Journal of the American College of Cardiology
© 2007 by the American College of Cardiology Foundation
Published by Elsevier Inc.

Vol. 50, No. 23, 2007
ISSN 0735-1097/07/$32.00
doi:10.1016/j.jacc.2007.08.033

Circulating Progenitor Cells Can Be Reliably Identified on the Basis of Aldehyde Dehydrogenase Activity

Thomas J. Povsic, MD, PHD,* Katherine L. Zavodni, BS,* Francine L. Kelly, BS,* Shoukang Zhu, PHD,* Pascal J. Goldschmidt-Clermont, MD, FACC,‡ Chunming Dong, MD,* Eric D. Peterson, MD, MPH, FACC†

Durham, North Carolina; and Miami, Florida

Objectives Our objective was to develop and assess a novel endogenous progenitor cell (EPC) assay based on aldehyde dehydrogenase (ALDH) activity, and to define the relationship of ALDH-bright ($ALDH^{br}$) cells with previously defined EPCs, patient age, and extent of coronary artery disease.

Background Accurate assessment of circulating EPCs is of significant interest, yet current assays have limitations. Progenitor cells display high levels of ALDH activity. An assay based on ALDH activity may offer a simple means for enumerating EPCs.

Methods We simultaneously determined the numbers of EPCs based on ALDH activity and cell surface expression of CD133, CD34, and vascular endothelial growth factor receptor-2 in 110 patients undergoing cardiac catheterization. We assessed the reproducibility of these estimates, correlation among EPC assays, and the association of $ALDH^{br}$ numbers with age and disease severity.

Results Aldehyde dehydrogenase-bright cells were easily identified in nonmobilized peripheral blood with median and mean frequencies of 0.041% and 0.074%, respectively. Aldehyde dehydrogenase-bright cells expressed CD34 or CD133 cell surface markers (57.0% and 27.1%, respectively), correlated closely with $CD133^{+}CD34^{+}$ cells ($r = 0.72$; $p < 0.001$), and differentiated into endothelial cells with greater efficiency than $CD133^{+}CD34^{+}$ cells. Aldehyde dehydrogenase-bright cell numbers were inversely associated with patient age and coronary disease severity.

Conclusions Aldehyde dehydrogenase activity represents a novel simplified method for quantifying EPCs. The correlation of $ALDH^{br}$ cells with clinical factors and outcomes warrants further study. (J Am Coll Cardiol 2007;50:2243–8)

The discovery of circulating endogenous progenitor cells (EPCs) capable of vascular repair (1) has broadened our conception of atherosclerosis pathophysiology and treatment (2). Most studies have found an inverse correlation between EPC number and cardiac risk factors (3,4), disease severity (5), and risk for future cardiovascular events (6,7), although these findings are not universal (8,9). This body of work suggests that accurate EPC enumeration may be clinically useful for assessment of vascular reparative capacity and patient risk stratification; however, EPC enumeration currently requires prompt cell culture or fluorescence-activated cell sorting (FACS) analysis.

Cytosolic aldehyde dehydrogenase (ALDH) has been identified as the enzymatic activity responsible for the resistance of progenitor cells to chemotherapeutic agents (10) and can be used to select for a highly enriched population of progenitor cells in bone marrow (11,12) and umbilical cord (13) sources. In addition, ALDH activity is present at high levels in progenitor precursors of a variety of mature cell lines (14–16) and may play an important role in maintaining the progenitor cell phenotype (17).

Recently, reagents for the dependable enumeration of aldehyde dehydrogenase-bright ($ALDH^{br}$) cells in bone marrow and mobilized blood using conventional FACS techniques and requiring only a single color channel have become available. We explored whether or not ALDH activity could be used as a novel and simpler tool to assess circulating EPC numbers in peripheral blood.

Methods

Patient enrollment and mononuclear cell isolation. Patients undergoing elective left heart catheterization were ap-

From the *Division of Cardiology and †Duke Clinical Research Institute, Duke University Medical Center, Durham, North Carolina; and the ‡Miller School of Medicine, University of Miami, Miami, Florida. Supported in part by National Heart, Lung, and Blood Institute grant K-18 HL081419-01A1, grants from the Duke Clinical Research Institute, a Society of Geriatric Cardiology Merck Geriatric Cardiology Research Award, and Medtronic, through the Medtronic-Duke Strategic Alliance. Aldecount kits were provided by Aldagen, Durham, North Carolina.

Manuscript received April 9, 2007; revised manuscript received August 23, 2007, accepted August 27, 2007.

JACC Vol. 50, No. 23, 2007
December 4, 2007:2243–8

Abbreviations and Acronyms

ALDH = aldehyde dehydrogenase
ALDH^{br} = aldehyde dehydrogenase-bright
CAD = coronary artery disease
EPC = endogenous progenitor cell
FACS = fluorescence-activated cell sorting
MNC = mononuclear cell
VEGFR = vascular endothelial growth factor receptor

proached for consent, and after arterial sheath insertion 30-ml peripheral blood was removed and stored in ethylenediaminetetraacetic acid tubes. Arterial blood was subjected to centrifugation (2,000 $g \times 10$ min), and the buffy coat was treated with 10 volumes of erythrocyte lysis buffer (Aldagen, Durham, North Carolina). The mononuclear cells (MNCs) were isolated by centrifugation (250 $g \times 5$ min), washed with phosphate-buffered saline-1% bovine serum albumin (Invitrogen, Carlsbad, California), and aliquoted for analysis.

Enumeration of ALDH^{br} cells. Mononuclear cells (2×10^6) were aliquoted to an Aldecount tube (Aldagen Inc., Durham, North Carolina), incubated with BODIPY-AAD at 37°C for 30 min, and maintained on ice to retain the fluorescent byproduct. A control reaction was performed in the presence of diethylaminobenzaldehyde. Cells were spun at 250 $g \times 5$ min, the reaction buffer removed, and the cells suspended in assay buffer on ice until FACS analysis was performed.

Analysis of EPCs based on cell surface marker expression. Mononuclear cells (4×10^6 cells) were aliquoted, incubated with Fc receptor (FcR) blocking reagent (Miltenyi Biotec, Auburn, California) for 10 min, and incubated with CD133-APC (Miltenyi Biotec), CD34-FITC (Miltenyi Biotec), and VEGFR-2-PE (R&D Systems, Minneapolis, Minnesota) for 60 min at 4°C. Dead and dying cells were excluded using staining with 7-AAD (1 μg/10^6 cells; Invitrogen). Directly conjugated isotype control antibodies were used to set baseline fluorescence levels.

FACS analysis. Flow cytometry was performed by trained technicians blinded to patient identity using an LSR II flow cytometer (BD Biosciences, San Jose, California) and analyzed using FlowJo software (Treestar, Costa Mesa, California). Compensation was performed daily using BD CompBeads incubated with each antibody.

Analysis was performed by gating lymphocytes and monocytes on the basis of light-scattering properties. The EPCs were expressed as a percentage of this monocyte/lymphocyte gate.

Expansion of ALDH^{br} cells into endothelial cells. Bone marrow was purchased from Cambrex (Walkersville, Maryland), depleted of erythrocytes using erythrocyte lysis buffer, labeled with Aldefluor reagent according to the manufacturer's directions, and sorted on a modified FACS-ARIA cell sorter (BD Biosciences) at 4°C.

The CD133/CD34 selection was performed using CD133-MACS beads (Miltenyi Biochemical). After elution from the beads, cells were incubated with PE- conjugated anti-CD34 and sorted on a modified FACS-ARIA cell sorter. The EPCs (5,000) were plated into fibronectin-coated 4-well chamber slides (BD Biosciences) and incubated in M199 medium containing 20% fetal calf serum, 10 ng/ml recombinant human vascular endothelial growth factor, 1 ng/ml b-fibroblast growth factor, and 2 ng/ml insulin-like growth factor 1. The numbers of adherent elliptical endothelial cells were quantitated after 5 days. The averages of 10 experiments are shown.

Duke Cardiovascular Databank. The methods of the Duke Cardiovascular Databank have been previously described (18,19). In brief, all patients who have undergone cardiac catheterization, percutaneous coronary intervention, or cardiac surgery have had their demographic, clinical, angiographic, and procedural data entered into a standard database. All cardiac catheterization procedures are systematically reviewed in a standardized fashion by 2 operators. Coronary disease severity was summarized as number of diseased coronary vessels, defined by at least 1 narrowing ≥75%.

Statistical analysis. The median and mean numbers (with standard deviations [SD]) of EPCs determined using each assay are reported, as are the mean numbers (±SD) of ALDH^{br} cells expressing specific cell surface markers. A Pearson correlation coefficient between independent EPC assessments was determined to assess the precision and temporal stability of EPC assays. To compare these correlations, the absolute difference between each matched pair of determinations was calculated and these differences compared using a Mann-Whitney test.

A D'Agostino and Pearson omnibus normality test was used to determine the normality of each distribution. Given non-normal distributions, Spearman correlations are reported for associations between EPC types as well as the association of ALDH^{br} cells with age. Analysis of variance and analysis for linear trend were performed to assess association of ALDH^{br} cells with extent of coronary artery disease (CAD).

GraphPad Prism 4.0 (GraftPad Software, San Diego, California) was used for all analyses.

Results

ALDH assay and characterization of ALDH^{br} cells. Analysis of ALDH activity was readily performed and resulted in the straightforward identification of cells characterized by high levels of ALDH activity and low side-scatter (Fig. 1). This population is not observed in the presence of diethylaminobenzaldehyde, a potent inhibitor of ALDH.

We characterized ALDH^{br} cells by counterstaining for CD133, CD34, and vascular endothelial growth factor receptor (VEGFR)-2 expression in 21 patients. As shown in Figure 2, 57.0 ± 21.9% of ALDH^{br} cells express CD34, with lower numbers of ALDH^{br} cells expressing CD133 (mean 27.1 ± 14.1%). Of those ALDH^{br} cells that were positive for CD133$^+$ expression, the majority also expressed

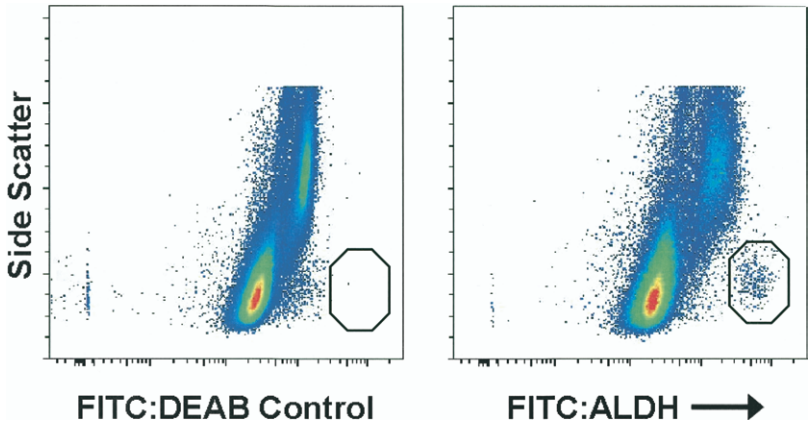

Figure 1 **EPC Identification Based on ALDH Activity**

A discrete group of cells, absent in the diethylaminobenzaldehyde (DEAB) control sample **(left)**, with high aldehyde dehydrogenase (ALDH) activity and low side-scatter, is identified in the right lower quadrant **(right)**. EPC = endogenous progenitor cell; FITC = fluorescein isothiocyanate.

CD34 (78.4 ± 27.2%). In contrast, these markers are expressed in only a very small percentage of cells lacking ALDH activity (percentage of $ALDH^{low}$ cells expressing CD34 0.18 ± 0.12%, expressing CD133 0.21 ± 0.18%, and expressing CD133 and CD34 0.015 ± 0.014%; $p < 0.0001$ for comparison of each $ALDH^{br}$ subpopulation with $ALDH^{low}$ cells expressing the same marker[s]). A high proportion of $CD133^+CD34^+$ cells also exhibit high levels of ALDH activity (63.5 ± 25.9%). These findings indicate that there is substantial, but not complete, overlap of the $ALDH^{br}$ and $CD133^+CD34^+$ cell populations. Interestingly, $ALDH^{br}$ cells did not express VEGFR-2 (data not shown).

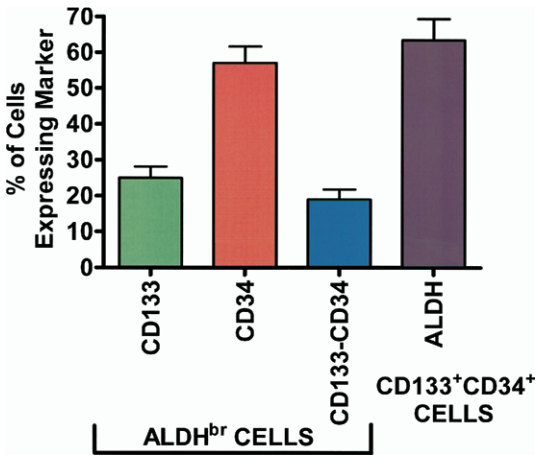

Figure 2 **Characterization of $ALDH^{br}$ Cells**

Aldehyde dehydrogenase-bright ($ALDH^{br}$) cells were subjected to staining for expression of select endogenous progenitor cell markers (n = 21). The **purple column** indicates the percentage of $CD133^+CD34^+$ cells also expressing elevated ALDH activity.

To assess test precision, we correlated EPC frequencies determined in independent samples. Whereas the intrapatient reproducibility of EPC count was high with each technique, it was slightly greater for the ALDH assay ($r = 0.98$, $n = 25$ for $ALDH^{br}$ cells; vs. $r = 0.94$, $n = 19$ for $CD133^+CD34^+$ cells; p = NS for comparison of paired differences).

We also assessed the temporal stability of EPCs by enumerating $ALDH^{br}$ and $CD133^+CD34^+$ cells in peripheral blood samples drawn from volunteers at 24-h intervals. Temporal stability was high for both assays ($r = 0.87$, $n = 18$ for $ALDH^{br}$ cells; vs. $r = 0.83$, $n = 18$ for $CD133^+CD34^+$ cells; p = NS for comparison of the paired differences).

Determining the endothelial potential of $ALDH^{br}$ cells. To determine the potential of $ALDH^{br}$ cells to contribute to vascular repair, we cultured isolated $ALDH^{br}$ cells and stained the differentiated cells with endothelial-specific markers. Aldehyde dehydrogenase-bright progeny displayed robust staining with acetylated low-density lipoprotein, Ulex lectin, and antibodies to CD31, von Willebrand factor, and VEGFR-2 (data not shown), demonstrating that $ALDH^{br}$ cells readily differentiate into endothelial cells. We also compared the capacity of $ALDH^{br}$ cells to generate endothelial cells, with EPCs defined using conventional markers. When equal numbers of $CD133^+CD34^+$ and $ALDH^{br}$ cells were cultured, the numbers of endothelial cells derived from $ALDH^{br}$ cells (10 experiments) exceeded those obtained from $CD133^+CD34^+$ cells by a factor of 25 (mean number of endothelial cells 80.5 ± 2.98 versus 2.3 ± 1.08, respectively).

Distribution of EPC numbers. We next determined the numbers of $ALDH^{br}$ cells in a prospectively collected series of 110 patients undergoing cardiac catheterization, while simultaneously assessing the numbers of MNCs expressing CD133, CD34, and VEGFR-2. Patient characteristics are

Table 1 Patient Characteristics

Factor	Mean
Age (yrs)	63.5
Race (Caucasian)	85.1%
Gender (male)	58.2%
Hypertension	73.6%
Hyperlipidemia	68.2%
Diabetes	26.4%
Ongoing tobacco use	25.7%
Prior PCI	18.2%
Prior CABG	11.8%
Extent of CAD	
0 vessel	44.9%
1 vessel	16.8%
Multivessel	38.3%

CABG = coronary artery bypass grafting; CAD = coronary artery disease; PCI = percutaneous coronary intervention.

shown in Table 1. Extent of coronary artery disease was evenly distributed, with about 45% having no significant CAD and 38% having multivessel disease (Table 1).

The numbers of ALDH^{br} cells were not normally distributed (D'Agostino and Pearson omnibus normality test: $p < 0.0001$), with a mean of 0.0743 ± 0.067% and median of 0.0510% (interquartile range [IQR] 0.026 to 0.105) of MNCs displaying high levels of ALDH activity. These numbers were comparable in magnitude with those observed for the numbers of CD133$^+$CD34$^+$ cells (mean 0.053 ± 0.043% and median 0.0413% [IQR 0.021 to 0.072%]), whereas the numbers of CD34$^+$-VEGFR-2$^+$ cells were significantly lower (mean 0.0134 ± 0.027%, median 0.0055% [IQR 0.0018 to 0.014%]).

Correlation among EPC assay methodologies. We determined the association of ALDH^{br} cells with EPCs identified based on expression of CD133, CD34, and VEGFR-2. We observed a strong correlation between EPC cell numbers identified using the ALDH assay relative to those enumerated based on expression of CD133, CD34, and CD133/CD34 ($r = 0.25$, 0.55, and 0.72, respectively) (Figs. 3A to 3C). In contrast, ALDH^{br} cell numbers failed to correlate with the numbers of cells expressing VEGFR-2 or VEGFR-2 and CD34, commonly used markers for EPC determination (Fig 3D).

Correlation between ALDH assay findings and patient age and CAD severity. To assess the relationship between chronic vascular injury and ALDH^{br} cells, we examined the association between the numbers of ALDH^{br} cells and patient age and extent of CAD. As shown in Figure 4, the numbers of ALDH^{br} cells varied inversely with patient age ($p < 0.005$). Patients were then divided into those with normal or minimal disease with no significantly obstructed vessels, single-vessel disease, and advanced multivessel CAD (Fig 4). We found a stepwise depletion of ALDH^{br} cells in patients directly related to the extent of CAD.

Discussion

Since the initial description of endogenous circulating progenitor cells capable of vascular repair (1), the number of published reports identifying and enumerating these cells

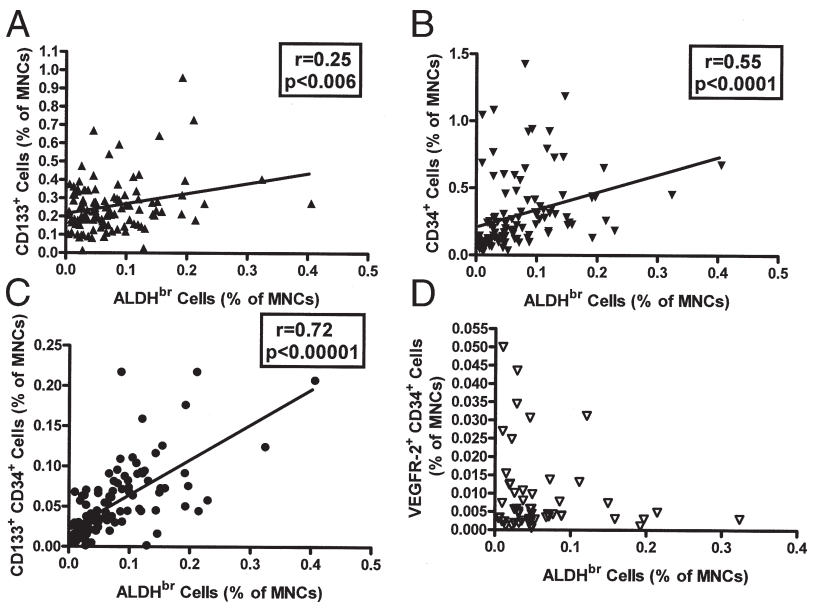

Figure 3 Correlation Between ALDH^{br} Cell Numbers and EPCs Expressing Specific Cell Surface Markers

Correlation of aldehyde dehydrogenase-bright (ALDH^{br}) cells with CD133$^+$ cells **(A)**, CD34$^+$ cells **(B)**, CD133$^+$CD34$^+$ cells **(C)**, and vascular endothelial growth factor receptor (VEGFR)-2$^+$CD34$^+$ cells **(D)**. Spearman correlation coefficients are shown. EPCs = endogenous progenitor cells; MNCs = mononuclear cells.

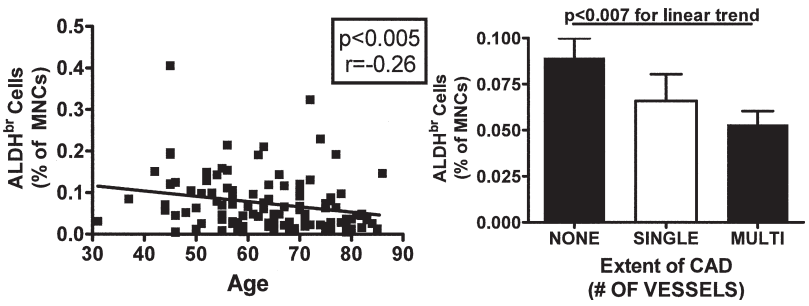

Figure 4 **Correlation of ALDH^{br} Cell Number and Patient Age and Extent of CAD**

Aldehyde dehydrogenase-bright (ALDH^{br}) cells are inversely correlated with patient age (n = 110; $p < 0.01$) and extent of coronary artery disease (CAD) (n = 110; p value for analysis of variance analysis and analysis for linear trend). MNCs = mononuclear cells.

has dramatically escalated. The use of EPC levels to predict cardiac risk has been explored in 2 reports demonstrating a relationship between EPC numbers and subsequent clinical outcomes (6,7), suggesting that EPC enumeration may be clinically useful for patient risk stratification; however, the methodologies required for EPC enumeration require proficiency with cell culture or complex FACS analysis using multiple cell surface markers.

Aldehyde dehydrogenase is a key enzymatic activity expressed at high levels in progenitor cells. Not only may ALDH activity be a fundamental property of the progenitor cell phenotype (17), but also selection on the basis of ALDH level may identify cells with greater proliferative and reparative capacity than progenitor cells selected on the basis of other markers (12,20). In addition, ALDH^{br} cells are capable of differentiation into a variety of phenotypes (15,16). We therefore explored whether ALDH activity may serve as a simpler method for identification of EPCs with high proliferative and reparative capacity.

We have now demonstrated that ALDH^{br} cells can be reliably enumerated using a simple gating strategy with a single fluorescence channel in blood samples from cardiac patients and that ALDH^{br} cells transform into endothelial cells with greater efficiency than other EPCs. These properties make the identification of ALDH^{br} cells a promising methodology for EPC enumeration in wider clinical contexts.

A substantial portion of ALDH^{br} cells express CD34 and CD133 markers, and there is strong correlation between the numbers of ALDH^{br} cells and the numbers of CD133^{+}/CD34^{+} EPCs. We do not find a similar correlation with cells expressing CD34^{+}VEGFR-2^{+}, a combination of markers commonly used to identify EPCs. These observations suggest that ALDH^{br} and CD133^{+}/CD34^{+} EPCs represent populations with substantial overlap, whereas VEGFR-2^{+} cells, including CD34^{+}VEGFR-2^{+} EPCs, identify a distinct progenitor cell population. Indeed, evidence suggests that different but widely published EPC assays identify cell types which fail to correlate with one another and likely represent distinct populations (21,22). Because ALDH activity selects for an early progenitor cell phenotype, and because VEGFR-2 is a marker of mature endothelial cells, the numbers of ALDH^{br} cells may more accurately reflect the levels of progenitors capable of long-term self-replication (11).

We found a close correlation between the frequency of ALDH^{br} cells and patients' age and a stepwise association with CAD severity. These observations suggest that ALDH^{br} cell depletion due to a combination of time and chronic vascular injury may play an important role in the loss of vascular reparative capacity preceding the development of clinically apparent vascular disease.

The identity of circulating ALDH^{br} cells and their ability to contribute to vascular repair is at present not completely known; however, several lines of evidence point to the importance of these cells in vascular health. First, we have shown that bone marrow ALDH^{br} cells are readily cultured into cells expressing multiple endothelial markers and show a greater capability for endothelial cell reconstitution than EPCs identified on the basis of CD133 and CD34 expression, findings recently corroborated by the demonstration that the ALDH^{br} population of bone marrow cells is markedly enriched in EPCs (23). Although bone marrow and unmobilized circulating progenitors may differ, significant evidence suggests that ALDH^{br} cells from a variety of sources, including marrow, umbilical cord blood, and mobilized blood, is highly concentrated in progenitor cells of multiple phenotypes, including endothelial cells (13,16,23,24). Second, the correlation of ALDH^{br} cells with CD133^{+}CD34^{+} cells and the high levels of cross-expression of CD34 and CD133 in the ALDH^{br} cell population suggest that the ALDH assay identifies a population that contains within it a high proportion of cells previously identified as EPCs. Finally, the correlation of ALDH^{br} cells with extent of vascular disease suggests that ALDH activity identifies cells which play an important role in maintaining vascular integrity.

Whether or not enumeration of ALDH^{br} cells in patients more accurately assesses reparative capacity and risk for future cardiovascular events awaits further validation. Ulti-

mately, the utility of enumerating ALDH^{br} EPCs will depend on the association of these cells with clinical outcomes; however, the simplicity and reliability of this technique might allow extension of EPC analysis to a wider range of clinical settings, expanding the contexts in which EPC biology might be explored.

Conclusions

The measurement of ALDH activity in blood MNCs is a promising technique for the accurate, reproducible enumeration of circulating EPCs. Aldehyde dehydrogenase-bright cells can be enumerated with greater simplicity than other EPC types, correlate strongly with some, but not all, previously identified EPCs, and are depleted in elderly patients with coronary disease.

The degree to which enumeration of ALDH^{br} progenitor cells reflects reparative capacity, as well as the association of ALDH^{br} cells with clinical outcomes, will define the usefulness of enumerating these cells for risk stratification of patients as well as further define the value of such cells for treatment of vascular disease.

Acknowledgments

The authors acknowledge Tracy Gentry and Darren Hickerson of Aldagen for help with isolation of sorted ALDH^{br} cells.

Reprint requests and correspondence: Dr. Thomas J. Povsic, Box 3126, Duke University Medical Center, Durham, North Carolina 27710. E-mail: povsi001@mc.duke.edu.

REFERENCES

1. Asahara T, Murohara T, Sullivan A, et al. Isolation of putative progenitor endothelial cells for angiogenesis. Science 1997;275:964–7.
2. Rauscher FM, Goldschmidt-Clermont PJ, Davis BH, et al. Aging, progenitor cell exhaustion, and atherosclerosis. Circulation 2003;108:457–63.
3. Hill JM, Zalos G, Halcox JPJ, et al. Circulating endothelial progenitor cells, vascular function, and cardiovascular risk. N Engl J Med 2003;348:593–600.
4. Vasa M, Fichtlscherer S, Aicher A, et al. Number and migratory activity of circulating endothelial progenitor cells inversely correlate with risk factors for coronary artery disease. Circ Res 2001;89:e1–7.
5. Kunz G, Liang G, Cuculi G, et al. Circulating endothelial progenitor cells predict coronary artery disease severity. Am Heart J 2006;152:190–5.
6. Schmidt-Lucke C, Rossig L, Fichtlscherer S, et al. Reduced number of circulating endothelial progenitor cells predicts future cardiovascular events: proof of concept for the clinical importance of endogenous vascular repair. Circulation 2005;111:2981–7.
7. Werner N, Kosiol S, Schiegl T, et al. Circulating endothelial progenitor cells and cardiovascular outcomes. N Engl J Med 2005;353:999–1007.
8. Guven H, Shepherd RM, Bach RG, Capoccia BJ, Link DC. The number of endothelial progenitor cell colonies in the blood is increased in patients with angiographically significant coronary artery disease. J Am Coll Cardiol 2006;48:1579–87.
9. Hristov M, Fach C, Becker C, et al. Reduced numbers of circulating endothelial progenitor cells in patients with coronary artery disease associated with long-term statin treatment. Atherosclerosis 2007;192:413–20.
10. Colvin OM. Pharmacological purging of bone marrow. In: Thomas ED, Blume KG, Forman SJ, editors. Hematopoietic Cell Transplantation. Malden, MA: Blackwell Sciences, 1999:217–24.
11. Hess DA, Meyerrose TE, Wirthlin L, et al. Functional characterization of highly purified human hematopoietic repopulating cells isolated according to aldehyde dehydrogenase activity. Blood 2004;104:1648–55.
12. Storms RW, Green PD, Safford KM, et al. Distinct hematopoietic progenitor compartments are delineated by the expression of aldehyde dehydrogenase and CD34. Blood 2005;106:95–102.
13. Pearce DJ, Taussig D, Simpson C, et al. Characterization of cells with a high aldehyde dehydrogenase activity from cord blood and acute myeloid leukemia samples. Stem Cells 2005;23:752–60.
14. Cai J, Cheng A, Luo Y, et al. Membrane properties of rat embryonic multipotent neural stem cells. J Neurochem 2004;88:212–26.
15. Corti S, Locatelli F, Papadimitriou D, et al. Identification of a primitive brain-derived neural stem cell population based on aldehyde dehydrogenase activity. Stem Cells 2006;24:975–85.
16. Laird DJ, De Tomaso AW, Weissman IL. Stem cells are units of natural selection in a colonial ascidian. Cell 2005;123:1351–60.
17. Chute JP, Muramoto GG, Whitesides J, et al. Inhibition of aldehyde dehydrogenase and retinoid signaling induces the expansion of human hematopoietic stem cells. Proc Natl Acad Sci U S A 2006;103:11707–12.
18. Califf RM, Harrell FE Jr., Lee KL, et al. The evolution of medical and surgical therapy for coronary artery disease. A 15-year perspective. JAMA 1989;261:2077–86.
19. Mark D, Nelson C, Califf R, et al. Continuing evolution of therapy for coronary artery disease. Initial results from the era of coronary angioplasty. Circulation 1994;89:2015–25.
20. Hess DA, Wirthlin L, Craft TP, et al. Selection based on CD133 and high aldehyde dehydrogenase activity isolates long-term reconstituting human hematopoietic stem cells. Blood 2006;107:2162–9.
21. Powell TM, Paul JD, Hill JM, et al. Granulocyte colony-stimulating factor mobilizes functional endothelial progenitor cells in patients with coronary artery disease. Arterioscler Thromb Vasc Biol 2005;25:296–301.
22. George J, Shmilovich H, Deutsch V, Miller H, Keren G, Roth A. Comparative analysis of methods for assessment of circulating endothelial progenitor cells. Tissue Eng 2006;12:331–5.
23. Gentry T, Foster S, Winstead L, Deibert E, Fiordalisi M, Balber A. Simultaneous isolation of human BM hematopoietic, endothelial and mesenchymal progenitor cells by flow sorting based on aldehyde dehydrogenase activity: implications for cell therapy. Cytotherapy 2007;9:259–74.
24. Corti S, Locatelli F, Papadimitriou D, et al. Transplanted ALDHhiSSClo neural stem cells generate motor neurons and delay disease progression of nmd mice, an animal model of SMARD1. Hum Mol Genet 2006;15:167–87.