Identification and Isolation of Endothelial Cells Based on Their Increased Uptake of Acetylated-Low Density Lipoprotein

JOHN C. VOYTA,* DAVID P. VIA,‡ CATHERINE E. BUTTERFIELD,* and BRUCE R. ZETTER*
*Departments of Surgery and Physiology, The Children's Hospital and Harvard Medical School, Boston, Massachusetts 02115; and ‡Department of Medicine, Baylor College of Medicine, Houston, Texas 77030

ABSTRACT Acetylated-low density lipoprotein (Ac-LDL) is taken up by macrophages and endothelial cells via the "scavenger cell pathway" of LDL metabolism. In this report, aortic and microvascular endothelial cells internalized and degraded 7–15 times more [^{125}I]-Ac-LDL than did smooth muscle cells or pericytes. Bound [^{125}I]-Ac-LDL was displaced by unlabeled Ac-LDL, but not unmodified LDL. The ability to identify endothelial cells based on their increased metabolism of Ac-LDL was examined using Ac-LDL labeled with the fluorescent probe 1,1′-dioctadecyl-3,3,3′,3′-tetramethyl-indocarbocyanine perchlorate (Dil-Ac-LDL). When cells were incubated with 10 μg/ml Dil-Ac-LDL for 4 h at 37°C and subsequently examined by fluorescence microscopy, capillary and aortic endothelial cells were brilliantly fluorescent whereas the fluorescent intensity of retinal pericytes and smooth muscle cells was only slightly above background levels. Dil-Ac-LDL at the concentration used for labeling cells had no effect on endothelial cell growth rate. When primary cultures of bovine adrenal capillary cells were labeled with 10 μg/ml of Dil-Ac-LDL for 4 h at 37°C, then trypsinized and subjected to fluorescence-activated cell sorting, pure cultures of capillary endothelial cells could be obtained. Utilizing this method, large numbers of early passage microvascular endothelial cells can be obtained in significantly less time than with conventional methods.

A major problem in the study of microvascular endothelial cells is the identification of the desired cell population and the subsequent isolation of pure cultures. Our currently used method of establishing pure cultures of capillary endothelial cells involves many weeks of "weeding out" nonendothelial cells (1). The weeding technique involves the assumption that the morphology of capillary endothelial cells is similar to other endothelial cells and is thus directed at isolating colonies with those characteristics. Several markers for endothelial cells are routinely used for confirmation that established cell lines are of endothelial origin. These include the presence of factor VIII related antigen (2, 3) and angiotensin converting enzyme (4, 5). Microvascular endothelial cells differ from large vessel endothelial cells in their requirement for additional growth factors and modified surfaces for optimal growth (1), and their response to tumor factors (1, 6).

The receptor-mediated uptake of low density lipoprotein (LDL[1]) by cells has been studied in detail (for a review see reference 7). An alternative pathway for the metabolism of chemically modified lipoproteins has also been described (8) and has been termed the "scavenger cell pathway" of LDL metabolism, due to its occurrence in rodent and canine macrophages (9–11) and human monocytes (12). Various chemical methods for modification of LDL have been used to modify the charge of amino groups on LDL including acetylation (8), acetoacetylation (9), and malondialdehyde treatment (12). These modified lipoproteins are taken up by

[1] *Abbreviations used in this paper:* Ac, acetylated; BAEC, bovine aortic endothelial cell; BASMC, bovine aortic smooth muscle cell; BCEC, bovine capillary endothelial cell; BRP, bovine retinal pericyte; DiI, 1,1′-dioctadecyl-3,3,3′,3,-tetramethyl-indocarbocyanine perchlorate; DME, Dulbecco's modified Eagle's medium; LDL, low density lipoprotein; VIII R:Ag, factor 8 related antigen.

THE JOURNAL OF CELL BIOLOGY · VOLUME 99 DECEMBER 1984 2034–2040

cells by a receptor (10), which differs from the receptor for unmodified LDL in terms of specificity, regulation of cholesterol levels (10, 13), and biochemical properties[2] (13). In addition to monocytes and macrophages, bovine aortic endothelial cells have been demonstrated to metabolize acetylated LDL, but not normal LDL, at an accelerated rate compared with other cell types (14).

Flow cytometric instrumentation allows one to analyze and sort complex cell populations (for a review see reference 15). Using flow cytometry, it is possible to sort cell mixtures based on the differential labeling of various cell types in the mixture. Cells may be specifically labeled based on the differential expression of antigens or on metabolic differences. Lipoproteins labeled with the fluorescent probe 1,1′-dioctadecyl-3,3,3′,3′-tetramethyl-indocarbocyanine perchlorate (DiI) have been utilized to visualize the uptake of lipoproteins by macrophages and arterial foam cells (16, 17). DiI is a highly lipophilic molecule that can be noncovalently incorporated into lipoproteins and thus has no effect on surface charge. Once a lipoprotein that contains DiI is taken up by a cell, the lipoprotein molecule is acted upon by lysosomal enzymes and the DiI accumulates in lysosomal membranes. Thus, cells that metabolize lipoproteins at different rates will accumulate varying amounts of DiI. In this paper we demonstrate that capillary as well as aortic endothelial cells, but not pericytes or smooth muscle cells possess the scavenger pathway for acetylated-low density lipoprotein (Ac-LDL) metabolism. We further demonstrate that DiI-Ac-LDL can be used to metabolically label endothelial cells thus facilitating their identification by fluorescence microscopy and isolation by fluorescence-activated cell sorting.

MATERIALS AND METHODS

Lipoprotein Isolation and Modification: LDL (d 1.019–1.063 g ml^{-1}) and lipoprotein deficient serum (d 1.21 g ml^{-1}) were obtained from the serum of fasted human donors by sequential ultracentrifugation using standard techniques (18). LDL was acetylated using acetic anhydride as described (10) and then labeled with the fluorescent probe DiI (Molecular Probes, Junction City, OR) as described by the method of Pitas et al. (16). Ac-LDL was iodinated using [^{125}I] (Amersham Corp., Arlington Heights, IL) as described (19). All lipoprotein solutions were exhaustively dialyzed against 0.15 M NaCl, 0.05 M Tris, 0.002 M EDTA, pH 7.4 and filter sterilized prior to use.

Cell Culture: Bovine aortic endothelial cells (BAECs) and bovine aortic smooth muscle cells (BASMCs) were isolated by standard techniques (20, 21). BAECs and BASMCs were grown in Dulbecco's modified Eagle's medium (DME), 10% calf serum, 2 mM glutamine, 100 U ml^{-1} penicillin, and 100 U ml^{-1} streptomycin. Bovine capillary endothelial cell (BCECs) from bovine adrenals were isolated by the technique of Folkman et al. (1) and by the cell sorting method presented in this paper. Bovine retinal pericyte (BRPs) were provided by Dr. Patricia D'Amore (Department of Surgical Research, Children's Hospital, Boston, MA)

Primary cultures of BCECs were prepared for cell sorting using a modification of the method described by Folkman et al. (1). The adrenal cortex from calves were minced and treated with 0.75% collagenase (Worthington, Malvern, PA), 0.5% bovine serum albumin in phosphate buffered saline, pH 7.0 for 15 min at room temperature while shaking. The digest was filtered through a 110-μm Nytex filter, centrifuged at 900 rpm for 10 min at room temperature, washed once with DME containing 10% calf serum, and plated onto gelatin coated plates (1) in BCEC growth media. BCEC growth media was 45% DME, 45% mouse sarcoma conditioned DME, 10% calf serum and 100 μg ml^{-1} heparin (Hepar, Franklin, OH), 2 mM glutamine, 100 U ml^{-1} penicillin, and 100 U ml^{-1} streptomycin. Heparin was added to the BCEC media to suppress the proliferation of nonendothelial cell types[3] (22).

The growth rate of endothelial cells was assessed as follows: confluent cells were trypsinized and plated in 24 well plates (10,000 per well) in 0.5 ml media. BAECs were grown in DME, 10% calf serum. BCECs were grown in the BCEC growth media detailed above. The cells were trypsinized at the intervals indicated in the figure and counted using a Coulter Counter.

Metabolism of [^{125}I]-Ac-LDL by Vascular Cells: Cultures of BAECs, BCECs, BASMCs, and BRPs were plated into 35-mm dishes and allowed to grow to the densities indicated in the legend of Fig. 1. The media was removed and [^{125}I]-Ac-LDL was added to the cells in DME containing 10% lipoprotein deficient serum and incubated for 4 h at 37°C. The internalization and degradation of [^{125}I]-Ac-LDL was measured as described by Craig et al. (19).

Fluorescence Microscopy: For fluorescence microscopy, cells were grown on glass coverslips or on the plastic surface of 35-mm tissue culture dishes. Cells were incubated with 10 μg ml^{-1} DiI-Ac-LDL at 37°C in normal media for 4 h. The media was then removed and the cells were washed once with probe-free media for 10 min, rinsed with PBS, and then fixed with 10% buffered formalin phosphate (Fisher, Pittsburgh, PA) for 5 min. Coverslips were inverted over a drop of 10% PBS in glycerol prior to viewing. 1 ml of 10% PBS in glycerol was added to the 35-mm dishes prior to viewing.

The presence of factor VIII related antigen (VIII R:Ag) in endothelial cells was demonstrated using commercially available rabbit anti-human factor VIII (Calbiochem Corp., La Jolla, CA). Cells were first fixed with −20°C methanol for 5 min, then incubated with the first antibody (1-100 dilution of anti-factor VIII R:Ag antibody) and then a 1:500 dilution of rhodamine-labeled goat anti-rabbit antibody (Capell Laboratories, Cochranville, PA).

Cells were examined with a Zeiss Photoscope II using a 25 X Zeiss Plan-Neofluor objective. DiI was visualized using the standard rhodamine excitation/emission filter combinations. Tri-X Pan film and an exposure setting of ASA 1600 was used.

Cell Sorting: Prior to cell sorting, cells were labeled with 10 μg ml^{-1} DiI-Ac-LDL for 4 h at 37°C. The labeled cells were washed once with PBS and then trypsinized to produce single cell suspensions. The trypsin was neutralized by washing the cells once in DME containing 10% calf serum. Prior to sorting, the cells were resuspended in serum-free DME. Cells and collection tubes were kept chilled on ice prior to and during the sorting procedure.

DiI-Ac-LDL-labeled endothelial cells were sorted from other cell types using a Becton-Dickson FACS IV cell sorter (Mountain View, CA). The 514-nm line of an argon laser was used for excitation. The fluorescence emission above 550 nm was collected. Sampling gates were set using stained BAECs and BASMCs as "positive" and "negative" controls. The scatter gates were set to minimize the contribution of cell pairs and the fluorescence gates were set to eliminate the more highly fluorescent macrophages. Cells were collected into tubes containing 10% calf serum. After the sort was complete, the cells were pelleted, washed once, and plated at various dilutions in gelatin-coated 24 well plates in BCEC growth media. All cell sorting was performed in the cell sorting facilities of the Department of Genetics, Children's Hospital (Boston, MA) under the direction of Dr. Marc Lalande and Mr. Robert Hoffman.

RESULTS

The presence of a scavenger pathway of lipoprotein metabolism in capillary and aortic endothelial cells was examined by incubating cultures with [^{125}I]-Ac-LDL. As shown in Fig. 1 *a*, BCECs and BAECs degraded Ac-LDL at a rate 7–15 times higher than both smooth muscle cells and pericytes. Confluent BAECs degraded [^{125}I]-Ac-LDL at a higher rate than preconfluent BAECs as expected from previous studies (14). BCECs were examined only at one density. The specificity of lipoprotein degradation was examined by conducting incubations in the presence of a 20-fold excess of unlabeled LDL or Ac-LDL (Fig. 1 *b*). Ac-LDL but not LDL reduced lipoprotein metabolism by >90%.

To visualize the interaction of Ac-LDL with endothelial cells, cultures were incubated with DiI-Ac-LDL and examined by fluorescence microscopy. Pure cultures of both BCECs and BAECs were brightly stained (Fig. 2). The fluorescence was predominantly punctate with a perinuclear distribution. Low background fluorescence was observed in cultures of smooth muscle cells or pericytes. In mixed cultures of endothelial cells and other cells, endothelial cells were easily distinguished (Fig. 3). When primary cultures of BCECs were stained with DiI-Ac-LDL and examined, colonies of endothelial cells were

[2] Via, D. P., H. A. Dresel, and A. M. Gotto, Jr., submitted for publication.

[3] Orlidge, A., and P. D'Amore, manuscript in preparation.

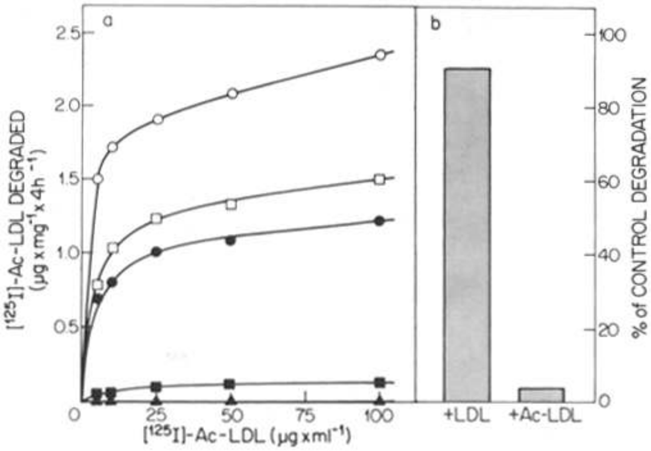

FIGURE 1 Metabolism of $[^{125}I]$-Ac-LDL by vascular cells. (*a*) $[^{125}I]$-Ac-LDL was incubated with BAECs (○, ●), BCECs (□), BASMCs (■), and BRPs (▲) for 4 h at 37°C. The media was processed as described in Materials and Methods. The results obtained from the average of triplicate incubations for free $[^{125}I]$ in the media is plotted. The number of cells per 35-mm dish were: confluent BAECs, 5.7×10^5 (○); subconfluent BAECs, 3×10^5 (●); BCECs, 4.9×10^5 (□); BASMCs, 2.8×10^5 (■); BRPs, 5.5×10^4 (▲). (*b*) Confluent BAECs were incubated with 20 μg ml^{-1} $[^{125}I]$-Ac-LDL in the presence of 400 μg ml^{-1} unlabeled LDL or Ac-LDL. As shown, Ac-LDL, but not LDL, competes for the $[^{125}I]$-Ac-LDL binding. This demonstrates the specificity of the receptor.

brightly fluorescent, while all other cell types, except macrophages, exhibited varying levels of background uptake. Macrophages, when present could be easily distinguished from endothelial cells by their shape, granular contents, and much greater fluorescent intensity.

The effect of DiI-Ac-LDL uptake on endothelial cell growth is shown in Fig. 4. No inhibition of cell growth was observed over a period of 7 d when BAECs were labeled with DiI-Ac-LDL prior to trypsinization and plating for the growth curve. Thus the fluorescent probe DiI that accumulates in the cells, is not cytotoxic.

After staining with DiI-Ac-LDL, cultures of cells were analyzed and sorted using a FACS IV cell sorter. As expected, a strong signal was obtained with BAECs while BASMCs gave a weaker fluorescence intensity (Fig. 5 *a*). The data from these analyses were used to set scatter and fluorescence sampling gates for analysis and sorting of the primary culture. The profile of an 8-d old primary culture from a collagenase digest of bovine adrenal cortex labeled with DiI-Ac-LDL is seen in Fig. 5 *b*. Quantitation of the profile indicates that the DiI-Ac-LDL-labeled endothelial cells represented ~7% of this cell mixture. The endothelial cells were separated from those cells exhibiting lower levels of fluorescence by sorting under sterile conditions and were subsequently grown in culture (BCEC-S1s). Initially these cultures were ~95% endothelial cells based on visual examination. Allowing the cells to grow in culture for 2 wk at this point resulted in higher numbers of cells for sorting. The disadvantage to the 2 wk of growth was that the nonendothelial cell types appeared to proliferate at a higher rate. After two weeks, the cultures were again stained with DiI-Ac-LDL and resorted. The cultures from the second sort were completely free of nonendothelial cell types (BCEC-S2s). To obtain sufficient numbers of the desired cell type for plating and growth within 2 wk, ~5×10^6 cells of the primary culture were sorted. For subsequent sorts, 1×10^6 BCEC-S1s and 5×10^5 BCEC-S2s were used. The percent endothelial cells in each preparation at the time of the sort is summarized in Table I.

The cultures obtained from the second sort (BCEC-S2s) were grown in wells of 24 well plates until confluent and then expanded for growth and marker antigen analysis. The BCEC-S2s had a doubling time of 4.2 d as compared with 3.7 d for BCECs isolated by the "weeding and feeding" protocol. Fig. 6 shows micrographs of these cells. As shown, all of the cells take up high amounts of DiI-Ac-LDL and are positive for the presence of factor VIII R:Ag. DiI-Ac-LDL consistently labeled endothelial cells much more uniformly than did antibodies against factor VIII R:Ag.

DISCUSSION

In this report, we have demonstrated that capillary endothelial cells degrade iodinated Ac-LDL at a much higher rate than other microvascular cells. Capillary endothelial cells metabolize Ac-LDL at the same high rate as shown previously for aortic endothelial cells (14). Microvascular pericytes degrade much lower levels, in common with those shown for aortic smooth muscle cells (14).

On the basis of this differential uptake of iodinated Ac-LDL, we have used fluorescent-labeled Ac-LDL to metabolically label capillary endothelial cells, facilitating both their identication and isolation. When primary cultures derived from bovine adrenocorticoid microvessels were labeled with DiI-Ac-LDL, only the endothelial cells were labeled. We have successfully used this labeling procedure in conjunction with fluorescence activated cell sorting, to isolate pure capillary endothelial cells from mixed primary cultures derived from bovine adrenal cortex.

The fluorescent probe used, DiI, was very well suited for these studies. Due to its lipophilic nature, once the DiI-Ac-LDL enters the cell and is degraded by lysosomes, it accumulates in the lysosomal membranes (16). The emission spectrum of this fluorescent probe does not overlap with cell autofluorescence and is readily visualized with standard filter sets for rhodamine fluorescence. Furthermore, we have demonstrated that DiI-Ac-LDL has no inhibitory effect on the growth rate of cells that internalize it.

Labeling endothelial cells by the method presented here has numerous advantages over other previously reported endothelial cell specific markers such as anti-factor VIII R:Ag (2, 3) and anti-angiotensin converting enzymes (4, 5). The primary advantage is that the method is highly reproducible and all endothelial cells are labeled. Also, the labeling is performed on live cells with no fixation or permeabilization required for optimal labeling. Furthermore, because the probe is incorporated into lysosomal membranes, the label is not removed from the cells during trypsinization.

Isolating endothelial cells by cell sorting has distinct advantages over other isolation techniques. The technique is fast compared with established procedures. After two sorts and an elapsed time of 4–5 wk we obtained pure microvascular endothelial cell populations from primary cultures that initially contained <10% endothelial cells. In contrast, earlier methods (1) required 3–4 mo to obtain pure cultures. When primary cultures contain a lower percentage of endothelial cells, additional sorts at 2-wk intervals should allow pure endothelial cell cultures to be rapidly obtained. Using this technology, no visual biases such as morphology or growth characteristics are involved in the selection process. Another

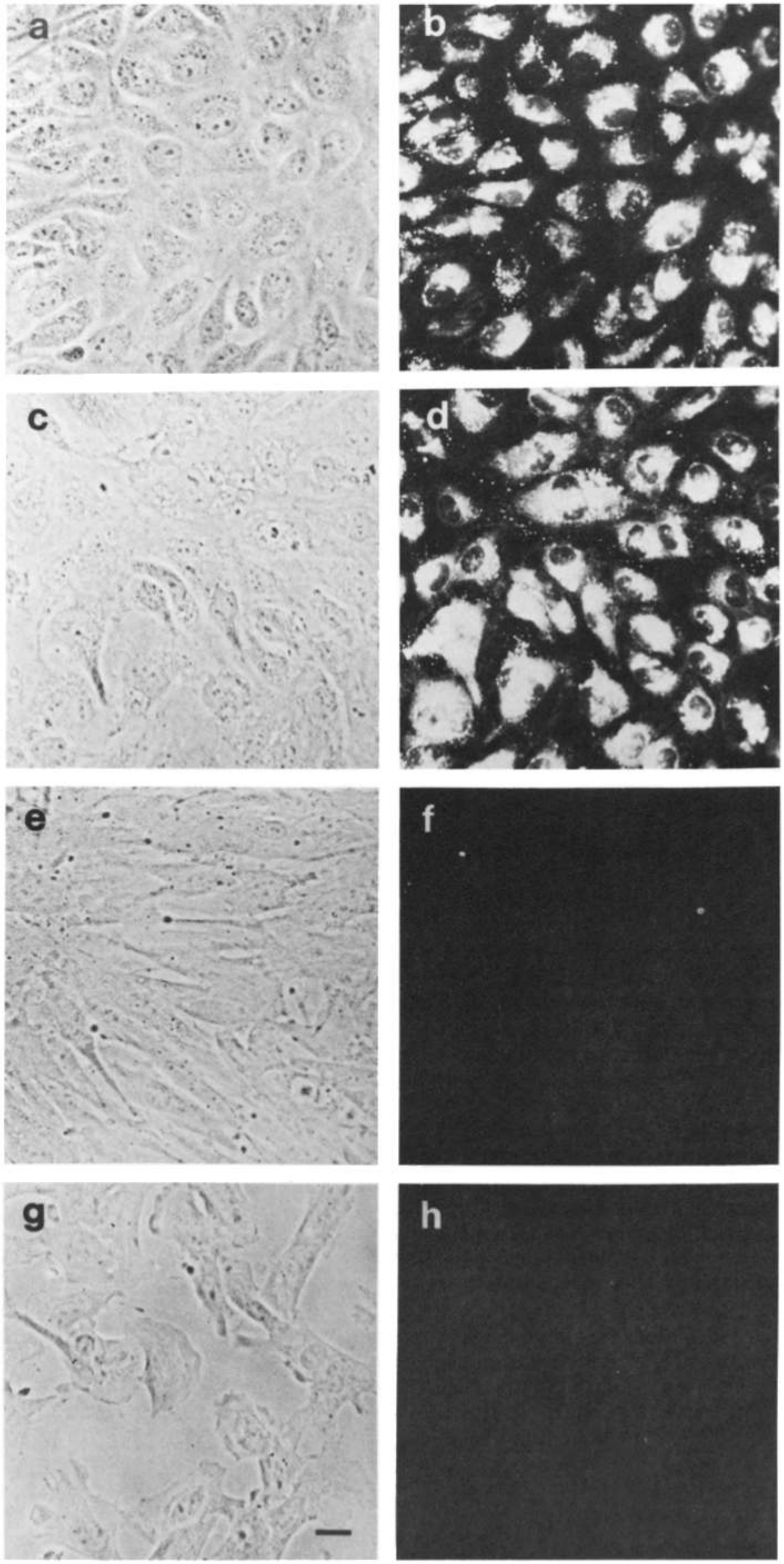

FIGURE 2 Labeling of vascular cells with DiI-Ac-LDL. Phase-contrast (*a*, *c*, *e*, *g*) and fluorescence (*b*, *d*, *f*, *h*) micrographs of BAECs (*a* and *b*), BCECs (*c* and *d*), BASMCs (*e* and *f*), and BRPs (*g* and *h*) that have been incubated with 10 $\mu g\ ml^{-1}$ DiI-Ac-LDL for 4 h at 37°C. The cells were visualized using a standard rhodamine excitation:emission filter set. Note the abundance of punctate perinuclear fluorescence in the BAECs (*b*) and BCECs (*d*). Only background fluorescence is observed in the BASMCs (*f*) and BRPs (*h*). Bar, 20 μm. × 250.

advantage is that if desired, a heterogeneous population of endothelial cells derived from multiple microvessels can be obtained for further studies. The cells obtained may be cloned if desired by flow cytometry and single cell deposition or by serial dilution.

Auerbach et al. (23) first demonstrated the feasibility of using a cell sorter to separate endothelial cells from other cell types. They utilized a monoclonal antibody against the cell surface marker, angiotensin converting enzyme to differentiate endothelial cells from other cell types. This method had limitations imposed by the facts that angiotensin converting enzyme is not expressed until after cells reach confluence (24) and their monoclonal antibody bound to some nonendothelial cell types with Fc receptors. In contrast, endothelial cells at all densities degrade Ac-LDL at elevated rates compared with other cell types.

DiI-Ac-LDL is potentially useful in other situations where endothelial cell identification is desired. Ac-LDL has been shown to be quickly cleared by endothelial cells of the liver following injection into mice (25, 26). We have used DiI-Ac-LDL as an aid in histological identification of endothelial cells in frozen sections of murine liver following intravenous

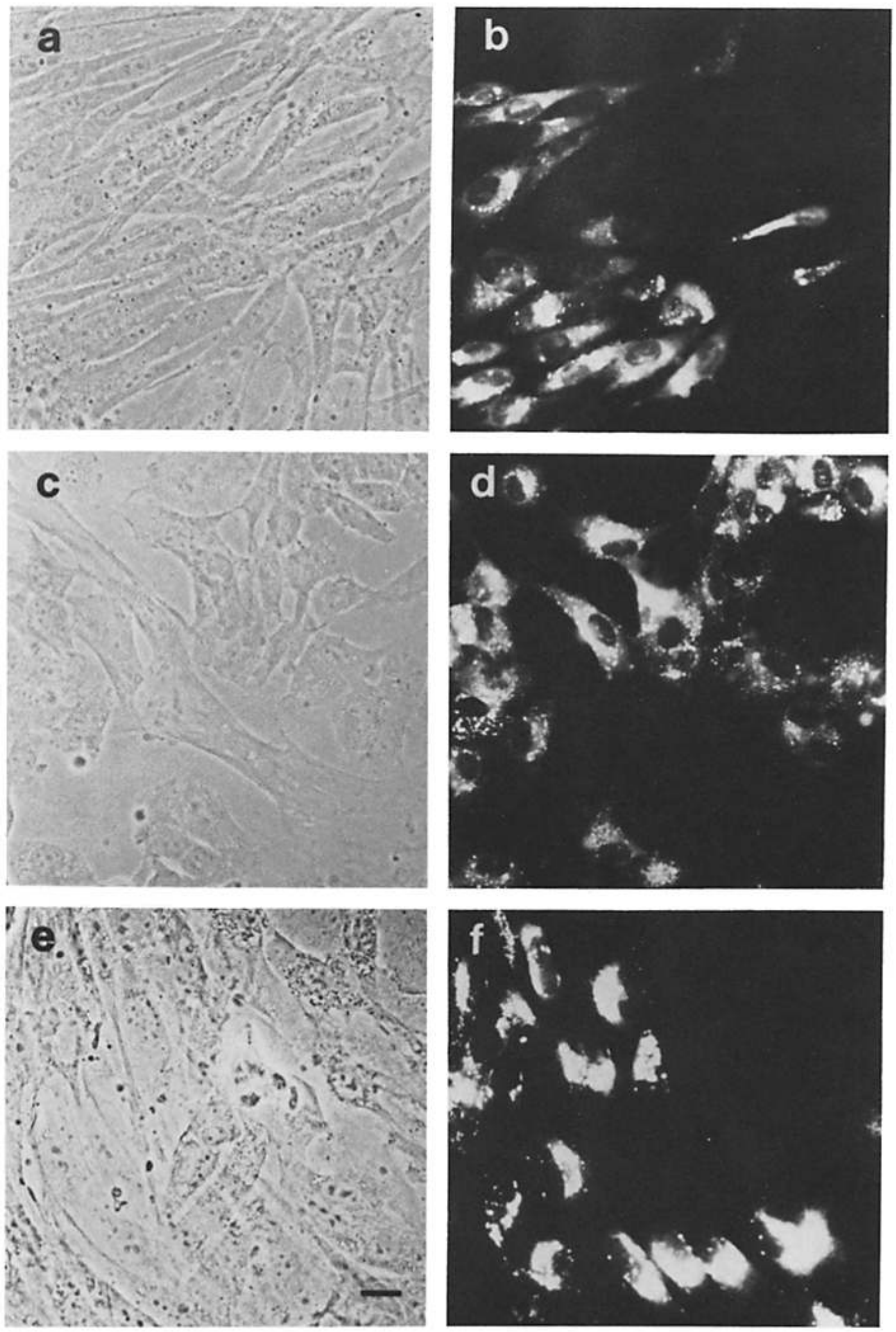

FIGURE 3 Visualization of endothelial cells in mixed cultures after labeling with DiI-Ac-LDL. Phase-contrast (*a, c, e*) and fluorescence (*b, d, f*) micrographs of mixed cultures of endothelial cells and other vascular cells that have been incubated with 10μg ml^{-1} DiI-Ac-LDL for 4 h at 37°C. Mixed cultures of BAECs and BASMCs (*a* and *b*), and BAECs and BRPs (*c* and *d*) are shown. *e* and *f* are of a primary adrenocorticoid microvessel culture. In all cases, only cells that appear to be endothelial are labeled with the DiI-Ac-LDL. Bar, 20 μm. × 250.

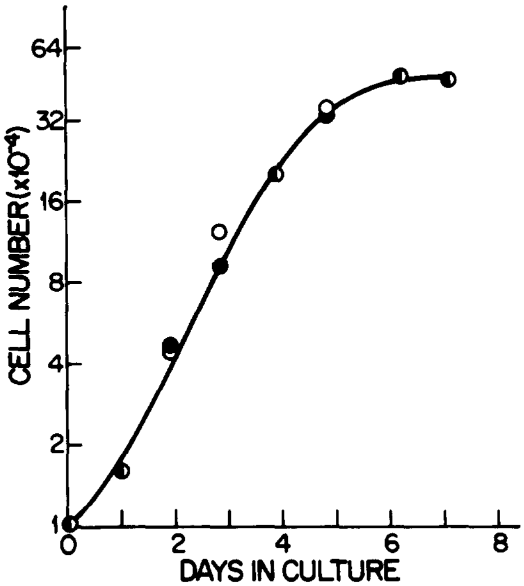

FIGURE 4 Labeling cells with Dil-Ac-LDL has no effect on endothelial cell viability. Confluent BAECs (P-8) were incubated with (●) and without (○) 20 μg ml^{-1} in DME, 10% calf serum for 4 h at 37°C. They were then trypsinized and plated for a growth curve as described in Materials and Methods. The averages of quadruplicate wells are plotted.

TABLE I

Cell Sorter Analysis of Various Dil-Ac-LDL Labeled Cultures

Cell preparation	Endothelial	Other*
	%	%
BAEC	100	0
BASMC	0	100
Primary culture (8 d)‡	7	93
BCEC-S1 (22 d)§	35	65
BCEC-S2	100‖	0

* Corresponds to all cells that take up only background levels of Dil-Ac-LDL.
‡ The cells were sorted 8 d after plating the collagenase digest.
§ The cells obtained from sorting the primary culture (BCEC-Sls) were allowed to grow for 2 wk. They were then labeled with Dil-Ac-LDL and resorted.
‖ This value was obtained by visual examination of Dil-Ac-LDL labeled cultures.

injection of the lipoprotein. We have also used the fluorescent lipoprotein to aid in identification of capillary fragments in collagenase-digested tissues. Preliminary studies indicate that DiI-Ac-LDL can be used to label capillary endothelial cells in the chick chorioallantoic membrane.[4] The techniques reported here could also be used to quickly obtain endothelial cells in cases where large numbers of endothelial cells are needed in short times, such as to serve as a lining for vascular grafts (27, 28).

In summary, we have demonstrated that labeling endothelial cells with DiI-Ac-LDL is an improved method for specifically visualizing endothelial cells without affecting cell viability. Utilizing this labeling procedure in conjunction with cell sorting technology, we have increased the speed and efficiency of techniques used for the isolation of a pure cultures of microvascular endothelial cells.

[4] Netland, P. A., J. C. Voyta, and B. R. Zetter, unpublished results.

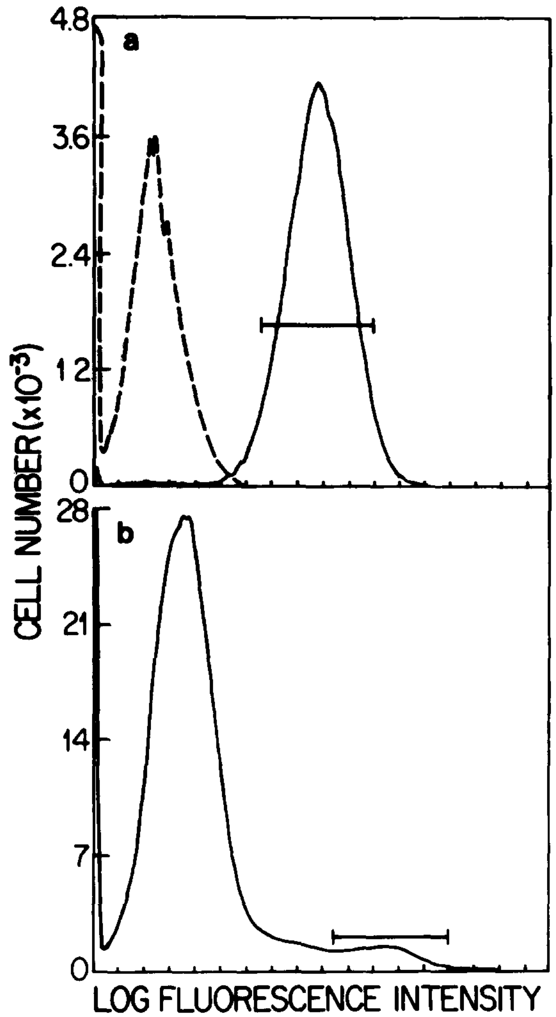

FIGURE 5 Cell sorting of bovine adrenal capillary endothelial cells. Pure established cultures of BAECs and BASMCs were labeled with 10 μg ml^{-1} Dil-Ac-LDL for 4 h at 37°C. The cells were then trypsinized to produce a single cell suspension and subjected to cell sorter analysis. The analysis of pure cultures of BAECs (——) and BASMCs (- - - -) are superimposed in *a*. *b* illustrates the analysis of a labeled primary adrenocorticoid microvessel culture similar to that shown in Fig. 3, e and *f*. The brightly fluorescent endothelial cells indicated by the bar were sorted from the less fluorescent cells and grown in culture. These cells correspond to the BCEC-S1s in Table I.

The authors thank Dr. Judah Folkman for his continuous support and encouragement throughout these studies and Dr. Patricia D'Amore for her critical review of the manuscript. We also acknowledge Dr. Marc Lalande and Mr. Robert Hoffman for their assistance with the cell sorting and Dr. Alicia Orlidge for suggesting that we add heparin to our primary cultures. Bonnie Troped provided expert assistance with preparation of the manuscript and Annette Fanslow provided expert technical assistance.

Funding was provided by grants from the Texas Heart Association (D. P. Via), the National Cancer Institute (B. R. Zetter) (R01 CA37393), and the Monsanto Corporation. B. R. Zetter is the recipient of a Faculty Research Award (#263) from the American Cancer Society.

Received for publication 29 May 1984, and in revised form 1 August 1984.

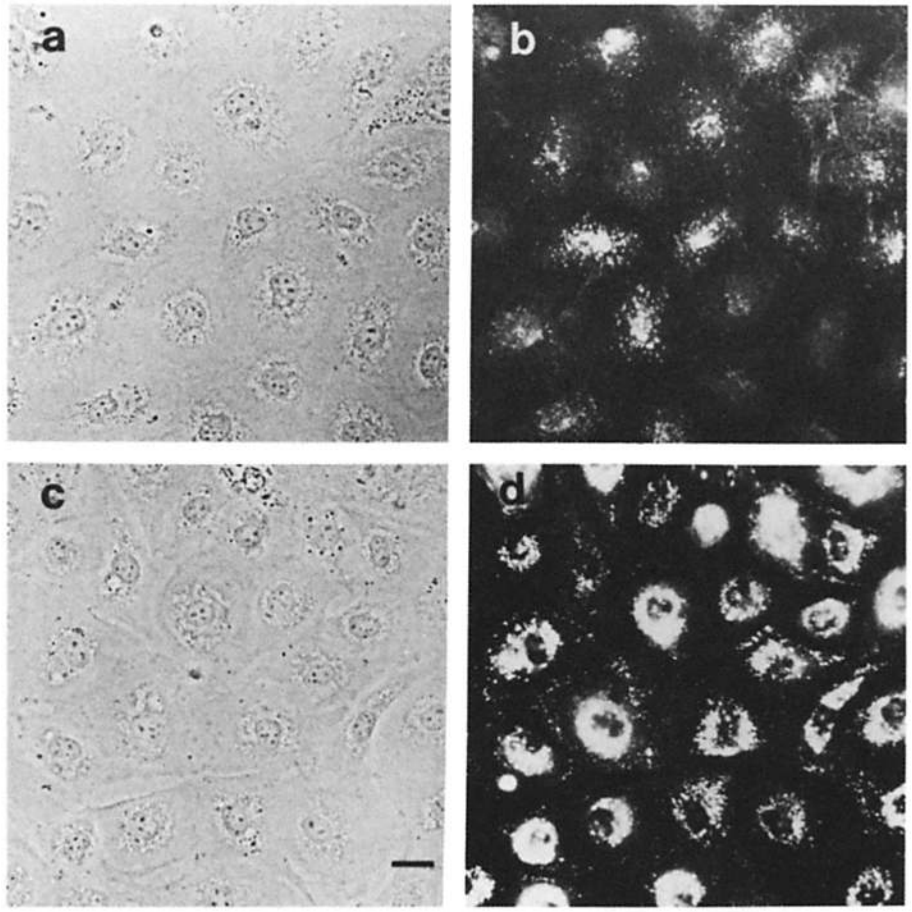

FIGURE 6 Factor VIII R:Ag and DiI-Ac-LDL staining of endothelial cells isolated by cell sorting. Phase-contrast (*a* and *c*) and fluorescence (*b* and *d*) micrographs of BCEC-S2s labeled with rabbit anti-human factor VIII R:Ag (*a* and *b*) and DiI-Ac-LDL (*c* and *d*) as described in Materials and Methods. These cells were isolated by cell sorting on the basis of their elevated metabolism of DiI-Ac-LDL. The labeling pattern with DiI-Ac-LDL appears to be more uniform than that observed with anti-factor VIII R:Ag. Bar, 20 μm. × 250.

REFERENCES

1. Folkman, J., C. C. Haudenschild, and B. R. Zetter. 1979. Long-term culture of capillary endothelial cells. *Proc. Natl. Acad. Sci. USA.* 76:5217–5221.
2. Hoyer, L. W., R. P. de los Santos, and J. R. Hoyer. 1973. Antihemophilic factor antigen: localization in endothelial cells by immunofluorescent microscopy. *J. Clin. Invest.* 52:2737–2744.
3. Jaffe, E. A., L. W. Hoyer, and R. L. Nachman. 1973. Synthesis of antihemophilic factor antigen by cultured human endothelial cells. *J. Clin. Invest.* 52:2757–2764.
4. Ryan, U. S., J. W. Ryan, C. Whitaker, and A. Chiu. 1976. Localization of angiotensin converting enzyme (kininase II). II. Immunocytochemistry and immunofluorescence. *Tissue Cell.* 8:125–145.
5. Johnson, A. R., and E. G. Erdos. 1977. Metabolism of vasoactive peptides by human endothelial cells in culture. Angiotensin I converting enzyme (kinnase II) and angiotensinase. *J. Clin. Invest.* 59:684–695.
6. Zetter, B. R. 1980. Migration of capillary endothelial cells is stimulated by tumor-derived factors. *Nature (Lond.).* 285:41–43.
7. Goldstein, J. L., and M. S. Brown. 1977. Atherosclerosis: The low-density lipoprotein receptor hypothesis. *Metabolism.* 26:1257–1275.
8. Basu, S. K., J. L. Goldstein, R. G. W. Anderson, and M. S. Brown. 1976. Degradation of cationized low density lipoprotein and regulation of cholesterol metabolism in homozygous familial hypercholesterolemia fibroblasts. *Proc. Natl. Acad. Sci. USA.* 73:3178–3182.
9. Mahley, R. W., T. L. Innerarity, K. H. Weisgraber, and S. Y. Oh. 1979. Altered metabolism (*in vivo* and *in vitro*) of plasma lipoproteins after selective chemical modification of lysine residues of the lipoprotein. *J. Clin. Invest.* 64:743–750.
10. Goldstein, J. L., Y. K. Ho, S. K. Basu, and M. S. Brown. 1979. Binding site on macrophages that mediates uptake and degradation of acetylated low density lipoprotein, producing massive cholesterol deposition. *Proc. Natl. Acad. Sci. USA.* 76:333–337.
11. Brown, M. S., S. K. Basu, J. R. Falck, Y. K. Ho, and J. L. Goldstein. 1980. The scavenger cell pathway for lipoprotein degradation: Specificity of the binding site that mediates the uptake of negatively-charged LDL by macrophages. *J. Supramol. Struct.* 15:67–81.
12. Fogelman, A. M., I. Schechter, J. Seager, M. Hokom, J. S. Child, and P. A. Edwards. 1980. Malondialdehyde alteration of low density lipoproteins leads to chosterylester accumulation in human monocyte-macrophages. *Proc. Natl. Acad. Sci. USA.* 77:2214–2218.
13. Via, D. P., H. A. Dresel, and A. M. Gotto, Jr. 1982. Isolation and characterization of the murine macrophage acetyl-LDL receptor. *Arteriosclerosis.* 2:414*a*. (Abstr.)
14. Stein, O., and Y. Stein. 1980. Bovine aortic endothelial cells display macrophage-like properties towards acetylated [^{125}I]-labelled low density lipoprotein. *Biochim. Biophys. Acta.* 620:631–635.
15. Shapiro, H. M. 1983. Multistation multiparameter flow cytometry: a critical review and rationale. *Cytometry.* 3:227–243.
16. Pitas, R. E., T. L. Innerarity, J. N. Weinstein, and R. W. Mahley. 1981. Acetoacetylated lipoproteins used to distinguish fibroblasts from macrophages in vitro by fluorescence microscopy. *Arteriosclerosis.* 1:177–185.
17. Pitas, R. E., T. L. Innerarity, and R. W. Mahley. 1983. Foam cells in explants of atherosclerotic rabbit aortas have receptors for β-very low density lipoproteins and modified low density lipoproteins. *Arteriosclerosis.* 3:2–12.
18. Havel, R. J., H. A. Eder, and J. G. Bragdon. 1955. The distribution and chemical composition of ultracentrifugally separated lipoproteins in human serum. *J. Clin. Invest.* 34:1345–1353.
19. Craig, I. F., D. P. Via, B. C. Sherrill, L. A. Sklar, A. M. Gotto, Jr., and L. C. Smith. 1982. Incorporation of defined cholesteryl esters into lipoproteins using cholesteryl ester-rich microemulsions. *J. Biol. Chem.* 257:330–335.
20. Coughlin, S. R., M. A. Moskowitz, B. R. Zetter, H. N. Antoniades, and L. Levine. 1980. Platelet-dependent stimulation of prostacyclin synthesis by platelet-derived growth factor. *Nature (Lond.).* 288:600–602.
21. Ross, R. 1971. The smooth muscle cell. II. Growth of smooth muscle in culture and formation of elastic fibers. *J. Cell Biol.* 50:172–186.
22. Castellot, J. J., Jr., M. L. Addonizio, R. Rosenberg, and M. J. Karnofsky. 1981. Cultured endothelial cells produce a heparinlike inhibitor of smooth muscle cell growth. *J. Cell Biol.* 90:372–379.
23. Auerbach, R., L. Alby, J. Grieves, J. Joseph, C. Lindgren, L. W. Morrissey, Y. A. Sidky, M. Tu, and S. L. Watt. 1982. Monoclonal antibody against angiotensin-converting enzyme: its use as a marker for murine, bovine and human endothelial cells. *Proc. Natl. Acad. Sci. USA.* 79:7891–7895.
24. DelVecchio, P. J., and J. R. Smith. 1981. Expression of angiotensin-converting enzyme activity in cultured pulmonary artery endothelial cells. *J. Cell. Physiol.* 108:337–345.
25. Nagelkerke, J. F., K. P. Barto, and T. J. C. van Berkel. 1983. *In vivo* and *in vitro* uptake and degradation of acetylated low density lipoprotein by rat liver endothelial, Kupffer, and parenchymal cells. *J. Biol. Chem.* 258:12221–12227.
26. Pitas, R. E., J. Boyles, R. W. Mahley, and D. M. Bissell. 1983. Sinusoidal endothelial cells mediate the rapid clearance of acetoacetylated low density lipoproteins by rat liver. *Fed. Proc.* 42:1819*a* (Abstr.).
27. Herring, M. B., R. Dilley, R. A. Jersild, L. Boxer, A. Gardner, and J. Glover. 1979. Seeding arterial prostheses with vascular endothelium. The nature of the lining. *Ann. Surg.* 190:84–90.
28. Stanley, J. C. 1980. Endothelial cell seeding of prosthetic vascular grafts: early experimental studies with autologous canine endothelium. *Arch. Surg.* 115:929–933.