Endothelial Cells from Umbilical Vein and a Hemangioendothelioma Secrete Basement Membrane Largely to the Exclusion of Interstitial Procollagens

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The biosynthesis of extracellular matrix proteins by primary cultures of endothelial cells from human umbilical vein, and by clones from a murine hemangioendothelioma, was studied and compared to that reported for endothelium cultured from other sources. Umbilical vein endothelial cells secreted two glycoproteins—fibronectin and thrombospondin—which comprise the major proportion of the protein in the culture media of bovine aortic, venous, and corneal endothelial cells. These biosynthetic products were absent from hemangioendothelioma cultures. However, in contrast to bovine endothelium from large vessels and cornea, which secrete primarily Type III procollagen into the culture medium, both the umbilical vein and hemangioendothelioma cultures secrete Type IV (basement membrane) procollagen. In addition, EC, a novel endothelial collagen type that has been characterized in bovine endothelial cell supernates, was not present in the umbilical vein or tumor-derived endothelium.

The production of basement membrane procollagen as the major collagen type in the medium of these cultures probably reflects the nature of the vascular bed from which the endothelial cells originated, rather than differences in species or in cellular isolation and subculture. We suggest that endothelial cells from different vascular environments could display variations in growth, migration, morphology, and response to exogenous blood-borne factors as a result of their relationship to an extracellular matrix/subendothelium composed of diverse structural glycoproteins.

(Arteriosclerosis 2:27-36, January/February 1982)
that endothelial cells secrete components that are found in plasma (fibronectin or cold-insoluble globulin), in platelets (thrombospondin), and in association with the coagulation cascade (Factor VIII-associated antigen). Certain structural components of basement membranes, including laminin and Type IV procollagen, have been identified in endothelial cells in culture. In addition, endothelial cells from several different sources have been shown to produce interstitial procollagens as well as a novel collagen type termed "EC." We have proposed that the differences reported among endothelial cells in procollagen type synthesis reflect, in part, the vascular environment from which the cells were derived. Secondarily, cellular isolation and the procedures used to subculture cells may affect collagen production quantitatively. In previous studies we have reported that bovine endothelia from aorta, vena cava, and cornea secrete primarily Type III procollagen, an interstitial type distributed preferentially in vascular and other distensible tissues. In addition, EC collagen and the basement membrane-associated collagen Types IV and V were found in smaller amounts, the latter two specifically associated with the cell layer. However, two types of bovine endothelium displayed an altered biosynthetic profile consisting primarily of Type I collagen: sprouting endothelium from aorta and capillary endothelial cells, which undergo in vitro angiogenesis as described by Folkman and Haudenschild. We have extended our studies to endothelial cells from human umbilical vein (HUV) and from a hemangioendothelioma (HM) for several reasons. Unlike aortic endothelium, HUV cells rest on a continuous, morphologically defined basal lamina, suggesting that differences in collagen type synthesis could occur between these two cell types. HUV cells also do not display the property of sprouting in vitro. In addition, protein synthesis by cultures of malignant endothelial cells has not heretofore been reported. The appearance of a recent report correlating endothelial thromboreistance with the presence of Type V collagen on the luminal surface underscores the importance of platelet-collagen interaction in both thrombosis and hemostasis. Another collagen-related function that could be important in maintaining the integrity of the vascular wall is that of cellular adhesion to a sub-stratum, as reviewed by Kleinman et al. In this respect the murine hemangioendothelioma cultures appeared intriguing, as they were derived from a malignant, although nonmetastatic tumor. In addition, in the presence of exogenous prostacyclin, they displayed surface characteristics similar to those of HUV cells but showed significantly enhanced platelet adherence in the absence of this compound.

**Cell Culture**

**Methods**

Primary cultures of HUV endothelial cells, provided by Dr. J. Harlan (Department of Medicine, University of Washington, Seattle, Washington), were prepared by brief collagenase treatment of the luminal surface of normal-term umbilical cord veins, according to previously described techniques. The cells were established in Waymouth's medium, supplemented with antibiotics and 20% fetal calf serum (heat activated; Grand Island Biological Company, Grand Island, New York), and were maintained in a humidified atmosphere containing 5% CO₂/95% air at 37°C. The cultures consisted of homogeneous populations of endothelial cells which displayed the typical cobblestone morphology and were uniformly positive for Factor VIII-associated antigen by immunofluorescence staining. Confluent cultures were free of smooth muscle cells, both by morphologic criteria and by the absence of radiolabeled interstitial collagen Types (I and III), which are the primary secreted products of these cells. In this study only primary HUV cultures were used.

Hemangioendothelioma (HM) cultures were provided by Dr. J. C. Hoak (Department of Medicine, University of Iowa, Iowa City, Iowa). The cells were prepared from a transplantable tumor that originally developed spontaneously in a 129 mouse strain (Jackson Laboratory, Bar Harbor, Maine) and was subsequently carried in the same strain. The HM cells, when injected subcutaneously into mice, produced rapidly growing, nonmetastatic tumors that appeared morphologically identical to those generated by transplantation among the mice. The animals developed severe thrombocytopenia, microangiopathic anemia, and thrombosis within the vascular channels of the tumor and usually died within 3 weeks after the tumor appeared.

The HM cells were grown in Waymouth's medium supplemented with antibiotics and 20% fetal calf serum (Armour Corporation, Kankakee, Illinois) in a humidified atmosphere of 5% CO₂/95% air at 37°C and were passaged weekly at a split ratio of 1:3 or 1:4. The cultures used in this study were derived from a single clone and were metabolically labeled between passages 15-25.

**Metabolic Labeling**

Cultures were preincubated for 30 to 60 minutes in proline-free Waymouth's medium containing sodium ascorbate (50 μg/ml) and β-aminopropionitrile (64 μg/ml) prior to the addition of L-[2,3-3H]proline (50 μCi/ml; 16–35 Ci/m mole; New England Nuclear, Boston, Massachusetts) in an aliquot of fresh medium. The cells were metabolically labeled for 22 hours and the culture medium initially processed as previously described.
Characterization of Culture Medium Proteins

Initial characterization of radiolabeled protein secreted into the culture medium by HUV and HM cells was performed following precipitation with 10% trichloroacetic acid and/or 50% ammonium sulfate (w/v), as outlined in previous studies. Collagenase (Form III, Advance Biofactures, Lynbrook, New York) digestion was performed as described. Affinity-purified antibodies to human Types I and IV collagen, human CIG, and bovine Type III procollagen were utilized. Antiseras against mouse laminin and Type IV procollagen (a gift from Dr. G. R. Martin, National Institutes of Health, Bethesda, Maryland), rat Type I procollagen, human Type V collagen, and bovine thrombospondin were also incubated with the culture media. These antibodies were highly specific and crossreacted with the appropriate human or mouse antigen by enzyme-linked immunosorbent assay.

Other Procedures

Following ammonium sulfate precipitation, culture medium proteins were fractionated by chromatography on DEAE-cellulose at 4°C in 6 M urea, 50 mM Tris-HCl, pH 8.0 buffer containing protease inhibitors, as previously described. Gradient elution was performed from 0–200 mM NaCl in 400 ml.

Radiolabeled proteins were resolved by sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE) in the presence of 0.5M urea and were visualized by fluorescence autoradiography, as previously described (see also reference list in ref. 20).

Results

Protein Synthesis by HUV and HM Cells

A summary of the levels of protein secreted into the culture medium by HUV and HM cells, based on the incorporation of [3H] proline over a 22-hour period, is shown in table 1. In comparison to cultures of bovine aortic endothelial (BAE) cells, HUV cells exhibited similar rates of protein secretion (approximately 4 × 10⁶ dpm per 10⁶ cells) and very similar levels of collagen synthesis (approximately 4% of the total culture medium protein). In contrast, cultures of HM cells exhibited a significantly higher rate of protein secretion when labeled under similar conditions (24 × 10⁶ dpm/10⁶ cells), but the percent of total protein that was collagenous was very similar (approximately 6%).

Characterization of Collagen and Other Proteins Secreted by HUV Cells

Radiolabeled proteins secreted by BAE and HUV cultures were initially precipitated using trichloroacetic acid and subsequently resolved by SDS-PAGE, as shown in figure 1. BAE cells (lane 1) secrete principally three glycoproteins, in the molecular weight range of 150,000–250,000 after reduction, which have been identified as fibronectin (FN), Type III procollagen, and thrombospondin (GP). HUV cells appeared to secrete at least two of these proteins, fibronectin and thrombospondin (lane 3), by the criterion of mobility on SDS-PAGE in both the presence and absence of reducing agent. Incubation of the culture media with bacterial collagenase, however, revealed differences between the two types of endothelial cells. In the HUV culture medium, three collagenase-sensitive bands that migrated between FN and GP were observed (figure 1, lanes 3 and 4). These three bands can be more clearly visualized in figure 2 (lane 2). In the absence of a reducing agent, they migrated on SDS-PAGE with an apparent molecular weight greater than 450,000 (figure 2, lane 1).

Further identification of proteins in HUV culture medium was performed using radioimmuno-precipitation. When dialyzed culture medium was incubated with affinity-purified antibodies to human Type IV collagen, followed by the addition of a second antibody, a doublet corresponding in molecular weight to two of the collagenase-sensitive bands was precipitated (figure 2, lane 3).
Figure 1. Comparison of proteins secreted into the culture medium by endothelial cells from bovine aorta and human umbilical vein. Cultures were incubated with [3H]proline for 22 hours under the same conditions, as described in Methods. Proteins from the culture media were precipitated using 10% trichloroacetic acid and were subsequently resolved by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) on composite 6% and 10% slab gels in the presence of dithiothreitol (DTT). Fluorescence autoradiogram shows trichloroacetic acid-insoluble protein both before and after incubation with bacterial collagenase.

Lane 1 = bovine aortic endothelial (BAE) culture medium protein-incubated control. Lane 2 = sample in lane 1 after collagenase digestion. Lane 3 = human umbilical vein (HUV) culture medium protein-incubated control. Lane 4 = sample in lane 3 after collagenase digestion. The major components secreted by BAE cells have been identified as fibronectin (FN), Type III procollagen (pro a1(III)), and a glycoprotein (GP) that has been characterized as platelet thrombospondin.

Figure 2. Characterization of proteins in HUV culture medium. [3H]proline-labeled protein from human umbilical vein (HUV) culture medium was precipitated using 10% trichloroacetic acid and was analyzed by SDS-PAGE under both reducing and nonreducing conditions. Aliquots of culture medium were also incubated with rabbit antibodies to human Type IV collagen and to plasma fibronectin. The radioimmune complexes were subsequently precipitated by addition of goat anti-rabbit IgG and were resolved by SDS-PAGE under reducing conditions. Visualization was by fluorescence autoradiography.

Lane 1 = HUV culture medium protein precipitated by 50% ammonium sulfate. Lane 2 = lane 1 after reduction with 50 mM DTT. Lane 3 = radioimmune precipitate of material in lane 2 following incubation with anti-Type IV collagen antibody. Lane 4 = radioimmune precipitate of material in lane 2 after incubation with anti-plasma fibronectin antibody. The position of migration of fibronectin (FN) under both reducing and nonreducing conditions is indicated.

The enhancement of the upper band over the lower in the doublet may reflect selective proteolytic digestion of the pro a2(V) chain during the course of the experiment; this lability has been described by others.22 Using this technique, we estimated that Type IV procollagen represented approximately 4% of the total radiolabeled protein in the culture medium of HUV cells (table 1). No counts per minute (cpm) above background were precipitated when antibodies to any other collagen types were used (not shown). The identity of the collagenase-sensitive component that was not precipitated by anti-Type IV collagen antibodies is under investigation.

Affinity-purified antibodies to human fibronectin selectively precipitated a protein corresponding to fibronectin in mobility on SDS-PAGE (figure 2, lane 4). However, only a fraction of the total cpm in fibronectin (as estimated by densitometric scanning of lane 2, figure 2) was precipitated. (Possible reasons for incomplete precipitation include a relatively low affinity antibody and the presence of other proteins in this fraction. Jaffe and Mosher6 have reported that fibronectin comprised only 15% of the protein secreted by HUV cells into the culture medium.) Other glycoproteins that were identified in HUV culture media included laminin and thrombospondin. Antilaminin antisera precipitated approximately 5% of the cpm in the medium, which, when analyzed by SDS-PAGE, were observed as two bands of approximately 200,000 and 400,000 molecular weight after reduction (not shown). In addition, antibovine thrombospondin IgG, which
has been shown to crossreact extensively with human thrombospondin,\(^7\) selectively precipitated a protein of \(M_r = 190,000\) after reduction, which comigrated with a thrombospondin standard (not shown). We estimated that thrombospondin comprised approximately 20% of the total labeled protein in HUV culture medium, based on radioimmune precipitation and titration.

The three major glycoproteins, Type IV procollagen, fibronectin, and thrombospondin, were further studied by ion-exchange chromatography on DEAE-cellulose. The elution profile consisted of two major peaks and one minor one, as shown in figure 3 A. SDS-PAGE analysis of the material contained within these three peaks is depicted in figure 3 B. Most of the proteins in peak I, which did not bind to the DEAE-cellulose, had molecular weights of less than 60,000. However, a disulfide-bonded component, which after reduction migrated as two chains of \(M_r = 185,000\) (using collagenous standards), was also evident in this fraction. This protein was identified as Type IV procollagen by its behavior in this chromatographic system\(^21\) and by specific precipitation with antibodies to human Type IV collagen.

In addition, preliminary one-dimensional mapping studies using CNBr cleavage indicated a high degree of similarity between the HUV procollagen and Type IV collagen isolated from human placenta by pepsin digestion. It was of interest that a novel collagen type (EC), which has been described in cultures of bovine endothelial cells derived from different tissues,\(^{1,22}\) was not seen following ion-exchange chromatography of HUV culture medium.

The major component in peak II was identified as fibronectin by its position of elution from DEAE-cellulose,\(^21\) by its comigration with human fibronectin on SDS-PAGE under both reducing and nonreducing conditions (figure 3 B), and by its specific precipitation by antibodies to human plasma fibronectin. This protein was also bound to a gelatin-Sepharose affinity column in a neutral pH, low ionic strength buffer and was subsequently eluted using 1 M urea and/or 0.5 M NaCl (not shown). This technique has been used extensively for the purification of both plasma and cellular fibronectins.\(^24\)

Peak III (figure 3 A) contained a single component that migrated very similarly to both bovine and human thrombospondin on SDS-PAGE under reducing and nonreducing conditions. The HUV protein in peak III was also precipitated by antibodies to bovine thrombospondin. Since the recovery of this protein was very low following ion-exchange chromatography (less than 4% of the total recovered cpm) as compared to its estimated level of 20% by radioimmune precipitation of the culture medium, it appeared that HUV thrombospondin was tightly bound to the DEAE-cellulose column.

Figure 3. Fractionation of proteins from HUV culture medium by DEAE-cellulose chromatography. Culture medium from human umbilical vein (HUV) cells, which had been incubated with \(^{3}H\) proline for 22 hours, was initially fractionated by ammonium sulfate precipitation as described in Methods (see figure 2, lanes 1 and 2). Proteins were resolved by chromatography on DEAE-cellulose at \(4^\circ C\) in 6 M urea, 50 mM Tris-HCl at pH 8 using a linear NaCl gradient from 0 to 200 mM in the presence of protease inhibitors. Pooled fractions were subsequently analyzed by SDS-PAGE and visualized by fluorescence autoradiography.

A. Elution profile from DEAE-cellulose. Arrow indicates inception of gradient. Pooled fractions are identified by Roman numerals. B. SDS-PAGE of pooled fractions from A, analyzed both in the presence and absence of DTT. Roman numerals correspond to peak fractions as indicated in A. Outer lanes contain standard proteins which have been identified as fibronectin (FN), Type I pro- and \(p_{\alpha(I)}\)-collagen chains, and the \(\alpha(I)\) collagen chain.
Characterization of Collagen and Other Proteins Secreted by HM Cultures

Precipitation of radiolabeled HM culture medium protein using trichloroacetic acid, followed by SDS-PAGE, revealed a single major high molecular weight component (figure 4, lanes 1 and 2) which was sensitive to bacterial collagenase (figure 4, lanes 5 and 6). After reduction, this collagenous protein migrated as two chains of approximately 185,000 and 175,000 molecular weight. This size difference between the mouse a1 (IV) and a2 (IV) chains is somewhat greater than the difference between the corresponding human chains and agrees closely with values reported for Type IV procollagen isolated from a transplantable mouse tumor by Timpl and co-workers (for a review, see ref. 22). This procollagen was further identified as Type IV by its specific precipitation from the culture medium by affinity-purified antibodies to human Type IV collagen and by specific antisera to mouse tumor Type IV procollagen (lanes 3 and 4). On the basis of radioimmunoprecipitation, we estimated that between 6% and 8% of the radiolabeled protein in the culture medium was Type IV procollagen (table 1). Antibodies to other collagen types did not precipitate any cpm above background.

Affinity-purified antibodies to human plasma fibronectin precipitated approximately 1% of the total cpm in HM culture medium. Components with molecular weights similar to those reported for laminin were not seen in HM culture medium after trichloroacetic acid precipitation or ion-exchange chromatography; however, approximately 10% of the cpm in the starting material was precipitated by anti-mouse laminin antisera. Although this number most probably represents an overestimate of the amount of laminin secreted by HM cells, the presence of two components of M, 400,000 and 200,000 after SDS-PAGE analysis of the radioimmune complex suggested that this protein was a biosynthetic product of this tumor system.

Chromatography of HM culture medium protein following ammonium sulfate precipitation (figure 4, lane 5) revealed a similar pattern with respect to the Type IV procollagen as was seen with HUV medium. As shown in figure 5, three fractions were pooled and subsequently analyzed by SDS-PAGE. Type IV procollagen did not bind to the DEAE-cellulose and was eluted in peak I. This protein was further purified by molecular sieve chromatography on Agarose A-5m, followed by reduction and alkylation. SDS-PAGE analysis of CNBr peptides from both the HM procollagen and mouse Type IV collagen containing a1 and a2 chains showed extensive similarities, further identifying the HM protein as Type IV procollagen (not shown). The presence of EC collagen, the major form of which does not bind to DEAE-cellulose, was not detected in HM culture medium.

Neither fibronectin nor thrombospondin was observed after ion-exchange chromatography of HM culture medium (figure 5). A major proportion of radiolabeled protein was eluted in peak II, which migrated as a broad band on SDS-PAGE with an M, of 72,000–80,000 after reduction (figure 5 B, peak II). The identity of these noncollagenous components is presently under investigation.

Peak III (figure 5) contained only a small proportion of the total cpm in the ammonium sulfate precipitate prior to chromatography; however, it was homogeneous when examined by SDS-PAGE.
and contained a protein of M, 43,000 after reduction. A protein that behaves very similarly on SDS-PAGE and on DEAE-cellulose has been described in bovine endothelial cells cultured from aorta, vena cava, and cornea, and in both bovine smooth muscle cells and human fetal fibroblasts. The identity and function of this 43K protein are presently not known.

Figure 5. Fractionation of hemangioendothelioma (HM) culture medium proteins by chromatography on DEAE-cellulose. [3H]proline-labeled proteins were initially fractionated by ammonium sulfate precipitation (see figure 4, lane 5) and were chromatographed on DEAE-cellulose at 4°C, as described in the legend to figure 3. Proteins in pooled fractions were subsequently analyzed by SDS-PAGE and visualized by fluorescence autoradiography.

A. Elution profile from DEAE-cellulose. Arrow indicates inception of gradient, and Roman numerals identify pooled fractions. B. SDS-PAGE analysis of proteins after DEAE-cellulose chromatography. Roman numerals refer to pooled fractions as indicated in A. The position of migration of standard human Type IV procollagen has been identified. Arrow shows mobility of bovine serum albumin after reduction (M, = 68,000).
Discussion

Endothelial cells cultured from human umbilical vein and a murine hemangiendothelioma were characterized with respect to the secretion of protein into the culture medium. While HUV cells secreted protein at a rate similar to that reported for bovine aortic, venous, and capillary endothelium, HM cells exhibited a fivefold enhanced rate of secretion, compared to the other endothelial cell types (table 1). However, with the exception of capillary endothelial cells that produce proportionately large amounts of collagen, both HUV and HM cells secreted relatively similar levels of collagen in comparison to the large vessel endothelium, representing 3% to 8% of the total protein in the culture medium. These values were based on incorporation of [3H]proline over a 22-hour period under serum-free conditions and were calculated by several different methods.

The collagen secreted by HUV and HM cells was found to consist largely if not exclusively of one genetic type, which was identified as Type IV procollagen by radioimmunoprecipitation, ion-exchange chromatography, and mobility on SDS-PAGE in the presence and absence of reducing agent. Peptide mapping studies further supported the conclusion that Type III procollagen and the novel collagen type EC, which are the major collagens secreted by other endothelial cells (see ref. 11 for a review), were not detectable in either HUV or HM culture media. Two other glycoproteins that comprise a considerably larger proportion of the endothelial cell secreted proteins — fibronectin and thrombospondin — were present in HUV culture media but absent (< 1% of total radiolabeled protein) from HM cultures. These proteins were identified by radioimmune precipitation and by ion-exchange chromatography following fractionation of the culture medium protein by ammonium sulfate in the presence of protease inhibitors.

Endothelial cells from human umbilical vein have been studied extensively in culture by several different groups. Jaffe and Mosher have characterized the secretion of fibronectin from four separate strains of these cells and reported a range of 25 to 77 μg fibronectin per culture flask over a 3-day period. In addition to the implication that endothelial cells serve as the principal source of plasma fibronectin, Maciag et al. have suggested that this protein serves as an attachment factor for HUV cells, thereby permitting clonal growth and survival in culture.

Another high molecular weight glycoprotein apparently identical with, or related to, platelet thrombospondin has been demonstrated immunologically in HUV cultures. This protein has been shown to be a constituent of platelet α-granules and is released upon thrombin-induced platelet aggregation. Thrombospondin has, in addition, been purified and extensively characterized from bovine aortic endothelial cells. In the present study, we have determined that antibodies to bovine thrombospondin selectively precipitated a component from HUV culture medium that had an apparent molecular weight very similar to that of the bovine protein. Although the function of thrombospondin is presently not known, possibilities include a role in the clotting process or as a cell attachment factor.

Earlier studies have implicated the synthesis of Type IV procollagen by HUV cells in culture. These identifications were based largely on immunofluorescence data and on posttranslational modifications, including the levels of hydroxylation of prolyl and lysyl residues, hydroxylysyl glycosylation, and apparent molecular size after pepsin treatment. We and others have shown, however, that both the type and level of posttranslational modification are variable among collagens of a single type. Additional criteria were therefore utilized in the present study.

The recovery of Type IV as the principal collagen type in the culture medium suggests that HUV and HM endothelial cells differ from the endothelial cells cultured from bovine aorta, vena cava, and cornea. The latter group of cells secreted primarily interstitial Type III procollagen, which could be recovered from both the culture medium and the cell layer, and the novel collagen Type EC. Although both Types IV and V collagen have been localized to the arterial subendothelium and to the extracellular matrix produced by bovine aortic endothelial cells in vitro, there is disagreement regarding the synthesis of Type III procollagen by these cells. We feel that this discrepancy is due to differences in tissue culture methodology and techniques for identification of procollagen. Such differences may also account for reports indicating that Type IV collagen is retained exclusively in the cell layer.

This study has also shown that endothelial cells cultured from a murine hemangiendothelioma secrete Type IV procollagen into the culture medium; however, neither fibronectin nor thrombospondin was detected. The absence of fibronectin in this malignant cell line correlates with the selective reduction of this cell-surface protein in transformed or tumor-cell-derived cultures (for a review, see ref. 24). From a recent ultrastructural study of capillary and cavernous hemangiomas in skin, it was concluded that hemangioma histogenesis paralleled very closely the development of embryonic and fetal capillaries. Such vasculogenesis occurred through early angioblastic anlagen and later by sprouting from preexisting vessels. Unlike many previously described tumors consisting of well-formed endothelial cells enclosed by continu-
ous, multilayered basal laminae, the murine hemangioendothelioma used in this study was composed of very immature, disorganized neoplastic endothelium. The thrombocytopenia and intravascular coagulation associated with the murine tumor could result from exposure of subendothelial collagen or other extracellular matrix components to the blood, as shown by Hoak et al. In this regard it was found that HM cells in culture were thrombogenic, exhibiting a level of platelet adherence of 67%, as compared to 74% for venous smooth muscle cells and 4% for HUV cells. This effect was reduced to control levels in the endothelial cultures by the addition of prostacyclin, but was only partially overgrown by the presence of HM cells, as Madri et al. have claimed that arterial endothelial thromboresistance was due to the presence of Type V collagen on the luminal surface. However, aggregated forms of both basement membrane (Type IV) and cell-surface-associated (Type V) collagen have been shown to elicit platelet aggregation in vitro. Although this issue is not yet resolved, the findings of Barnes et al. support the conclusion that the altered degree of platelet adherence of HM cells, as compared to that of HUV cells, was not due to Type IV procollagen but to another cell surface property.

Data on endothelial cell transformation have been sparse. Zetter et al. have described altered cell surface characteristics, fibronectin distribution, and platelet binding by aortic endothelium exposed to a chemical mutagen. In addition, HUV cultures transformed by SV40 DNA exhibited differences in growth potential, protein synthesis, and morphology and in the mode of procollogen secretion. The present report is the first that characterizes extracellular protein synthesis by malignant endothelial cells in culture, which share several of the properties described for transformed endothelium.

It is clear that recognizable differences exist among endothelial cells from various vascular beds. Morphologic differences between aortic and venule endothelium occur with respect to cell thickness, intercellular junctions, and the structure of the underlying basal lamina. Endothelial cells derived from human umbilical arteries and veins exhibited differences in the concentration of insulin receptors on the cell surface. In addition, mast cell heparin has been shown to stimulate migration of bovine capillary, but not aortic, endothelial cells. Several striking differences in endothelial and microvessel regeneration have suggested that interactions between endothelial cells and their extracellular matrix determine, at least in part, the migratory, proliferative, and biosynthetic responses of these cells to injury. In large vessels, reendothelialization occurs by migration of the cells from the edge of the lesion, while neovascularization as a result of soft tissue injury or in response to angiogenic (tumor) factors occurs through vascular budding and lumen formation, resulting in new capillary outgrowth. Although the involvement of collagens and/or other extracellular matrix components in these processes is not known, the biosynthesis of specific collagen types by endothelium from different vascular environments suggests a reciprocity between cell behavior and biosynthetic pattern.

The importance of endothelial extracellular matrix components including those in basement membranes has been emphasized by the work of Kramer et al., who have demonstrated the preferential adherence of certain tumor cells to vascular endothelium and endothelial extracellular matrices, processes that could be mediated by fibronectin or collagen. Moreover, Liotta et al. determined that tumor cell strains of increasing metastatic potential secreted higher levels of Type IV collagenase, a neutral protease that specifically degrades Type IV collagen with concomitant disruption of basement membrane. Such fragmentation, which is often associated with tumor extravasation, is thought to result in increased vascular permeability, as has been described for granulocyte infiltration during inflammation and in cellular detachment.

A recent report has described differences, based on sensitivity to collagenase, in the strength of endothelial cell attachment to the underlying aortic intima between pig, a species susceptible to atherosclerosis, and rat, which is normally not predisposed to the disease. These authors also found differences in cellular GAG synthesis and proposed that the composition of the extracellular matrix was partially responsible for the differential rates of endothelial regeneration after injury in these animals. Several proteins secreted by endothelial cells are candidates for mediating their attachment to the subendothelial layer, including fibronectin, laminin, and several collagen types (see ref. 15 for a review). Studies of endothelial cell attachment in vitro have indicated a preferential adhesion of sheep aortic endothelium to Type IV collagen. Further experiments in this area should elucidate the mechanism and specificity of endothelial cell attachment in different vascular beds and provide a rationale for exploring detachment and regeneration of endothelium in response to injury.

Acknowledgments

We thank our colleagues, Drs. Stephen Schwartz, John Harlan, and John Hoak, for their collaboration in this study. The assistance of Pam Pritzl, K. Stanness, Karin Gochoel, and Clarisse Martin is also gratefully acknowledged.
References


Index Terms: endothelial cells • endothelioma • extracellular matrix • procollagen synthesis
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doi: 10.1161/01.ATV.2.1.27

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