD34, and c-kit (Fig 2*A* and Fig S1). Major populations of Sca-1$^+$ cells and of CD34$^+$ cells and of double-positive Sca-1$^+$CD34$^+$ cells were readily identified (Fig 2*A*, *upper panel* and Fig S1, *upper row*), consistent with the immunohistochemistry data. Double staining indicated that all Sca-1$^+$ cells and all CD34$^+$ cells were negative for c-kit (Fig 2*A*, *middle and lower panels* and Fig S1, *middle and lower rows*). The percentages of Sca-1$^+$ and CD34$^+$ cells decreased significantly with age, whereas the percentage of c-kit$^+$ cells was constant over the same time period (Fig S2). The decrease in Sca-1$^+$ cells as a percentage of total cells from postnatal days 1 to 5, even though the percentage of c-kit cells remained constant, argued in favor of a role for Sca-1$^+$ cells in early postnatal development.

Double staining for Sca-1 and CD34 confirmed three subpopulations (Fig 2*A*, *upper panel* and Fig S1, *upper row*)—a double-positive Sca-1$^+$CD34$^+$ population (8%–10% of total) that was actively cycling (7.2% of cells in G_2/M phase; Fig S3), a single positive Sca-1$^+$CD34$^-$ (15%–20% of total), quiescent population (1.7% of cells in G_2/M phase; Fig S3), and a Sca-1 negative population of CD34$^+$ cells (3%–4% of total). Similar results were found by FACS analysis using two different commercially available anti-Sca-1 monoclonal antibodies (Table S1). Mast cells were readily identifiable by toluidine blue staining in the c-kit$^+$Sca-1$^-$ population but not in the c-kit$^-$Sca-1$^+$ population (Fig S4), in contrast to previous publications that adult peritoneal and bone marrow mast cells carry Sca-1 (Drew *et al*, 2002). Double staining for Sca-1 and for 6-α integrin further revealed a subpopulation of double positive cells (Fig S6), again consistent with the immunohistochemistry data.

Cytospin preparations of sorted Sca-1$^+$CD34$^+$ and Sca-1$^+$CD34$^-$ populations were stained with antibodies to the adipose-specific marker FABP4, to keratin 14, and to

Abbreviations: FABP4, fatty-acid binding protein 4; Sca-1, stem cell antigen-1

[1]Wolnicka-Glubisz A, Noonan F: Expression of the stem cell markers Sca-1, CD34, CD45 and c-kit in neonatal mouse skin. J Invest Dermatol 123:172, A29, 2004 (abstr).

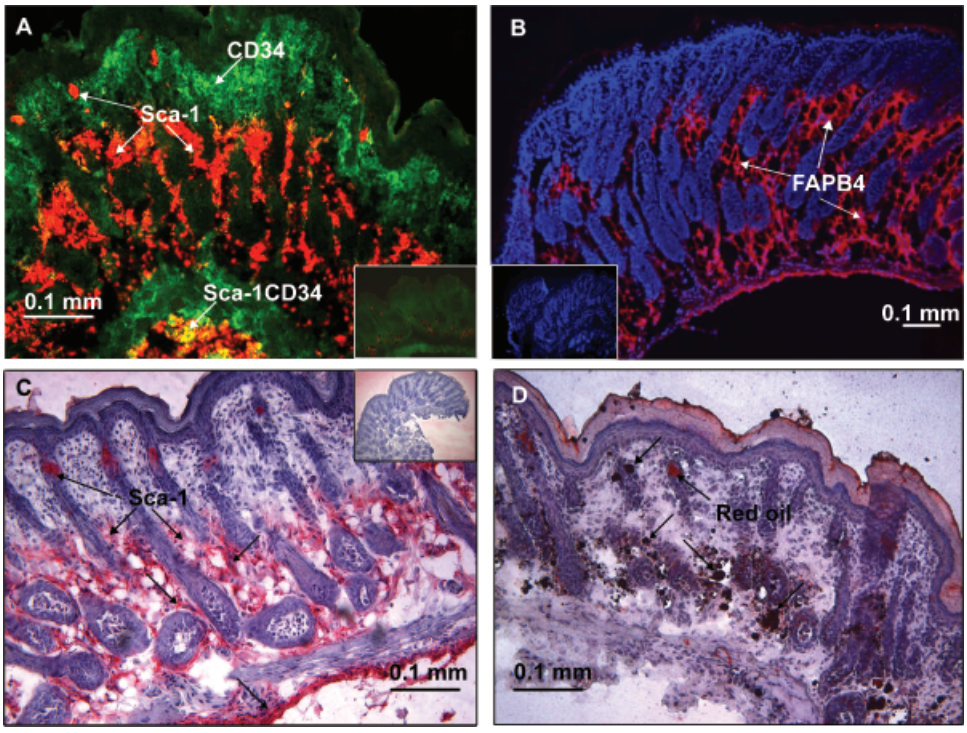

Figure 1
The adipocyte marker fatty-acid binding protein 4 (FABP4) co-localizes with stem cell antigen-1 (Sca-1) cells in the dermis and Sca-1$^+$CD34$^+$ cells in the hypodermis, whereas red oil co-localizes with Sca-1$^+$ cells only in the dermis and sebaceous glands in neonatal mouse skin. Frozen sections of neonatal mouse skin were stained with antibodies to Sca-1, CD34, or FABP4 and fat was visualized with red oil (for methods and antibodies, see Supplementary data and Table S1). (*A*) Double staining: Sca-1 (*red*) visualized in the dermis and in sebaceous glands (*arrows*); CD34 (*green*) in the upper dermis (*arrows*); double-stained Sca-1$^+$CD34$^+$ cells in the hypodermis (*yellow*; *arrows*); *inset*: negative control. (*B*) FABP4 staining (*red*) in the dermis and hypodermis; *inset*: negative control; DAPI (blue). (*C*) Sca-1 (*red*) visualized with Vector stain in the dermis, sebocytes, and hypodermis (*arrows*); *inset*: negative control. (*D*) Fat stained with red oil in the dermis and sebocytes (*arrows*).

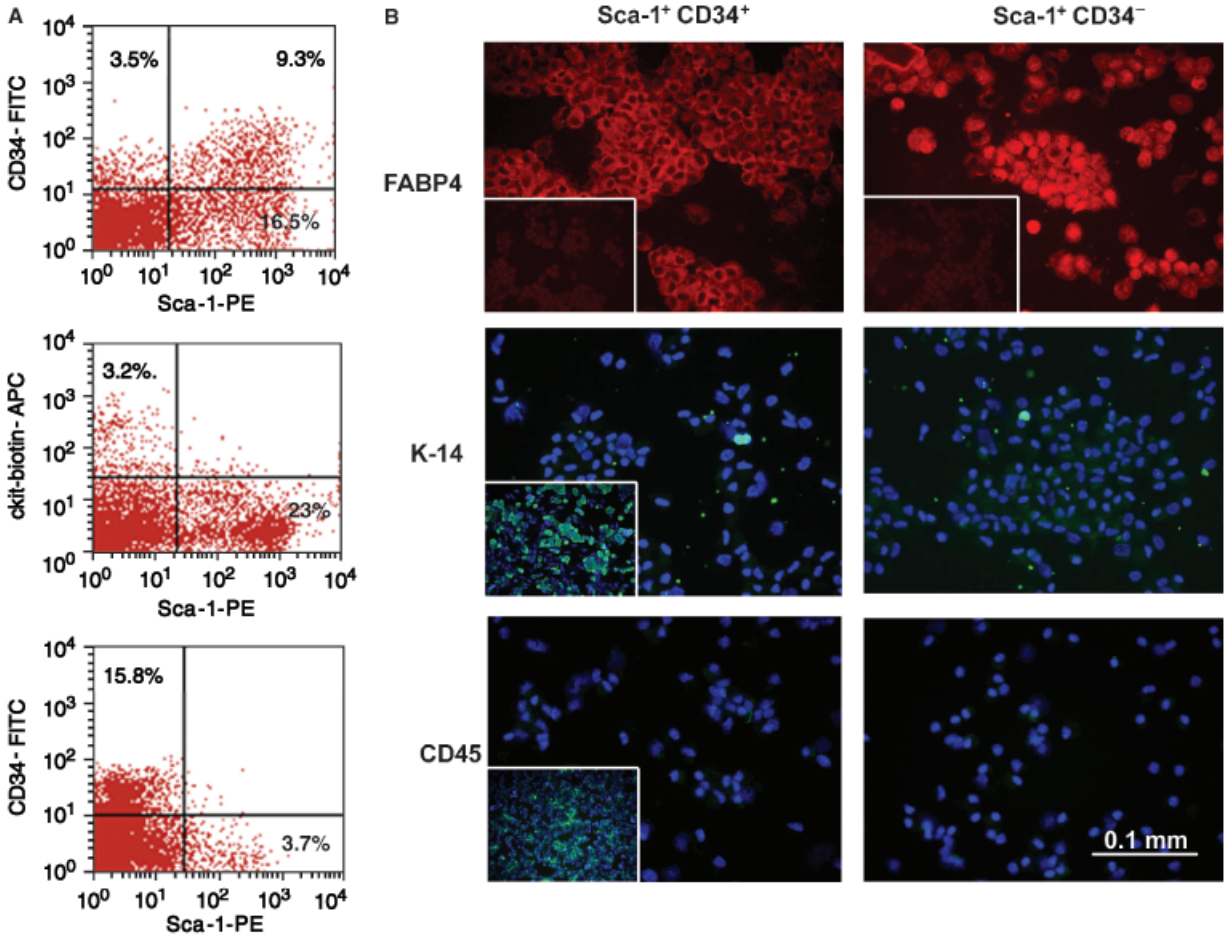

Figure 2
Stem cell antigen-1 (Sca-1)- and CD34-positive cells in neonatal mouse skin are negative for c-kit, for keratin 14, and for CD45. (*A*) FACS analysis of single-cell suspensions of neonatal mouse skin (for methods and antibodies, see Supplementary data and Table S1) double stained for Sca-1 and CD34 (*upper panel*), Sca-1 and c-kit (*middle panel*), and c-kit and CD34 (*lower panel*). Percentages of cells positive in each quadrant are indicated in the figure. Isotype controls were used for each antibody (for complete data, see Fig S1). (*B*) Cells were sorted for Sca-1$^+$CD34$^+$ (*left column*) and Sca-1$^+$CD34$^-$ (*right column*) and cytospins were stained for fatty-acid binding protein 4 (FABP4) (*red upper two panels*; *insets*: negative controls), for keratin 14 (*green middle two panels*; *inset*: positive control), or CD45 (*green middle two panels*; *inset*: positive control) DAPI (blue).

CD45, a lineage marker for hemopoietic cells. Both Sca-1$^+$ populations showed positive staining for FABP4 (Fig 2*B*, *upper two panels*) but were negative for keratin and for CD45 (Fig 2*B*, *middle and lower panels*), again consistent with an adipose phenotype. In contrast, Sca-1$^-$CD34$^+$ and Sca-1$^-$CD34$^-$ cells were negative for FABP4 (data not shown). 6-α integrin-positive cells were sorted and cytospins were double stained for Sca-1 and for keratin 14. Single positive cells were readily identified with each antibody but no double-stained cells, indicating that Sca-1$^+$ 6-α integrin$^+$ cells did not express keratin 14 (Fig S5*B*).

In conclusion, we have demonstrated that neonatal mouse dermis and hypodermis contain Sca-1$^+$ cells that do not express the hemopoietic stem cell marker c-kit, the lineage marker CD45, or keratin 14 but that do express the adipocyte marker FABP4. A role for Sca-1 in adipocyte development is supported by the observation that Ly-6A/E (Sca-1) null mice (Bonyadi *et al*, 2003) exhibited, in addition to long-term defects in osteoprogenitors, a deficiency in adipocyte colony-forming cells. Further, a CD34$^+$ stem cell that could differentiate into adipocytes was identified from human adipose stromal tissue (Gronthos *et al*, 2001). In this regard, a recent report[2] identified Sca-1$^+$CD34$^+$ dermal cells from neonatal mouse skin that matured into adipocytes or sebocytes on culture. The plasticity of the Sca-1$^+$ cells we have identified and whether they can give rise to cells of other lineages as described for Sca-1$^+$c-kit$^-$ skin-derived precursor cells located in the dermal papillae (Fernandes *et al*, 2004) or whether they are fully committed to the adipocyte lineage is of considerable interest.

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Supplementary Material

The following supplementary material is available for this article online.

Figure S1
Neonatal mouse skin contains c-kit negative cell populations expressing either Sca-1 alone or co-expressing Sca-1 and CD34.

Figure S2
Sca-1$^+$ and CD34$^+$ cells but not c-kit$^+$ cells decrease with age.

Figure S3
Double positive Sca-1$^+$CD34$^+$ cells are more proliferative than single positive Sca-1$^+$CD34$^-$ cells

Figure S4
Mast cells are contained in the c-kit$^+$Sca-1$^-$ and not in the c-kit$^-$Sca-1$^+$ population.

Figure S5
A Sca-1$^+$ 6-α integrin$^+$ subset of cells can be detected in skin and is keratin 14 negative.

Figure S6
A sub-population of Sca-1 positive cells is also positive for 6-α integrin.

Table S1
Summary of antibodies, sources, and dilutions used in these studies.

Supplemental Text
Materials and Methods

[2]Meidl S, Elbe-Burger A: The murine dermis contains cells with *in vitro* clonogenic potential. J Invest Dermatol 123:051, A9, 2004 (abstr).

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