Role of Laminin and Basement Membrane in the Morphological Differentiation of Human Endothelial Cells into Capillary-like Structures

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Abstract. We have defined a signal responsible for the morphological differentiation of human umbilical vein and human dermal microvascular endothelial cells in vitro. We find that human umbilical vein endothelial cells deprived of growth factors undergo morphological differentiation with tube formation after 6–12 wk, and that human dermal microvascular endothelial cells differentiate after 1 wk of growth factor deprivation. Here, we report that morphological differentiation of both types of endothelial cells is markedly accelerated by culture on a reconstituted gel composed of basement membrane proteins. Under these conditions, tube formation begins in 1–2 h and is complete by 24 h. The tubes are maintained for >2 wk. Little or no proliferation occurs under these conditions, although the cells, when trypsinized and replated on fibronectin-coated tissue culture dishes, resume division. Ultrastructurally, the tubes possess a lumen surrounded by endothelial cells attached to one another by junctional complexes. The cells possess Weibel–Palade bodies and factor VIII–related antigens, and take up acetylated low density lipoproteins. Tubule formation does not occur on tissue culture plastic coated with laminin or collagen IV, either alone or in combination, or on an agarose or a collagen I gel. However, endothelial cells cultured on a collagen I gel supplemented with laminin form tubules, while supplementation with collagen IV induces a lesser degree of tubule formation. Preincubation of endothelial cells with antibodies to laminin prevented tubule formation while antibodies to collagen IV were less inhibitory. Preincubation of endotheial cells with synthetic peptides derived from the laminin B1 chain that bind to the laminin cell surface receptor or incorporation of these peptides into the gel matrix blocked tubule formation, whereas control peptides did not. These observations indicate that endothelial cells can rapidly differentiate on a basement membrane–like matrix and that laminin is the principal factor in inducing this change.

It is well known that endothelial cells are capable of aggregating in vitro to form capillary-like structures (1, 3, 14, 16, 19). This process, which is thought to mimic the process by which endothelial cells form capillaries in vivo, requires specialized culture conditions, including the removal of growth factors, and takes place after 5–40 d. Maciag et al. (14) showed that endothelial cells derived from human umbilical veins also can be induced to differentiate and form similar tubes over a 4–6-wk period when cultured in the absence of mitogens. This process was accelerated when the human umbilical vein endothelial cells (HUVE cells)[1] were cultured on endothelial cell–derived extracellular matrix. Further support for an important role of the extracellular matrix in the induction of the tube structures came from studies on rat capillary endothelial cells that form tubules after 4–5 d of culture on a matrix consisting of collagens IV and V or on the basement membrane side of human amnion (16). In vivo, endothelial cells line the lumen of blood vessels and are normally in contact with an extracellular matrix of basement membrane. Basement membranes are composed of collagen IV, heparan sulfate proteoglycan, and the glycoproteins laminin and nidogen/entactin. While collagen has some adhesion-promoting activity, laminin has been demonstrated to have potent actions on cells: stimulating cell adhesion, growth, differentiation, and migration (6, 8, 10). Recently, an active site of laminin for some of these activities has been identified. It is comprised of five amino acids: tyrosine-isoleucine-glycine-serine-arginine (YIGSR) (4, 5).

The slow formation of tubes by cultured endothelial cells has made it difficult to identify the steps involved. Recently, we have noted, using endothelial cells cultured from umbilical vein and skin capillaries, that they rapidly undergo tubule formation when cultured on a reconstituted matrix of basement membrane components. Our studies indicate that the morphological differentiation of these cells is triggered by

1. *Abbreviations used in this paper*: ECGF, endothelial cell growth factor; HUVE cells, human umbilical vein endothelial cells; YIGSR, tyrosine-isoleucine-glycine-serine-arginine.

laminin and to a lesser extent by collagen IV. The differentiation of these cells is accompanied by a cessation of DNA synthesis, but these cells can be readily restored to a proliferating state.

Materials and Methods

Isolation and Culture of Endothelial Cells

Human Dermal Microvascular Endothelial Cells. Human microvascular endothelial cells were isolated from human neonatal foreskins by a modification of a previously described technique (17). Briefly, foreskins were cut into 3-mm squares and placed in PBS containing 0.3% trypsin (Sigma Chemical Co., St. Louis, MO) and 1% EDTA (Sigma Chemical Co.) at 37°C for 10 min. The skin segments were washed with Hank's balanced salt solution (HBSS) several times and placed in a petri dish in HBSS with the keratinized surface down. They were then individually compressed with the side of a scalpel blade to express microvascular fragments from the cut surfaces of the skin. The microvascular segments were passed through a 105-μm nylon mesh (Small Parts Inc., Miami, FL) and collected. The microvascular segments in 1 ml of HBSS were layered onto a 35% solution of Percoll in HBSS that had been spun at 30,000 *g* for 10 min at 4°C in an SS-34 rotor on a centrifuge (model RC2-B; Sorvall Instruments, Newton, CT). The gradient was then spun at 400 *g* for 15 min at room temperature. The fraction with a density <1.048 g/ml, which was rich in microvascular fragments, was removed. Those portions of the gradient containing the microvascular segments were applied to a human fibronectin-precoated (Advanced Biotechnologies, Silver Spring, MD) area 10 mm in diameter in the center of a 60-mm petri dish. The dishes were then incubated at 37°C in a moist incubator in 5% CO_2 overnight. Unattached cells were removed by washing with HBSS. The attached cells were then viewed with an inverted phase-contrast microscope. Nonendothelial cells were removed by detaching them with a 25-gauge sterile needle. Cells were cultured in medium 199 with 50% human serum, 100 μg/ml endotheial cell growth factor (ECGF; Meloy Laboratories Inc., Springfield, VA), 50 μg/ml heparin (Sigma Chemical Co.), 5×10^{-5} M dibutyryl cAMP (Sigma Chemical Co.), 3.3×10^{-5} M isobutylmethylxanthine (Calbiochem-Behring Corp., San Diego, CA), glutamine (Sigma Chemical Co.), 100 U/ml penicillin, 100 μg/ml streptomycin, and 250 μg/ml amphotericin B (Sigma Chemical Co). All cells were used at passages 2–4.

HUVE Cells. HUVE cells were isolated from fresh umbilical cords obtained by caesarean section by a modification of the technique of Jaffe et al. (9, 13). The umbilical vein was cannulated, and 0.1% collagenase in PBS was introduced and incubated for 20 min. The endothelial cells liberated by the collagenase were obtained by rinsing the umbilical vein with medium 199. The cells were washed with medium 199 three times and cultured in 75-cm^2 tissue culture flasks (Costar, Cambridge, MA) coated with fibronectin. Growth media consisted of medium 199 with 20% FCS (Hyclone Laboratories, Inc., Logan, UT), 50 μg/ml ECGF (Meloy Laboratories, Inc.), 50 μg/ml heparin (Sigma Chemical Co.), 100 U/ml penicillin, 100 μg/ml streptomycin, and 250 μg/ml amphotericin B (Sigma Chemical Co.). HUVE cells were passaged at confluence after treatment with trypsin–EDTA buffer (Biofluids Inc., Rockville, MD). All cells were used at passages 2–4.

Substrates Used for Cell Culture. Matrigel (Collaborative Research, Inc., Waltham, MA), an extract containing basement membrane components at 10 mg/ml, was applied to either a 35-mm tissue culture dish or a 24-well culture dish (Costar) and incubated at 37°C, which induced gelling. HUVE or human dermal microvascular endothelial cells in media were pipetted onto the gel. In some experiments, matrigel at 4°C was serially diluted 1:2, 1:4, and 1:8 with prechilled DME (Gibco Laboratories, Grand Island, NY) and 300 μl of the appropriate dilution was added to wells of a 24-well culture plate (Falcon Plastics, Cockeysville, MD), and then incubated at 37°C for 30 min to induce it to gel. Endothelial cells were then plated on the diluted matrix. Alternatively, matrigel was similarly diluted with a solution of collagen I (Collaborative Research, Inc.) (see below).

In other experiments, both types of endothelial cells were grown in tissue culture dishes coated with laminin (12), collagen I (Advanced Biomedical Technologies), and collagen IV (21) or extracellular matrix (Accurate Chemical & Scientific Corp., Westbury, NY) under standard conditions. The morphology and reorganization of the endothelial cells were monitored with a microscope (model IM-35; Carl Zeiss Inc., Thornwood, NY).

Matrix Reconstitution Experiments

Native collagen I gels were prepared by mixing together 1 vol of cold 10-fold concentrated PBS, 1 vol of 0.1 N NaCl, and 8 vol of cold collagen I solution (Collaborative Research, Inc.) in 0.02 N acetic acid. The mixture was kept on ice to prevent premature formation of a gel, and the solution was adjusted to pH 7.5. The initial concentration of collagen I in the gel was 1.84 mg/ml. Purified laminin (12) or collagen IV (21), at 1.7 and 1.0 mg/ml, respectively, in 0.15 M NaCl, 0.05 M Tris-HCl, pH 7.4, was added to the cold collagen I solution and mixed at the concentration indicated at 4°C. The mixtures were plated into 24-well dishes and incubated 30–60 min at 37°C. Endothelial cells were plated on the top of these reconstituted gels.

Antibody Blocking Studies

To further assess the roles of laminin and collagen IV in the formation of tubes, endothelial cells in suspension were preincubated with goat anti-laminin serum (1:5) or rabbit antiserum to collagen IV (1:4, 1:10) for 15 min at room temperature and then plated on matrigel and cultured overnight in the continuous presence of antibody (2, 22). Normal goat and normal rabbit serum were used as controls.

Laminin Peptide Experiments

The peptides (YIGSR, YIGSR-NH_2) were prepared using an automated synthesizer (OCS Laboratories Inc., Denton, TX) (5) and were incubated with endothelial cells for 1–2 h at room temperature at a variety of concentrations, and then plated on the top of matrigel. In some experiments, matrigel was mixed with various concentrations of the laminin peptides for 1 h at 4°C. The mixture was plated into a 24-well plate (0.3 ml/well) and was incubated for 30–60 min at 37°C to allow a gel to form. Endothelial cells were plated on the top of peptide-containing matrigel. Control peptides were included in parallel cultures in all experiments.

Immunofluorescence of Cultured Endothelial Cells

Immunofluorescent staining of endothelial cells for factor VIII–related antigen was performed on cell cultures fixed in 100% methanol for 10 min at 4°C. The cells were washed three times with PBS and then incubated with a 1:20 dilution of fluorescein-conjugated goat antihuman factor VIII antibody (Atlantic Antibodies, Scarborough, ME) for 30 min at room temperature. The specimens were washed three times with PBS and were examined with an inverted phase-immunofluorescence microscope (model IM-35; Carl Zeiss, Inc.). Endothelial cell cultures were incubated for 4 h at 37°C in medium 199 without either growth supplements or FCS but containing acetylated low density lipoprotein (10 μg/ml) labeled with 1,1′-dioctadecyl-1,3,3,3,3′-tetramethyl-indocarbocyanine-perchlorate (Biomedical Technologies, Inc., Cambridge, MA). The medium was then removed, and the cells were washed twice and visualized with standard rhodamine excitation/emission filters.

Electronmicroscopy of Endothelial Cells

Endothelial cells were washed with PBS and fixed with 2.5% glutaraldehyde in 100 μM cacodylate buffer, pH 7.4, for 1 h and postfixed in 1% osmium tetroxide for 1 h. The samples were dehydrated with ethanol and embedded in Epon 812, and 0.5-μm-thick sections were prepared and stained with toluidine blue. Thin sections were cut on an ultramicrotome. The sections were stained with uranyl acetate and lead citrate and were examined with an electron microscope (model 400; Philips Electronic Instruments, Inc., Mahwah, NJ).

Cell Growth Measurements

HUVE cells were plated on fibronectin- or matrigel-coated plastic dishes at an initial density of 3.7×10^5 cells per 35-mm dish and were grown under standard conditions at 37°C in 5% CO_2. Every other day, duplicate plates were trypsinized with 0.05% trypsin–0.02% EDTA mixture (M.A. Bioproducts, Walkersville, MD) or dispase (Collaborative Research, Inc.), and the cells were counted with a batch-counting chamber (Hausser Scientific, Blue Bell, PA) in a 1:20 dilution of 0.2% trypan blue. The viability of cells cultured on matrigel was 83 (original cell suspension), 80 (24 h), 81 (48 h), and 75% (72 h).

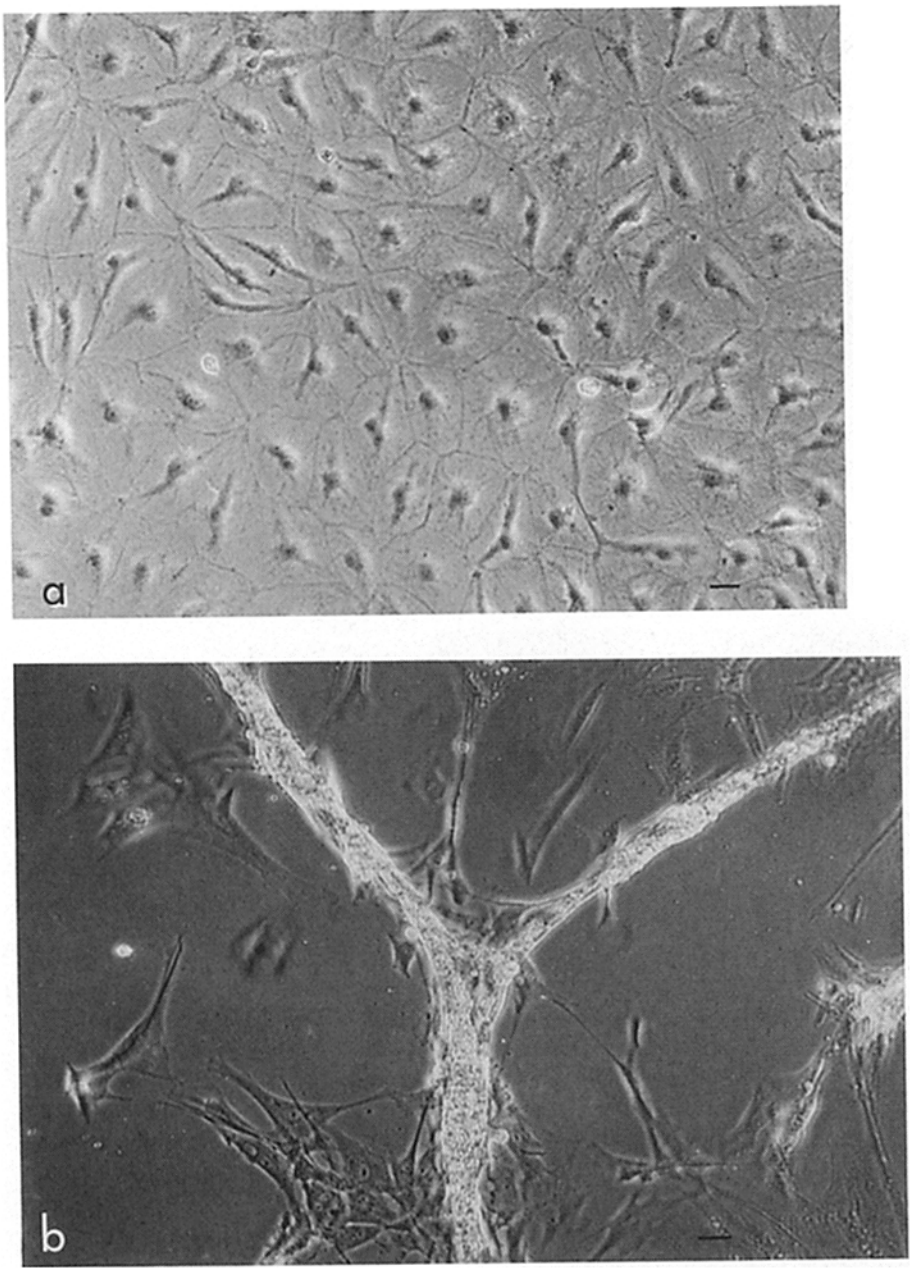

Figure 1. (*a*) Phase-contrast photomicrograph of a pure culture of human dermal microvascular endothelial cells. (*b*) Phase-contrast photomicrograph of HUVE cells forming a tubelike structure after 12 wk of culture in the absence of ECGF and heparin. Bars, 20 μm.

Results

Morphology

HUVE cells as well as human dermal microvascular endothelial cells assume a "cobblestone" pattern when cultured on fibronectin-coated tissue culture dishes in media supplemented with ECGF and heparin (Fig. 1 *a*). If ECGF and heparin are withdrawn, these cells cease proliferating and slowly form tubelike structures. Microvascular endothelial cells require a week to form these structures, whereas endothelial cells from large vessels require 6–12 wk (Fig. 1 *b*).

Since matrix components are known to influence the behavior of endothelial cells, we tested fibronectin, laminin, collagen I, or collagen IV, singly or in combination, as substrates for endothelial cells. Both types of endothelial cells

Table I. Comparison of Effects of Basement Membrane and Matrix Proteins on Endothelial Cell Differentiation

	Tube formation	
	HUVE	HDMEC*
Matrigel	+	+
Fibronectin	–	–
Laminin	–	–
Collagen I	–	–
Collagen IV	–	–
Extracellular matrix	–	–
Agarose gel	–	–
Fibronectin + laminin	–	–
Collagen I + collagen IV	–	–
Laminin + collagen IV	–	–

* *HDMEC*, human dermal microvascular endothelial cells.

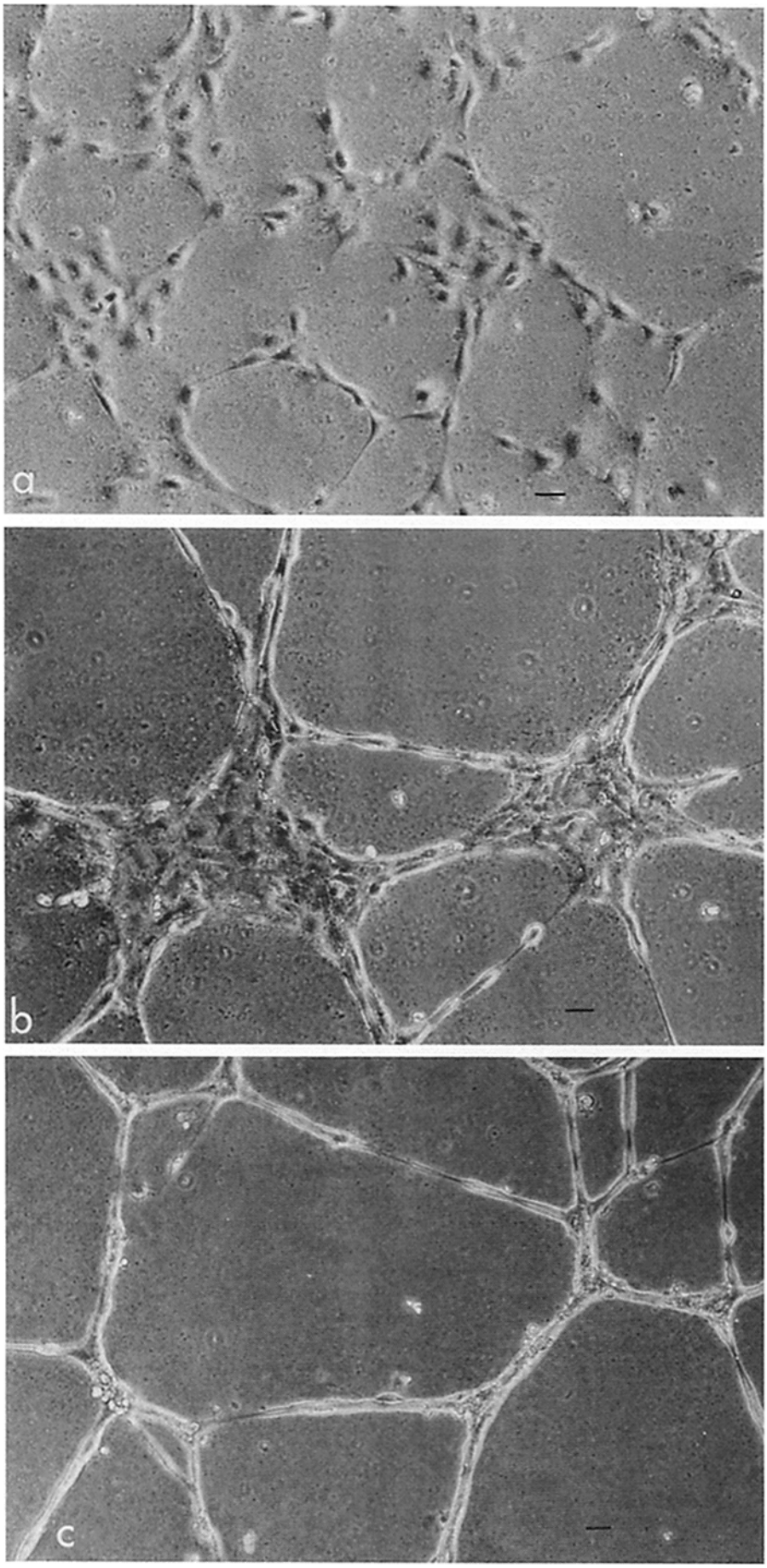

Figure 2. (*a*) HUVE cells cultured on matrigel for 1 h. The cells begin to align themselves end to end and to elongate. (*b*) HUVE cells cultured on matrigel for 8 h. Marked elongation is observed. (*c*) HUVE cells cultured on matrigel for 18 h. A complex network of anastomosing cells is observed. Bars, 20 μm.

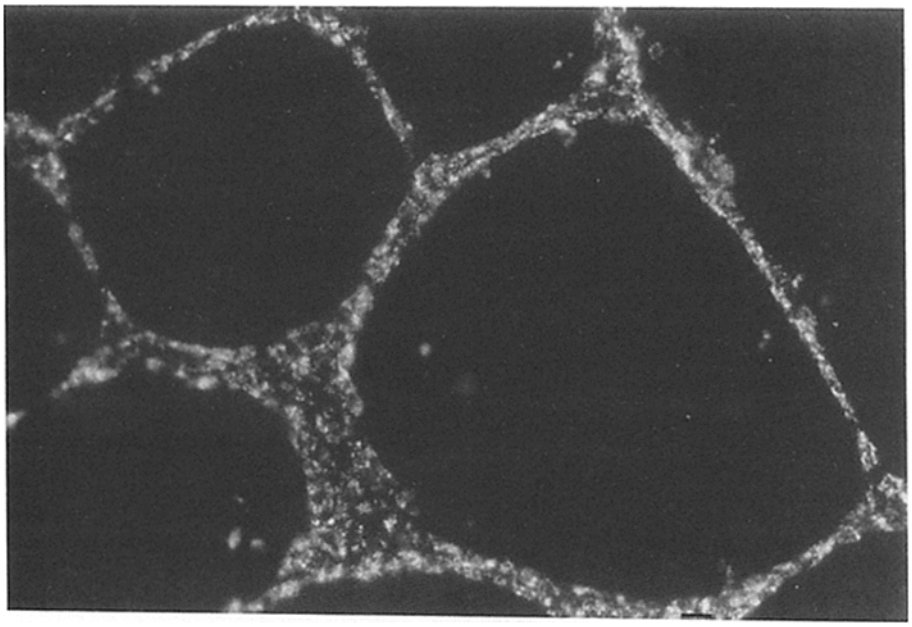

Figure 3. A phase–fluorescence photomicrograph of HUVE cells cultured on matrigel for 18 h. Showing uptake of acetylated low density lipoprotein. Bar, 20 μm.

assumed a cobblestone morphology typical of undifferentiated cells when plated on each of these substrates, as well as on commercially available extracellular matrix–coated dishes (Table I).

Due to the ability of basement membrane to stimulate differentiation, both types of cells were plated onto a gel formed of reconstituted basement membrane proteins (11) to which they attached rapidly. Within 1–2 h, elongated processes were observed (Fig. 2 *a*) and after 8 h the endothelial cell cultures showed abundant networks of branching and anastomosing cords of cells (Fig. 2 *b*). By light microscopy, most of these cords showed a central translucent structure along their long axis, suggesting the presence of a lumen. By 18 h, the endothelial cells had formed an interconnected network of anastomosing cells that by low power light microscopy had a "honeycomb" appearance (Fig. 2 *c*). The tubelike structures formed by the endothelial cells on matrigel persisted for ≥14 d of culture, after which the network tended to detach from the surface of the culture substrate, and elements of the network were found to be floating in the culture media. The formation of the tube structures was not dependent on ECGF or heparin in the culture medium, since tube formation took place in exactly the same way and with the same time course in their presence as in their absence.

The formation of tubes appeared to be relatively specific for endothelial cells since neither human dermal fibroblasts nor human smooth muscle cells formed tubes when cultured on matrigel.

Immunochemical Studies

The endothelial cells cultured on matrigel were examined by direct immunofluorescence for the expression of factor VIII–related antigen before, during, and after tube formation. The endothelial cells retained factor VIII reactivity under both conditions (data not shown). Furthermore, both HUVE cells and human dermal microvascular endothelial cells cultured on matrigel were metabolically active as indicated by their uptake of acetylated low density lipoprotein (Fig. 3).

Ultrastructural Studies

We performed transmission EM on cells cultured on matrigel for 18 h to examine the morphological characteristics of the tubes. Cross sections of tubelike structures of the surface of the matrigel revealed that the tubes contained a lumen surrounded by cells (Fig. 4 *a*). In some instances, lumen formation appeared to take place within individual cells (Fig. 4 *b*). The membranes of cells forming the lumen of the tubes were connected to one another by interdigitating junctional complexes (Fig. 4 *d*). Weibel–Palade bodies were observed in the cytoplasm of the cells forming the tubes (Fig. 4 *c*), confirming again that these were endothelial cells.

Proliferation Studies

We compared the growth of HUVE cells (3.7×10^5 cells per 35-mm dish) on fibronectin-coated tissue culture dishes with cells plated at the same density on matrigel. After 1 wk, the HUVE cells grown on fibronectin showed a fourfold increase in cell number, whereas those on matrigel showed no increase (Fig. 5). However, when differentiated endothelial cells were removed from matrigel and cultured on fibronectin-coated tissue culture dishes, they assumed a cobblestone morphology and resumed a normal rate of proliferation (data not shown).

Concentration and Component Dependence of Tube Formation

Various dilutions of matrigel were prepared in DME and allowed to polymerize. These studies showed rapid formation of tubes on matrigel diluted to 1:2, a reduced degree of tube formation at a 1:4 dilution, and very reduced tube formation at a 1:8 dilution. Matrigel was also diluted with a solution of collagen I at ratios of matrigel/collagen I of 3:1, 2:1, 1:1,

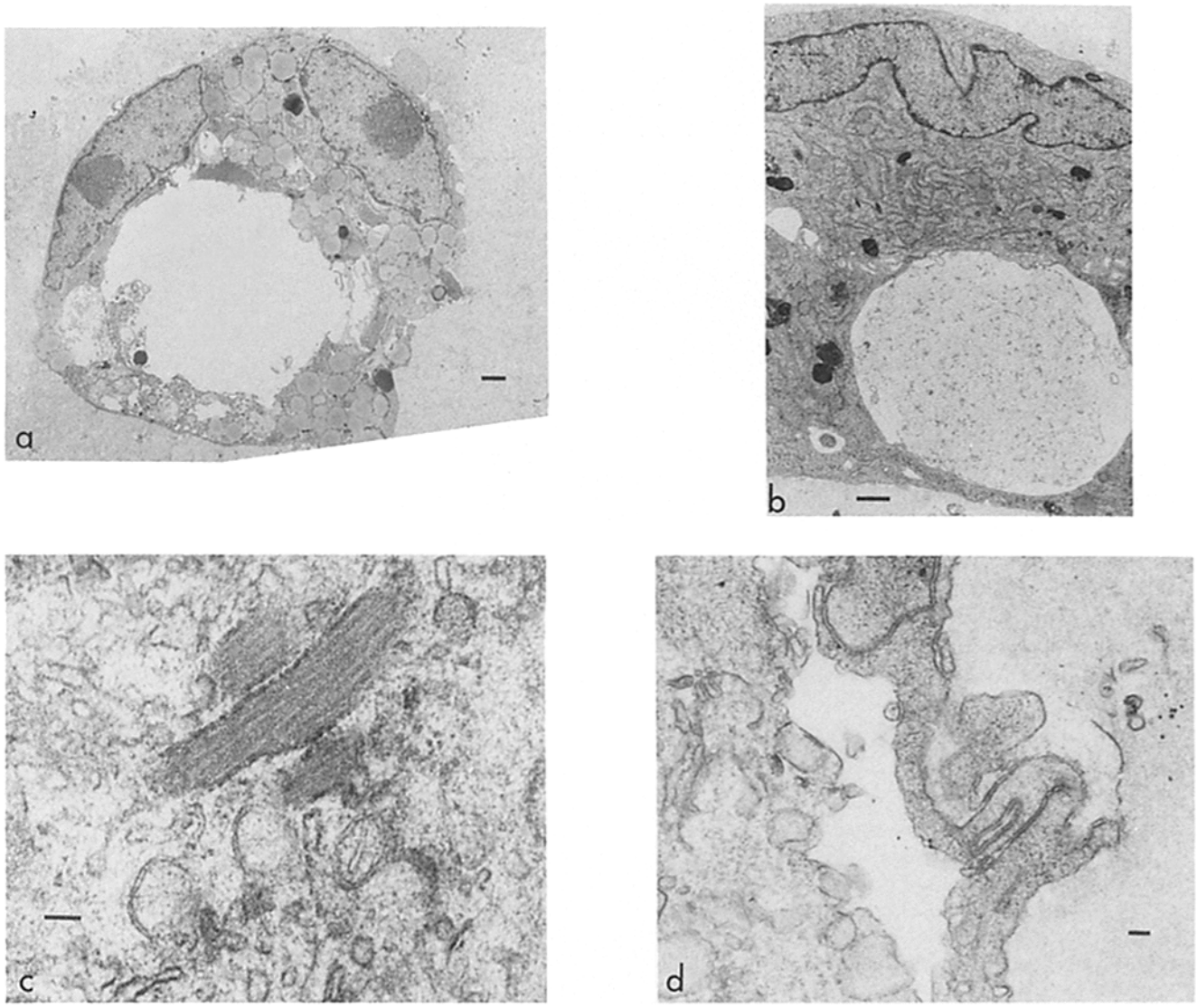

Figure 4. (*a*) Transmission electron micrograph of human dermal microvascular endothelial cells cultured on matrigel for 18 h. A cross section reveals lumen formation. Cells contain lipid-like inclusions. (*b*) Transmission electron micrograph of a human dermal microvascular endothelial cell showing early lumen formation. (*c*) Presence of Weibel–Palade bodies in a differentiated endothelial cell. (*d*) The differentiated endothelial cell shows interdigitating junctions. Bars: (*a* and *b*) 1 μm; (*c* and *d*) 100 nm.

1:2, 1:3, and 1:7. Matrigel diluted with collagen I supported endothelial cell tubule formation at ratios of 3:1 and 2:1, but at higher concentrations of collagen I, the endothelial cells grew in a cobblestone pattern in a monolayer without tubes. This result suggested either that collagen I was inducing the monolayer phenotype or that a critical component of the matrigel was being diluted. To further examine this issue, a collagen I solution was supplemented with laminin and collagen IV, singly or in combination and then allowed to gel. Endothelial cells cultured on a collagen I gel grew as a monolayer (Fig. 6 *a*). Collagen I gels supplemented with laminin supported tubule formation by endothelial cells (Fig. 6 *b*). Collagen I gels supplemented with type IV collagen showed less tubule formation (Fig. 6 *c*). Supplements of laminin plus collagen IV added to the collagen I gel supported tubule formation in a manner similar to that produced by collagen I gel supplemented with laminin alone. These studies indicate that laminin is the most active component in matrigel promoting tube formation.

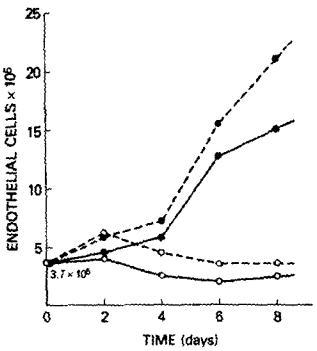

Figure 5. Proliferation of endothelial cells cultured on matrigel (○) or fibronectin-coated tissue culture plastic (●). Cells cultured on matrigel show no proliferation while those cultured on fibronectin show normal proliferation. (—) Adherent cells; (---) total cells.

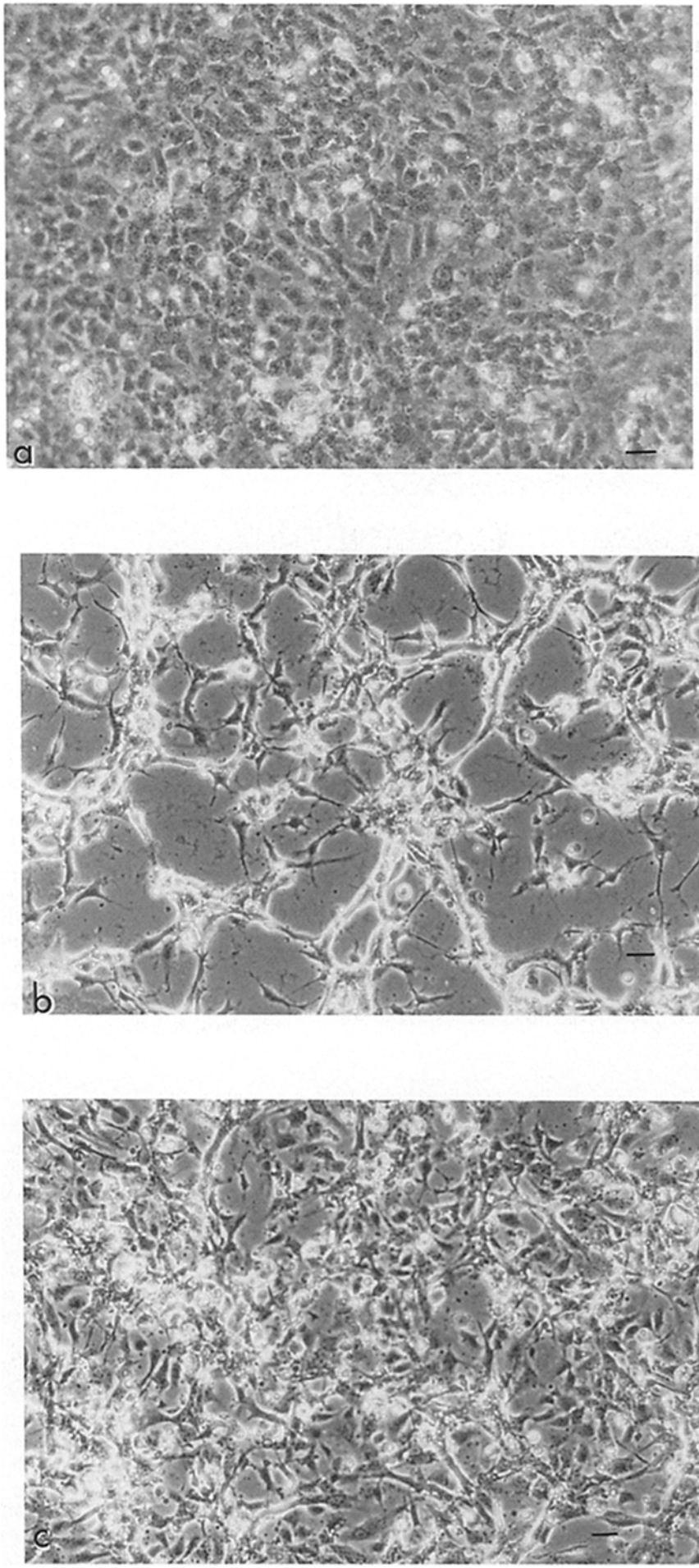

Figure 6. (*a*) HUVE cells cultured for 18 h on collagen I gel showing cobblestone morphology. (*b*) HUVE cells grown for 18 h on collagen I gel supplemented with laminin showing elongation and anastomosing network formation. (*c*) HUVE cells cultured for 18 h on collagen I gel supplemented with collagen IV. Minimal network formation is observed. Bars, 20 μm.

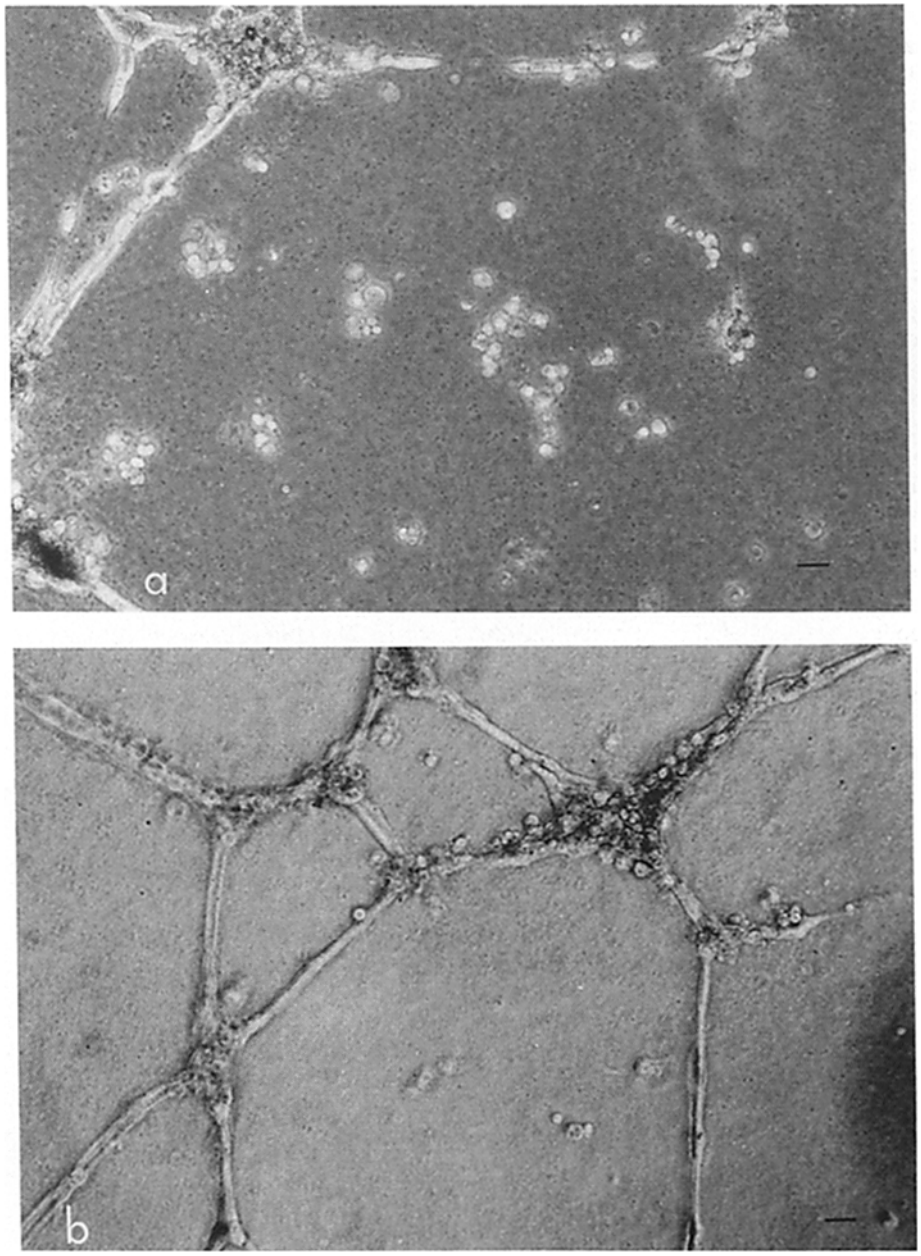

Figure 7. (*a*) HUVE cells cultured on matrigel in presence of synthetic peptide of laminin, YIGSR-NH_2 (200 μg/ml). Inhibition of tube formation is observed. (*b*) HUVE cells cultured on matrigel in presence of control peptide showing tube formation. Bars, 20 μm.

Antibody Blocking Studies

To further assess the roles of laminin and collagen IV in tube formation induced by matrigel, antibodies to either laminin or collagen IV were added along with the endothelial cells to the matrigel-coated dishes. Antibodies to laminin (1:5) caused a marked inhibiton of tube formation, whereas antibodies to collagen IV (1:4 and 1:10) were less inhibitory (data not shown). Preimmune sera had no effect on tubule formation on matrigel. Inhibition of tube formation by anti-laminin antibodies was not due to inhibition of endothelial cell attachment. Pretreatment of cells with a 1:5 dilution of antibody resulted in 70.6%, 1:10 in 62.5%, and 1:500 in 68.8% attachment.

Laminin Peptide Experiments

Because matrigel is known to be ~60% laminin and since anti-laminin antibodies blocked tube formation and laminin added to collagen I gel promoted tube formation, we tested the effects of various synthetic peptides, corresponding to sequences in laminin, on the tube formation process. We found that YIGSR-NH_2, either preincubated with endothelial cells or incorporated into the matrigel, inhibited tube formation in a dose-dependent fashion. At a concentration of 200 μg/ml there was moderate inhibition of tube formation (Fig. 7 *a*). At a concentration of 500 μg/ml, YIGSR-NH_2 completely inhibited tube formation by endothelial cells but also showed some inhibition of cell attachment. Studies with YIGSR showed less inhibition of tube formation, whereas other unrelated synthetic peptides produced no inhibition (Fig. 7 *b*). The amide form of YIGSR also shows greater activity than the nonamide form in other tests (5).

Discussion

Our results clearly indicate that matrigel, a reconstituted

basement membrane, is a substrate that promotes morphological differentiation of large and small vessel human endothelial cells into capillary-like structures. In comparison with other systems, the phenotype change is extremely rapid, with the initial alignment of cells occurring within 1–2 h in vitro. The cells forming the tubules retain characteristics of endothelial cells, namely the presence of factor VIII–related antigen and the ability to take up acetylated low density lipoprotein. These cells do not divide when cultured on matrigel, even in the presence of ECGF and heparin. However, they will resume their cobblestone morphology and begin to divide if they are subcultured on fibronectin. Ultrastructural studies confirm that the anastomosing cytoplasmic extensions of the morphologically differentiated endothelial cells contain a lumen completely encircled by one to two endothelial cells in cross section. The lumen contains various amounts of degenerated cytoplasm, suggesting that a very rapid remodeling of the cell takes place during tube formation. Viability studies of endothelial cells cultured on matrigel do not indicate that cell death plays a prominent role in tube formation. Moreover, these differentiated cells still retain the characteristic Weibel–Palade bodies of endothelial cells. This formation of tubes is apparently specific for endothelial cells, since neither fibroblasts nor smooth muscle cells undergo similar changes.

Our studies also indicate that laminin and perhaps collagen IV contained in the gel structure are the key ingredients in this rapid induction of angiogenesis. This is illustrated by the fact that neither an agar gel nor a collagen I gel is capable of inducing tube formation by endothelial cells. Endothelial cells cultured on collagen I gels supplemented with laminin or collagen IV do undergo tube formation, with laminin as the more potent stimulus. Tube formation on matrigel can be blocked by preincubating the cells with anti-laminin antibodies, while antibodies to collagen IV were less effective in this regard. Furthermore, a synthetic peptide corresponding to the receptor binding–cell attachment site (YIGSR) blocks tubule formation, presumably at the level of the laminin receptor.

Our observations that basement membrane proteins induce the differentiation of endothelial cells are in accord with other observations that this extracellular matrix is able to regulate cell differentiation. Hadley et al. (7), for example, found that Sertoli cells cultured on a reconstituted basement membrane formed monolayers of columnar cells closely resembling Sertoli cells in vivo. However, no attempt was made to define which of the constituents of the gel were important in inducing the morphological changes in these cells. Madison et al. (15) found that the basement membrane gel promoted peripheral nerve regeneration in vivo, and Reh et al. have documented transdifferentiation of retinal-pigmented epithelial cells to neurons by both laminin and matrigel (20). McGuire and Orkin (18) noted that endothelial cells from explants of rat aorta assumed a cobblestone morphology on collagen I but "grew in chains and sheets" from the edges of aortic explants on matrigel. Their photomicrograph depicting this phenomenon bears a striking resemblance to our cultures.

Other investigators previously have demonstrated that, under defined culture conditions, endothelial cells can be induced to undergo tube formation (1, 3, 14, 16, 19). In this regard, Folkman and Haudenschild (3) found that tube formation by capillary endothelial cells from a variety of sources began in areas that were confluent. In primary isolates, differentiation of capillary endothelial cultures occurred between 20 and 40 d of culture, although in later passage cells, tube formation occurred in 10 d or less. These investigators were not able to demonstrate tube formation by large vessel endothelial cells (HUVE cells) or by nonendothelial cells. However, it has been demonstrated that, by shifting HUVE cells from a proliferating to a nonproliferating state by withdrawing ECGF, HUVE cells organize into tubes over a 4–6-wk period (14). This study also suggested that matrix proteins could be important in endothelial cell differentiation because the morphological changes were accelerated by enzyme digestion of the fibronectin matrix. The importance of subendothelial matrix components in regulating the phenotypes of endothelial cells in vitro using rat epididymal fat pad capillary endothelial cells has also been demonstrated (16). These cells grown on interstitial collagens (types I or III) proliferated and formed some tubelike structures after 2–4 wk of culture. In contrast, using a combination of collagen IV and V as a culture substrate, the rat capillary endothelial cells formed tubelike structures after 4 d of culture. In our study, collagen IV was not a potent stimulus for angiogenesis. The differences between our results and those of Madri and Williams (16) may relate to differences in sources of endothelial cells (human vs. rat), culture conditions, and sources of collagen IV. In addition, they found that the rat capillary endothelial cells would also undergo differentiation after 4–5 d of culture on the basement membrane side of a cellular amnionic membrane (16).

The conversion of individual endothelial cells into tubelike structures in our culture system occurs very quickly. The major aspect is the assumption of an organized tube presumably caused by matrix proteins. Presumably, laminin triggers the morphological differentiation of the cells. The rapidity with which this happens on matrigel suggests that this system offers opportunities to define the molecular events in capillary formation.

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