HMEC-1: Establishment of an Immortalized Human Microvascular Endothelial Cell Line

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The study of human microvascular endothelial cells has been limited, because these cells are difficult to isolate in pure culture, are fastidious in their *in vitro* growth requirements, and have a very limited lifespan. In order to overcome these difficulties, we have transfected human dermal microvascular endothelial cells (HMEC) with a PBR-322–based plasmid containing the coding region for the simian virus 40 A gene product, large T antigen, and succeeded in immortalizing them. These cells, termed CDC/EU.HMEC-1 (HMEC-1), have been passaged 95 times to date and show no signs of senescence, whereas normal microvascular endothelial cells undergo senescence at passages 8–10. HMEC-1 exhibit typical cobblestone morphology when grown in monolayer culture, express and secrete von Willebrand's Factor, take up acteylated low-density lipoprotein, and rapidly form tubes when cultured on matrigel. HMEC-1 grow to densities three to seven times higher than microvascular endothelial cells and require much less stringent growth medium. HMEC-1 will grow in the absence of human serum, whereas microvascular endothelial cells require culture medium supplemented with 30% human serum. These cells express other cell-surface molecules typically associated with endothelial cells, including CD31 and CD36 and epitopes identified by monoclonal antibodies EN4 and PAL-E. They also express the cell adhesion molecules ICAM-1 and CD44 and following stimulation with interferon-γ express major histocompatibility complex class II antigens. HMEC-1 specifically bind lymphocytes in cell adhesion assays. Thus HMEC-1 is the first immortalized human microvascular endothelial cell line that retains the morphologic, phenotypic, and functional characteristics of normal human microvascular endothelial cells. *J Invest Dermatol 99:683–690, 1992*

In recent years it has become apparent that endothelial cells play critical roles in a large number of physiologic and pathophysiologic processes such as leukocyte trafficking, inflammation, wound healing, tumor metastasis, and angiogenesis [1–10]. Much of our understanding of the role of endothelial cells in these events stems from our ability to culture endothelial cells and grow large numbers of them in pure culture. Until now, almost all *in vitro* studies of human endothelial cells have relied on cells derived from human umbilical veins (HUVEC), because they are relatively easily isolated and cultured. However, most pathophysiologic events take place at the level of the microvasculature, which constitutes the vast majority of the human vascular compartment. In addition, it has become increasingly recognized that not all endothelial cells are alike, and that endothelial cells derived from the microvascular structures of specific tissues differ significantly from large-vessel endothelial cells [4,11–15]. Important differences between microvascular endothelial cells and large-vessel endothelial cells include 1) microvascular endothelial cells are much more fastidious in their *in vitro* culture requirements than large-vessel endothelial cells, indicating that significant biologic differences in both level of differentiation and function may exist [4,12,16,17]; 2) accordingly, microvascular endothelial cells undergo morphologic differentiation into capillary-like structures *in vitro* much more rapidly and readily than do large-vessel endothelial cells [4,18]; 3) microvascular endothelial cells have a different prostaglandin secretory profile from that of large-vessel endothelial cells [11]; 4) there are differences in the types and amounts of cell adhesion molecules expressed by microvascular and large-vessel endothelial cells [13,19,20,21]; and 5) at a functional level, the "homing" of leukocytes to specific lymphoid and non-lymphoid tissues occurs exclusively within the microvascular compartment and is mediated via differential and sequential expression of specific cell adhesion molecules that appear to be regulated in a tissue-specific fashion [1,22]. These and a number of other observations pointing to the heterogeneity of human endothelial cells have resulted in an increasing interest in the biology of microvascular endothelial cells. However, progress in this area has been substantially hampered by the extreme difficulty in isolating and culturing large numbers of pure microvascular endothelial cells from any organ. Thus, it would be ideal if one or more immortalized human microvascular endothelial cell lines were available for study. Herein, we report the transfection and immortalization of human dermal microvas-

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Abbreviations:
ELISA: enzyme-linked immunosorbent assay
FITC: fluorescein isothiocyanate
HMEC-1: human dermal microvascular endothelial cells
HUVEC: human umbilical vein endothelial cells
ICAM: intercellular adhesion molecule-1
LDL: low-density lipoprotein
MoAb: Monoclonal antibody
PBS: phosphate-buffered saline
TNFα: tumor necrosis factor-alpha
vWF: von Willebrand's factor

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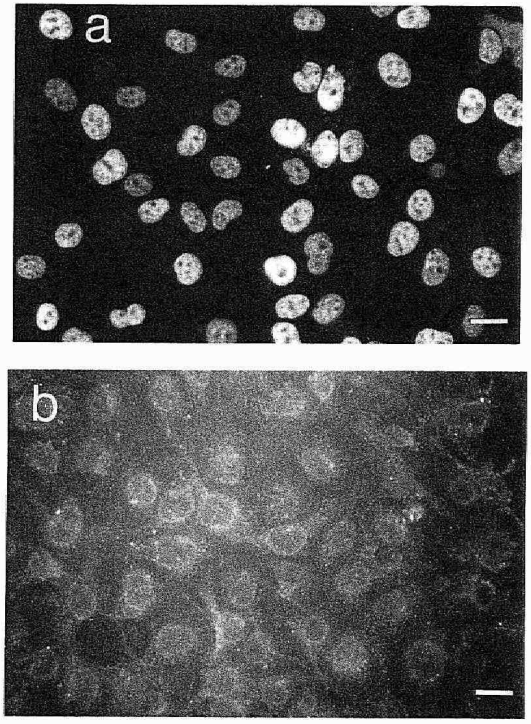

Figure 1. Direct immunofluorescence of cultured endothelial cells demonstrates the presence of SV40 large T antigen in the nuclei of HMEC-1 *(a)* but not in non-transfected microvascular endothelial cells *(b)*. Bar, 22 μm.

cular endothelial cells by simian virus 40 large T antigen and demonstrate that these cells retain the characteristics of endothelial cells.

MATERIALS AND METHODS

Isolation and Culture of Human Dermal Microvascular Endothelial Cells Human microvascular endothelial cells were isolated from six human foreskins as previously described [4].

Transfection of Human Microvascular Endothelial Cells A vector designated as pSVT was used. pSVT is a PBR322-based construct containing the sequences encoding the transforming protein SV40 large T, and its expression is driven by the Rous Sarcoma Virus long terminal repeat. pSVT was supplied by A. Srinivasan (Wistar Institute, Philadelphia, PA) [23,24]. Microvascular endothelial cells in passage 6 were used for transfection. In a modification of a standard technique [25], endothelial cells pooled from six donors were plated in 6-well culture dishes (Costar, Cambridge, MA) at 3.5×10^5 cells per well (approximately 50% confluent) and incubated overnight at 37°C with 5% CO_2. Five micrograms of pSVT DNA were added per well. The cells were incubated overnight at 37°C with 5% CO_2, washed, and cells from each well were transferred to individual 25-cm^2 culture flasks and observed for growth.

Karyotypic Analysis Karyotypic analysis was performed by Dr. Ward Peterson, Children's Hospital of Michigan.

Detection of SV40 Large T Antigen in Immortalized Microvascular Endothelial Cells Immortalized microvascular endothelial cells were analyzed for the expression of SV40 large T antigen by direct immunofluorescence and by enzyme-linked immunosorbent assay (ELISA). Cells tested included immortalized microvascular endothelial cells, normal microvascular endothelial cells, and SV-T2 cells, a murine Balb/c/3T3 cell line transfected with SV40 and expressing nuclear large T antigen. In immunofluorescence studies, all three cell types were cultured separately in LabTek chambers, fixed with 90% methanol at −20°C, and washed. The cells were then reacted with a 1 : 40 dilution of a mouse monoclonal antibody (MoAb) to SV40 large T antigen (MoAb 101) (American Type Culture Collection, Rockville, MD), or an isotype-matched irrelevant control antibody, at room temperature for 30 min, and then washed three times with phosphate-buffered saline (PBS). Bound antibody was detected by staining with a fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse IgG $(Fab')_2$ antibody for 30 min at room temperature. Following three final washes, the slides were viewed under a fluorescence microscope for characteristic nuclear fluorescence.

An ELISA for SV40 large T antigen was also performed using routine methods [26].

Characterization of Endothelial Cell Cultures Representative cultures of human microvascular endothelial cells and SV40T immortalized endothelial cells (CDC/EU.HMEC-1) were characterized in three ways. Cultures were evaluated by inverted phase-contrast microscopy to determine whether transfected cells maintained the characteristic cobblestone morphology of endothelial cells. Cells were also evaluated for expression of von Willebrand's factor (vWF) and binding of Ulex europaeus lectin by direct immunofluorescence. Cells were grown on glass slides and then fixed in 100% methanol for 10 min at −20°C. They were then incubated at room temperature in a humidified chamber with a 1 : 40 dilution of rabbit anti-human vWF (anti–factor VIII-associated antigen,

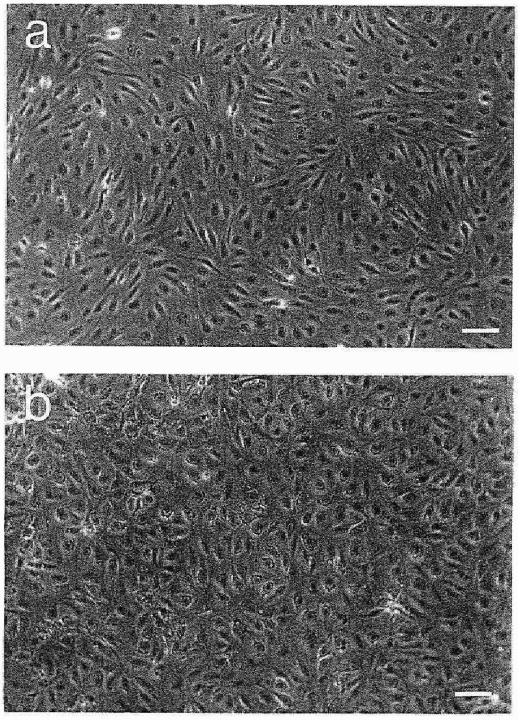

Figure 2. Both HMEC-1 *(a)* and non-transfected microvascular endothelial cells *(b)* demonstrate the typical cobblestone morphology of endothelial cells. Bar, 67 μm.

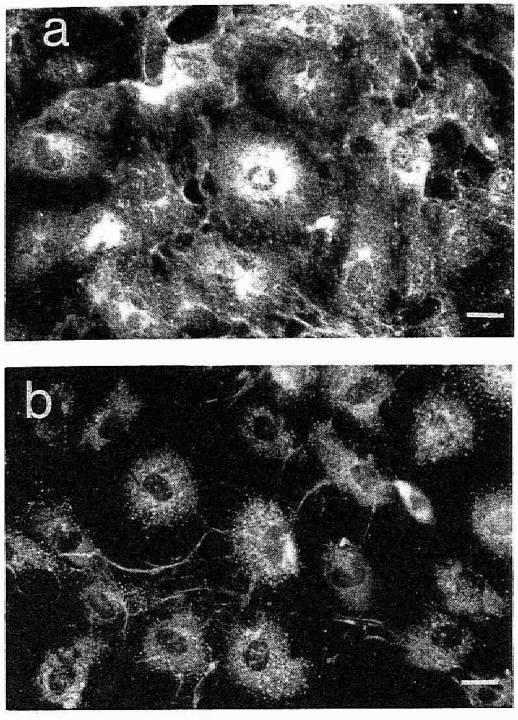

Figure 3. Direct immunofluorescence of HMEC-1 *(a)* and non-transfected microvascular endothelial cells *(b)* for vWF shows positive staining of both cell types. Bar, 22 μm.

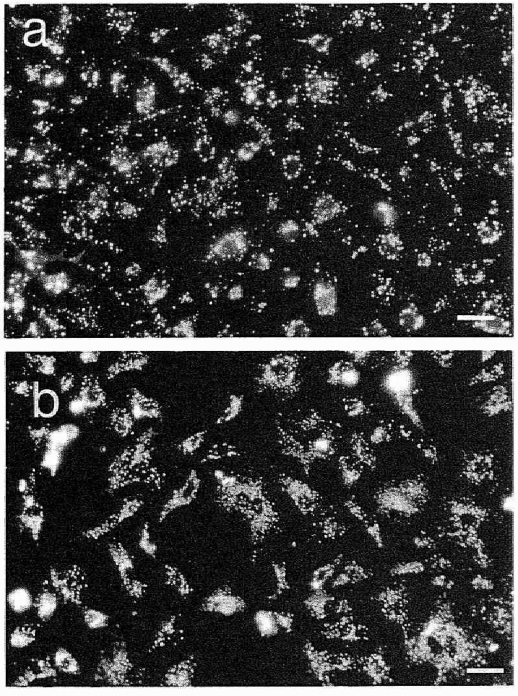

Figure 4. Direct immunofluorescence of HMEC-1 *(a)* and non-transfected microvascular endothelial cells *(b)* incubated for 4 h with acetylated LDL. Both cell types show uptake. Bar, 44 μm.

Behring Diagnostics, La Jolla, CA) for 30 min or with FITC-conjugated Ulex europaeus at a dilution of 1 : 100. Binding of the unconjugated rabbit anti-human vWF antibody was detected by incubation of cells with FITC-conjugated goat anti-rabbit IgG (Caltag, San Francisco, CA). Cells were examined by fluorescence microscopy.

Cells were also evaluated for their ability to take up acetylated low-density lipoprotein (LDL). Dil-AC-LDL is a biologic probe incorporated by living endothelial cells and, to a lesser extent, by monocytes or macrophages. Cells, unfixed, were incubated with acetylated LDL (10 μg/ml), labeled with 1,1′dioctadecyl-1,3,3,3′3′-tetramethyl indocarbocyanine perchlorate (Dil-AC-LDL) (Biomedical Technologies, Inc., Stoughton, MA) at 37°C in culture medium without human serum for 4 h. The medium was removed, and the cells were washed and visualized in a fluorescence microscope with standard rhodamine excitation emission filters.

Differentiation of Endothelial Cells and Growth in Soft Agar Matrigel, an extract of the EHS sarcoma that contains basement membrane components (Collaborative Research, Bedford, MA), was applied to glass slides and allowed to gel at 37°C. Normal human microvascular endothelial cells or HMEC-1 were then plated onto the matrigel and observed for 1–48 h for the formation of tube-like structures. HMEC-1 were also assessed for their ability to grow in soft agar by a previously described technique [27].

Cytokines and Antibodies Recombinant human interferon gamma and tumor necrosis factor alpha were purchased from Amgen Corporation (Thousand Oaks, CA). MoAb recognizing CD31 (clone 5.6E, mouse IgG_1) was purchased from AMAC

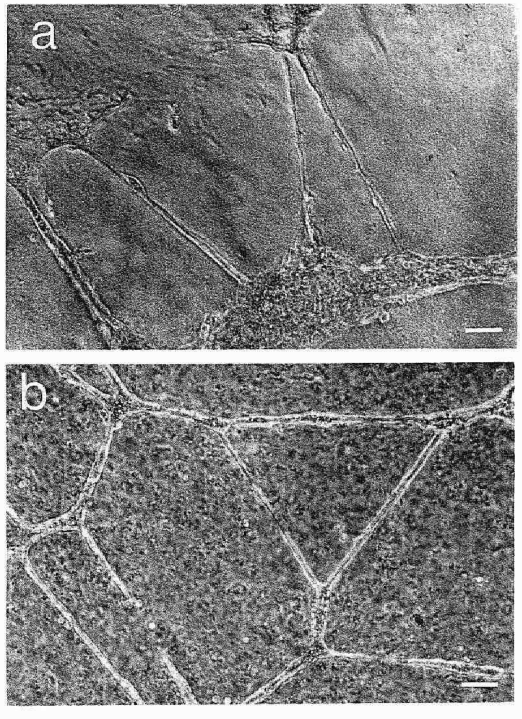

Figure 5. Phase-contrast photomicrographs of HMEC-1 *(a)* and microvascular endothelial cells *(b)* cultured on matrigel for 18 h show tube formation by both cell types. Bar, 67 μm.

(Westbrook, ME). MoAb EN4 and PAL-E (mouse IgG) recognizing human endothelium [28,29] were purchased from Sanbio (Uden, Holland). MoAb 101 recognizing SV40 large T was obtained from American Type Culture Collection. FITC-conjugated Ulex europaeus lectin was obtained from Sigma. MoAb to major histocompatibility complex class II was purchased from Becton Dickinson (Mountain View, CA). MoAb to ICAM-1 (84H10, mouse IgG_1) was a gift of Dr. Stephen Shaw, National Institutes of Health; MoAb to CD44 (A1G3, mouse IgG_1) a gift of Dr. Bart Haynes, Duke University; and MoAb to CD36 (OKM8 and OKM5, mouse IgG_1), a gift from Dr. Patricia Rao (R.W. Johnson Pharmaceutical Research Institute, Raritan, NJ).

Growth Studies The ability of HMEC-1 to grow in cell-culture medium supplemented with human serum was assessed. 1.4×10^6 cells were plated in gelatin-coated T-75 flasks and cultured in complete MCDB 131 medium supplemented with various concentrations of human serum (30%, 20%, 10%, 5%, 1%, and 0%) for 8 d at 37°C. Cells were then removed with trypsin-EDTA buffer, stained with trypan blue, and counted in a hemocytometer. Cells were routinely >99% viable. Untransformed microvascular endothelial cells were similarly studied.

Flow Cytometry Cell-surface molecules on untransformed microvascular endothelial cells and HMEC-1 were analyzed using direct immunofluorescence and flow cytometry as previously described [19,20].

ELISA Non-immortalized microvascular endothelial cells or HMEC-1 were analyzed for cell-surface expression of selected markers as previously described [20], having been plated on 96-well tissue culture plates 24 to 48 h before the assay.

Adherence Assay Untransformed microvascular endothelial cells or HMEC-1 were plated on 48-well tissue-culture plates and analyzed for their ability to adhere to purified T cells. T lymphocytes were isolated as previously described [30] and were labeled with ^{51}Cr (1 mCi/10^6 cells × 1 h) and layered onto monolayers of HDMEC or HMEC-1. After 30 min, the monolayers were washed and the percent adherence of T cells was calculated using the following formula:

$$\text{percent binding} = \frac{\text{adherent counts} - \text{background counts}}{\text{total added counts} - \text{background counts}} \times 100.$$

RESULTS

Transfection of Microvascular Endothelial Cells with SV40 Large T Following transfection of the pooled human dermal microvascular endothelial cells, there was a two-week period of dramatically decreasing viability. This was followed by the emergence of viable colonies in five different flasks. These colonies were expanded and cryopreserved, and cells from one flask (HMEC-1) were further evaluated. The frequency of transfection was not measured in these experiments.

Direct immunofluorescent examination of HMEC-1 with MoAb against SV40 large T revealed bright nuclear staining similar to that detected in the positive control SV-T2 cells; untransformed endothelial cells were uniformly negative for SV40 large T (Fig 1).

ELISA studies of all three cell types for the expression of large T antigen in cell-free lysates revealed its presence in HMEC-1 and SV-T2 but not in untransformed endothelial cells (data not shown).

Karyotype analysis revealed the cell line to be aneuoploid human male with chromosome counts in the near diploid range.

Phenotypic Characterization of HMEC-1 HMEC-1 assumed a cobblestone morphology when cultured on gelatin-coated tissue-culture dishes in MCDB 131 supplemented with 30% human serum. Their morphology was essentially indistinguishable from

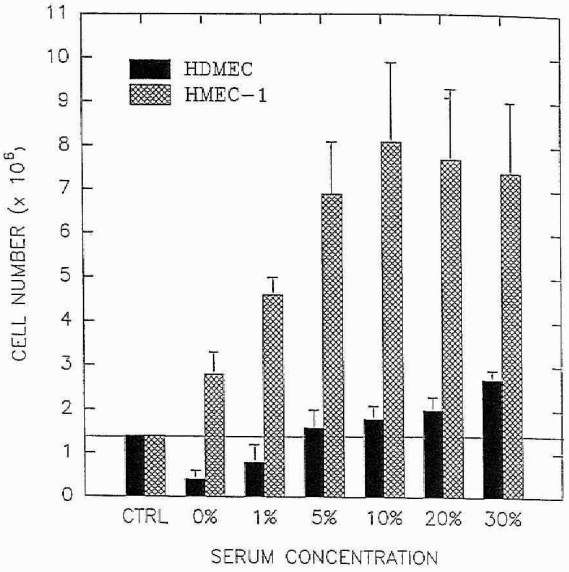

Figure 6. A bar graph comparing the growth of HMEC-1 and microvascular endothelial cells in MCDB 131 culture medium containing various concentrations of human serum. HMEC-1 replicate in the absence of serum and grow to higher density than microvascular endothelial cells, which require 30% human serum to undergo one population doubling in 8 d. Values represent the mean of the results of multiple experiments ± SD.

that of untransformed endothelial cells (Fig 2). However, when they were allowed to become hyperconfluent, the HMEC-1 were capable of growing to a higher density than normal endothelial cells, and the cells appeared smaller under these conditions. Thus, hyperconfluent endothelial cell cultures contained $3-5 \times 10^6$ cells/75 cm^2 flask, whereas HMEC-1 cultures contained up to 22.5×10^6 cells/75 cm^2 when grown to hyperconfluency. However, HMEC-1 retained their anchorage-dependent growth, grew only in monolayers, and did not grow in soft agar. Microvascular endothelial cells routinely became senescent by passage 8–10. Senescence is characterized by increase in cell volume, lack of cell division, and cell death. HMEC-1 has shown no signs of senescence. To date HMEC-1 has been passaged 95 times over the span of 500 d without any change in growth characteristics or morphology.

To determine whether HMEC-1 retained endothelial cell phenotypic characteristics, they were evaluated for vWF expression and uptake of acetylated LDL. HMEC-1 and normal endothelial cells both stained positively for vWF when examined by direct immunofluorescence (Fig 3). The cytoplasmic staining of non-immortalized endothelial cells was more granular than HMEC-1 but otherwise was quite similar. Both types of cells also demonstrated uptake of acetylated LDL after 4 h of exposure (Fig 4), and stained with FITC-conjugated Ulex europaeus (data not shown).

To determine whether HMEC-1 were capable of morphologic differentiation into tubes, they were cultured on matrigel. We have previously shown that endothelial cells will form capillary-like structures when cultured on this basement membrane-like matrix [4]. They attach rapidly, within 1–2 h elongated processes are observed, and after 8 h the endothelial cell cultures show abundant networks of branching and anastomosing cords of cells with a central lumen. HMEC-1 tube formation on matrigel paralleled that of non-immortalized endothelial cells. Both cell types demonstrated tube formation that was evident at 2 h. Well-developed networks of anastomosing tubes were present by 18 h (Fig 5).

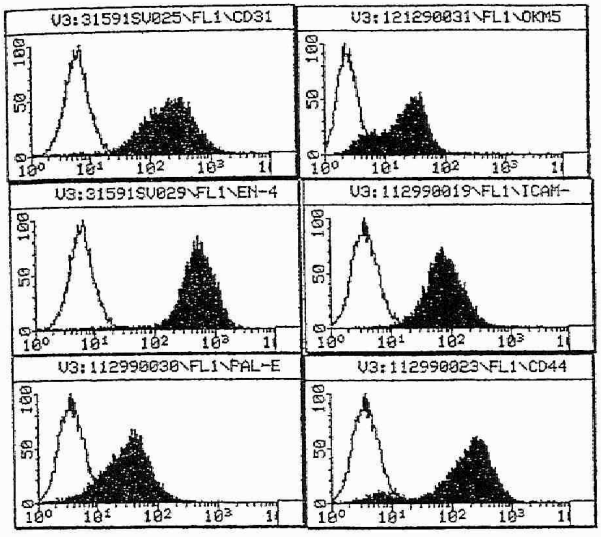

Figure 7. Flow-cytometric analysis of HMEC-1 demonstrates cell-surface expression of the endothelial cell markers CD31, EN4, and PAL-E, as well as cell adhesion molecules ICAM-1, CD36, and CD44.

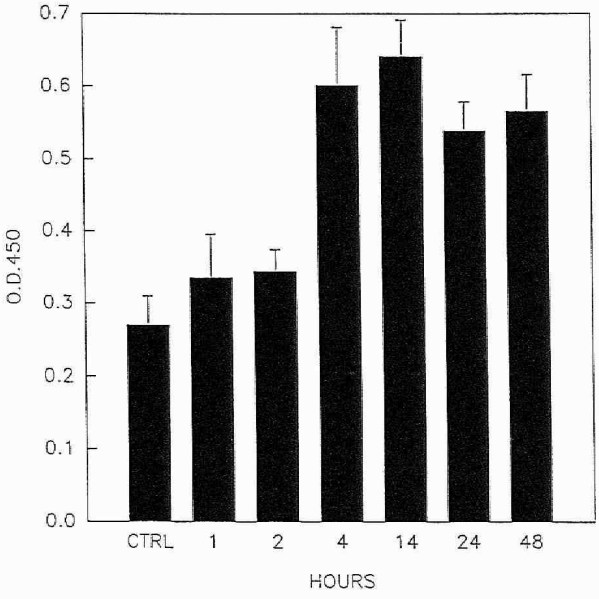

Figure 8. An ELISA study showing that HMEC-1 constitutive expression of ICAM-1 is increased by exposure to TNFα (100 u/ml) for 4 h. The upregulation of ICAM-1 is maintained through 48 h. Results represent the mean ± SD of four data points.

Proliferation Studies To determine the medium requirements for growth of HMEC-1, 1.4×10^6 HMEC-1 or non-immortalized endothelial cells were plated in flasks and cultured in MCDB 131 supplemented with various concentrations of human serum (Fig 6). After 8 d, cells were harvested and counted. Microvascular endothelial cells grown in medium containing 1% serum or less not only failed to proliferate, but also demonstrated a significant decrease in cell number. When grown in 5–20% serum, they did not die but did not substantially increase cell number. As previously noted [4], microvascular endothelial cells grew optimally in medium containing 30% serum undergoing a single population doubling after 8 d.

In contrast, HMEC-1 grew well even at extremely low serum concentrations. Maximal growth was seen at serum concentrations of 10%, at which cells underwent a nearly sixfold increase in cell number over 8 d. Cells grown in 0% or 1% serum also proliferated, undergoing a twofold and a threefold increase, respectively. Furthermore, HMEC-1 could be passaged long term in medium containing no serum. To date, these cells have been passaged more than 35 times in medium without serum without evidence of senescence.

Cell-Surface Molecule Expression To further define the phenotypic characteristics of HMEC-1, we examined their expression of other endothelial-cell–associated epitopes by flow-cytometric analysis (Fig 7). HMEC-1 expressed cell-surface CD31, an epitope shared by monocytes and endothelial cells. They also expressed cell-surface epitopes defined by MoAb EN4 and PAL-E in amounts similar to those seen on nonimmortalized endothelial cells. These antibodies have been previously shown to specifically stain endothelium in tissue sections. HMEC-1 also expresses cell-surface intercellular adhesion molecule-1 (ICAM-1) and CD44 as determined by flow-cytometric analysis. ICAM-1 expression on HMEC-1 was increased by exposure of the cells to tumor necrosis factor-alpha (TNFα) in a manner similar to that of normal human microvascular endothelial cells (Fig 8). Moreover, HMEC-1 express CD36, an epitope expressed by human microvascular endothelial cells *in vivo* and *in vitro* but not expressed by HUVEC (Fig 8) [20]. Endothelial cells do not constitutively express class II molecules. However, following stimulation with 100–1000 u/ml of IFN-γ for 72 h, HMEC-1 expressed class II antigen (Fig 9). This data demonstrates that immortalized HMEC-1 maintain an endothelial cell phenotype.

T Cell–HMEC-1 Adhesion Study To examine whether purified T cells would bind to monolayers of HMEC-1 and to determine whether this binding could be regulated by pro-inflammatory cytokines, we performed T cell–HMEC-1 binding studies (Fig 10). T cells bound to HMEC-1 (17.6% ± 3%) as well as to microvascular endothelial cells (27.1% ± 5%). T-cell binding was enhanced by 70% following pre-incubation of HMEC-1 with 100 u TNFα for 24 h. HDMEC showed an increase of 41% in T-cell binding following exposure to TNFα.

DISCUSSION

Endothelial cells are active participants in a variety of physiologic and pathologic processes. Much of our understanding regarding their importance in these processes was made possible by the development of techniques that allowed for the isolation and growth of endothelial cells from umbilical veins [31,32]. However, these large-vessel endothelial cells are limited in their usefulness because of their limited lifespan, their requirement for significant concentrations of serum for growth, and their lot-to-lot variability in functional assays. Furthermore, the vast majority of pathophysiologic events involving endothelial cells occur at the level of the microvasculature, and endothelial cells derived from large vessels exhibit distinct phenotypic and functional differences from small-vessel endothelial cells [4,5,11–22].

A number of cell lines of endothelial origin have been previously reported [33–49]. These have included a large number of murine cell lines as well as those from other non-human species. Some of these have retained many of the features usually associated with endothelial cells. Fewer human endothelial cell lines have been created, and most of these have been of large vessel origin. However, most of the human lines have experienced loss of many essential endothelial cell characteristics or have undergone crisis with subsequent death of the cell line. To our knowledge, no human

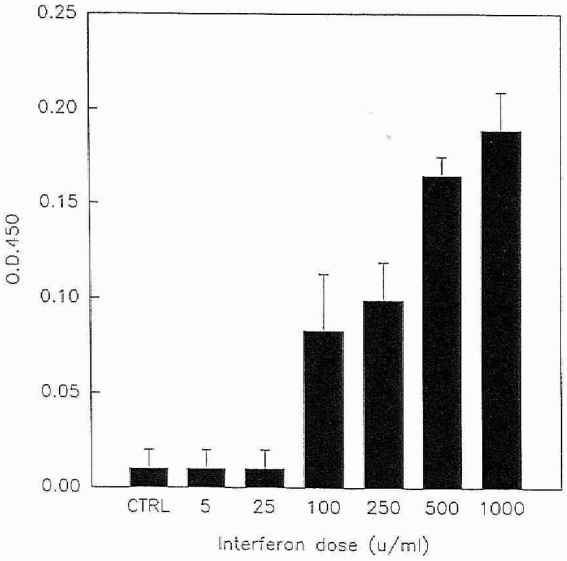

Figure 9. An ELISA analysis of class II major histocompatibility complex (HLA-DR) expression by HMEC-1. HMEC-1 do not constitutively express HLA-DR but can be induced to do so by incubation with IFN-γ for 72 h.

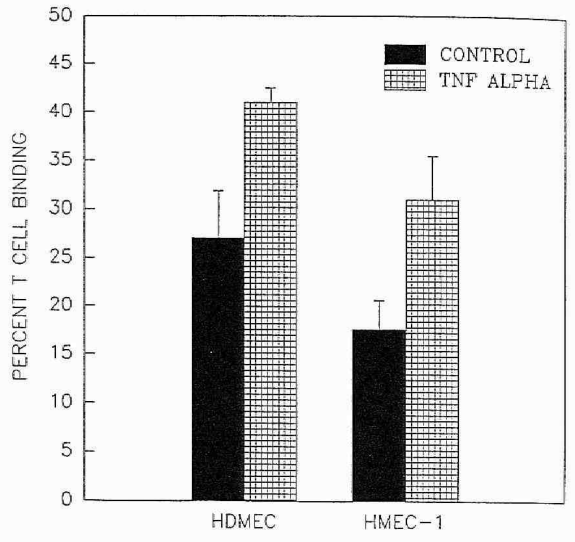

Figure 10. A bar graph demonstrating that HMEC-1 bind human T cells and that this binding can be enhanced by stimulation of HMEC-1 with TNFα (100 units, 24 h). Results represent the mean ± SD of three data points.

microvascular endothelial cell line derived from normal skin exists that retains the characteristics of endothelial cells after prolonged passage in culture.

To overcome the difficulties associated with the isolation, purification, and growth of microvascular endothelial cells, we transfected human dermal microvascular endothelial cells with SV40 large T and immortalized them. Immortalization of human microvascular endothelial cells has widespread utility in a number of different biologic systems, provided that the immortalized cells retain critical biologic features characteristic of the parent cell. Immortalized microvascular endothelial cells, capable of growth in defined media, would represent a unique reagent that would allow questions to be addressed concerning the biology and function of microvascular endothelial cells that are otherwise unapproachable using microvascular endothelial cells derived from multiple unknown donors, cultured in extremely nutrient rich media, and having a short life span.

Human microvascular endothelial cells transfected with SV40 large T have been successfully immortalized, in that they have been passaged more than 95 times over the span of approximately 500 d. In contrast, non-transfected microvascular endothelial cells cannot be propagated more than 10 passages. Furthermore, HMEC-1 have been passaged more than 35 times in medium without serum, and these cells grow as well as microvascular endothelial cells grown in 30% normal human serum. Although HMEC-1 failed to grow in an anchorage-independent manner, they grew to densities 3–7 times greater than microvascular endothelial cells. This data clearly demonstrates that HMEC-1 is immortalized and can serve as a continuously renewable cell line that can be grown in defined medium.

HMEC-1 would be of limited utility if they failed to maintain an endothelial phenotype. However, HMEC-1 express vWF and take up acetylated LDL. Furthermore, they stain with the endothelial-cell–specific lectin Ulex europaeus and form tube-like structures on matrigel. Flow-cytometric analysis demonstrated that these cells express a series of cell-surface epitopes previously shown to be endothelial cell specific. These include cell-surface CD31 and epitopes defined by MoAb EN4 and PAL-E. This data demonstrates that HMEC-1 retains endothelial cell phenotypic characteristics as defined by essentially all commonly accepted criteria.

We further examined the phenotype of HMEC-1 to determine whether they also retained microvascular endothelial cell characteristics. HMEC-1 express cell-surface CD36. This epitope is not expressed by HUVEC *in vivo* or *in vitro*, but is expressed by a variety of microvessels *in vivo* [50] and by human dermal microvascular endothelial cells *in vivo* and *in vitro* [20]. Unstimulated HMEC-1 also express high baseline levels of ICAM-1. This is a characteristic of microvascular endothelial cells, whereas cultured human umbilical vein endothelial cells generally express relatively low levels of ICAM-1, unless stimulated with interleukin 1 or TNF [19]. Furthermore, ICAM-1 expression on HMEC-1 can be upregulated by TNFα in a dose- or time-dependent fashion, similar to that of non-immortalized endothelial cells. This data suggests that HMEC-1 not only express an endothelial phenotype but also express a phenotype that retains characteristics of microvascular endothelial cells.

HMEC-1 behaves functionally in a manner similar to human microvascular and human umbilical vein endothelial cells. Unstimulated HMEC-1 bind purified populations of T cells, and this binding can be enhanced by stimulation with TNFα. Although differences in baseline binding of T cells were seen between HMEC-1 and non-immortalized microvascular endothelial cells in some experiments, the percent binding of T cells to HMEC-1 falls within the range of T-cell binding to microvascular endothelial cells and human umbilical vein endothelial cells seen in our laboratory and in previous published reports [51,52]. More importantly, HMEC-1 bind T cells in a regulatable manner and respond to pro-inflammatory cytokines comparably to non-transformed endothelial cells.

In summary, HMEC-1 represents a unique immortalized human microvascular endothelial cell line capable of growth in defined medium that will be useful in the dissection of the role of the microvascular endothelium in physiologic and pathophysiologic processes.

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ANNOUNCEMENT

The 42nd annual Symposium on the Biology of Skin will be "Genetics of Skin Disease." The symposium will be held at Snowmass Lodge in Snowmass, Colorado on July 24–28, 1993. The meeting will be held just before the annual meeting of the Society for Pediatric Dermatology. Those wishing to present a poster at the SBS may submit an abstract to the Symposium Director on a standard SID or ESDR abstract form. All correspondence regarding participation in this meeting, and all abstract submissions, should be addressed to: David A. Norris, M.D., Symposium on the Biology of Skin, Department of Dermatology B-144, University of Colorado School of Medicine, 4200 East Ninth Avenue, Denver, Colorado 80262