Expression of the CD34 Gene in Vascular Endothelial Cells

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All seven of a set of CD34 monoclonal antibodies that recognize epitopes on an ≈110 Kd glycoprotein on human hemopoietic progenitor cells also bind to vascular endothelium. Capillaries of most tissues are CD34 positive, as are umbilical artery and, to a lesser extent, vein, but the endothelium of most large vessels and the endothelium of placental sinuses are not. Angioblastoma cells and parafollicular mesenchymal cells in fetal skin are also CD34 positive, as are some stromal elements. An ≈110 Kd protein can be identified by Western blot analysis with CD34 antibodies in detergent extracts of freshly isolated umbilical vessel endothelial cells, and CD34 mRNA is present in cultured umbilical vein cells as well as other tissues rich in vascular endothelium (breast, placenta). These data indicate that the binding of CD34 antibodies to vascular endothelium is to the CD34 gene product, and not to crossreactive epitopes. Despite the presence of CD34 mRNA in cultured, proliferating endothelial cells, the latter do not bind CD34 antibodies. In addition, CD34 antigen cannot be upregulated by growth factors. We conclude that under these conditions, CD34 protein is downregulated or processed into another form that is unreactive with CD34 antibodies. Electron microscopy of umbilical artery, breast, and kidney capillary vessels reveals that in all three sites, CD34 molecules are concentrated on membrane processes, many of which interdigitate between adjacent endothelial cells. However, well-established endothelial cell contacts with tight junctions are CD34 negative. CD34 may function as an adhesion molecule on both endothelial cells and hematopoietic progenitors.

THE CD34 MONOCLONAL antibodies recognize a cell surface antigen of ≈110 Kd that is expressed selectively on human hematopoietic progenitor cells, including myeloid and lymphoid progenitors,[1-6] primitive, marrow stroma-dependent progenitor cells,[7] and cells capable of reconstituting lethally irradiated baboons.[8] This unique specificity provides an opportunity to exploit positive selection of stem cells for growth factor regulation studies, gene transfection, and transplantation. The biochemical structure and partial amino acid sequence of the CD34 molecule have been described in some detail.[5,9] A full length cDNA has been cloned and sequenced (DL Simmons, personal communication, December 1988), and the gene has been mapped to chromosome 1q.[10] However, neither the structural and sequence data nor functional studies with antibodies have so far been able to determine the role of the CD34 antigen in early hematopoiesis, which remains an enigma.

The CD34 monoclonal antibodies MY10 and BI-3C5 have also been observed to bind to capillary endothelia in some normal tissues and tumors.[1,5] Obviously, this observation has some implications for the possible function of the CD34 antigen, but it is unclear if this reactivity is a consistent feature of CD34 antibodies and if it represents binding to the CD34 gene product or a crossreactive epitope. The latter possibility gains credence from the observation that most CD34 epitopes are carbohydrate-dependent.[5,6,9] In order to resolve this issue and also to further pursue the possible function of the CD34 protein, we have analyzed a series of CD34 monoclonal antibodies for their reactivity with endothelial cells of different tissues, determined the topographical localization of bound antibody, and screened vascular endothelial cells for CD34 mRNA and protein.

MATERIALS AND METHODS

Antibodies, tissues, and staining. Established and candidate CD34 monoclonal antibodies used in this study were MY10,[1] 12.8,[4] BI-3C5,[2] TUK3, p115.2,[6] and ICH3.[5] These antibodies were raised against the leukemia cell lines KG1/KG1a and were all submitted for verification as CD34 reagents to the recent Fourth Leukocyte Antigen Workshop in Vienna, Austria.[6] An additional CD34 antibody, QBEND10, was also included in this study. This reagent was raised against placental endothelial membrane vesicles, as will be described in detail elsewhere.[12] Other monoclonal antibodies used included the anti-von Willebrand factor or factor VIII related antigen (vWF:Ag)[13] to delineate tissue endothelial cells; anti-smooth muscle actin,[14] BF-G6 anti-embryonic myosin,[15] HMB45 anti-melanocyte[16]; 2F11 anti-neurofilament protein and V9 anti-vimentin 57 Kd intermediate filament (Dako, Bucks, UK); 9.4[17] specific for the pan-hematopoietic T200/CD45 molecule, RR1/1,[18] which reacts with the cell adhesion molecule I CAM 1; and Ki-67, which stains the nuclei of all dividing cells.[19]

Fetal tissue was obtained from abortions through the Royal Marsden Hospital Foetal Tissue Bank (Dr L Wong) and with the approval of the Hospital Ethics Committee. Adult tissue sections were provided from mammoplasty operations or postmortems and were provided by Dr B Gusterson (Royal Marsden Hospital, London, UK) and Dr G Cattoretti (Department of Anatomical Pathology and Cytology, Instituto Nazionale Tumori, Milan, Italy).

Immunohistochemical staining was done with the APAAP technique[20] with a kit obtained from Dako, on cytospins and cryostat tissue sections, air dried overnight and fixed in acetone (5 minutes at room temperature). Breast tissue sections were obtained from

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specimens fixed in methacarn (methanol:inhibisol:acetic acid, 60:30: 10[21] and were paraffin-embedded; before immunostaining, the sections were dewaxed as described.[21]

Electron microscopy. Samples of human umbilical artery and vein were fixed in 4% paraformaldehyde plus 0.05% glutaraldehyde in phosphate buffer, and small pieces of human breast and human kidney were fixed in 4% paraformaldehyde in phosphate buffered saline (PBS). Both tissues were embedded by the progressive lowering of temperature method: 30% ethanol at 0°C, 50% ethanol at −18°C, 75% ethanol at −35°C, 100% ethanol at −35°C, and 100% ethanol at −50°C for 1 hour each, followed by 100% ethanol overnight. Embedding in lowicryl HM20 followed manufacturers' recommendations. Samples were UV-polymerized in a Reichert CSAuto (Cambridge, UK) at −50°C for 72 hours.

Ultrathin sections were immunostained with the CD34 antibody QBEND10 diluted 1:100 in PBS (18 hours) and, after three PBS washes, were incubated with 10 nm gold (umbilical artery or vein) and 5 nm gold (human breast) conjugated to goat anti-rat immunoglobulins (Janssen, ICN Biomedicals, High Wycombe, UK) diluted 1:20 in PBS (90 minutes). The 5 nm gold was silver-enhanced for 6 minutes using IntenSe M (Janssen). Sections were double-stained in an LKB Ultrostainer (Selsdon, UK). Control sections were incubated in the absence of the primary antibody or with a control antibody.

Endothelial cells. Human endothelial cells were released from washed umbilical cord artery and vein using Collagenase (Worthington, NJ) essentially as described previously.[22] The detached cells were flushed from the blood vessels and washed, and aliquots were stained with vWF:Ag or anti-T200 (CD45) antibodies to assess the purity of the endothelial cell preparations.

Immunoblotting. Cell lysates of KG1a cells and fresh endothelial cells were prepared and subjected to either sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and Western blotting directly or immunoprecipitation with the anti-CD34 antibody TUK3. This reagent was used in all immunoprecipitations, since it most efficiently immunoprecipitates native and desialylated forms of the CD34 molecule.[6,23]

Immune complexes thus made from KG1a or endothelial cells were absorbed onto protein A-Sepharose 4B (Pharmacia, Uppsala, Sweden), washed, and analyzed by SDS-PAGE.[5,9] A duplicate pair of immune complexes were incubated with Vibrio cholera neuraminidase (BDH Chemicals, Poole, UK) for 30 minutes at 37°C before analysis by SDS-PAGE. Separated proteins were electrophoretically transferred to nitrocellulose,[24] and the immunoblots were developed using QBEND10 followed by rabbit anti-mouse immunoglobulin and ^{125}I-Protein A (Amersham International, Oakville, Ontario, Canada), as described in detail elsewhere.[5] QBEND10 was chosen in preference to other CD34 antibodies because it binds with equally high efficiency to both native and desialylated CD34 antigen in immunoblots.[25]

Cell culture. Human umbilical vein endothelial (HUVE) cells were maintained in culture under standard conditions, on 1% gelatin coated flasks with 199 medium (GIBCO, Uxbridge, UK) supplemented with 20% fetal calf serum (FCS), 90 μg/mL heparin (Sigma, Poole, UK), and 20 μg/mL endothelia growth supplement (EGS) (Sigma). We used 10 to 15 passage old HUVE cells in this study. In some experiments, HUVE cells were grown on Matrigel (Flow, Rickmansworth, UK) coated plates in the presence of various growth factors (see text).

mRNA analysis. Poly-A RNA from cultured HUVE cells, umbilical cord, normal breast biopsy, and placenta was isolated, Northern-blotted by standard methods,[24] and hybridized to a human CD34 cDNA probe provided by Dr DL Simmons (Institute of Molecular Medicine, Oxford, UK).

Transfection of COS cells. A cDNA coding for the CD34 antigen, cloned in a eukaryote expression vector, was transfected into COS-1 cells[26] by the DEAE dextran procedure, as described.[27] The transient expression of the transfected cDNA was tested 72 hours after transfection by APAAP staining of acetone-fixed cytospin preparations and by flow cytometry of viable, unfixed cells using fluorescent antibodies.

RESULTS

Verification of CD34 antibodies. The prototypic CD34 antibodies MY10[1] and BI-3C5,[2,3] together with 12.8[4] and ICH3,[5] appear to react with the same ≈110 Kd glycoprotein on KG1a cells and CD34 reactive fresh leukemia samples. These reagents, together with the more recently produced TUK3, p115.2, and QBEND10, were assessed as part of the Fourth Leucocyte Antigen Workshop.[6] All antibodies immunoprecipitated the same ≈110 Kd structure from radioiodinated KG1a cell lysates, albeit with different efficiencies.[6] Antibodies MY10, BI-3C5, ICH3, and QBEND10 identify CD34 in immunoblots, but only QBEND10 and, to a lesser extent, ICH3 detect the desialylated CD34 structure in this assay.[6,25]

A definitive test for the CD34 specificity is to assess the reactivity of candidate antibodies with COS cells that have been transfected with a full length CD34 cDNA in an expression vector. The CD34 cDNA was, in fact, cloned in this way using the antibody MY10 as a marker for protein expression. Therefore, we screened all of the above antibodies against CD34 cDNA-transfected COS cells and assessed antibody binding by both APAAP staining of acetone fixed cells and flow cytometry of viable cells. All seven antibodies had unambiguous CD34 reactivity, as shown in Fig 1. As anticipated, only a proportion of COS cells were CD34 positive (5% to 30% in different experiments).

No binding of CD34 antibodies was observed in nontransfected COS cells or in COS cells transfected with an irrelevant cDNA (CD3δ). Neither COS cells transfected with CD34 cDNA nor sham transfected COS cells stain with isotype specific controls (IgM, IgG_1, IgG_{2a}, IgG_3) followed by fluorescein-labeled anti-mouse IgG/M.

Immunohistochemical analysis of CD34 antigen in tissues. The reactivity of the CD34 certified panel of antibodies with various fetal and adult (normal, inflammatory, and neoplastic) tissues was assessed and compared with that of the endothelial marker vWF:Ag (Table 1).

All seven CD34 monoclonal antibodies (MoAbs) gave a similar pattern of reactivity, although binding to stromal elements was variable. Although CD34 antibodies had strong reactivity with vascular endothelial cells, the binding of vWF:Ag and CD34 antibodies were not concordant.

Capillaries from most tissues were CD34 positive (+), with the exception of fetal liver and adult central nervous system (CNS) structures, where only a small percentage of capillaries were labeled. Large vessels of the umbilical cord (especially the artery, Fig 2A) and of the fetal foot were CD34+; conversely, large vessels in fetal liver and in adult kidney and hypophysis were mostly CD34−, though vWF:Ag+. Striking differences in CD34 expression were observed in the placenta between fetal capillaries and endo-

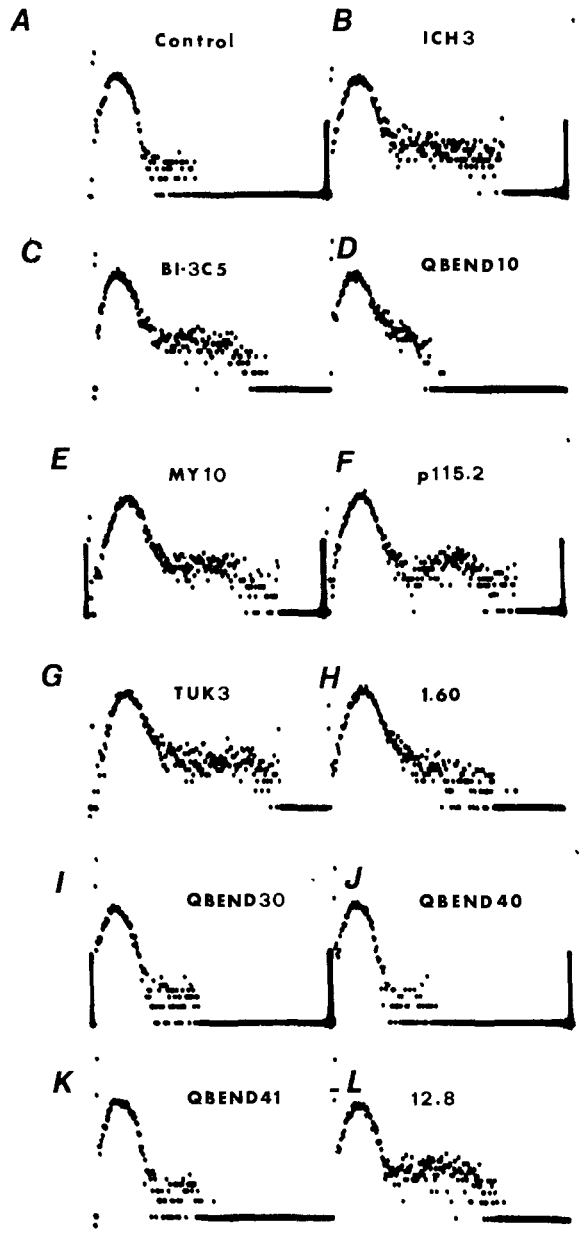


Fig 1. Analysis by flow cytometry of CD34 antibody binding to COS cells transfected with CD34 cDNA. Axes: horizontal, relative fluorescence intensity; vertical, relative cell number. ICH3, BI-3C5, QBEND10, MY10, p115.2, TUK3, 1.60, and 12.8 are CD34 antibodies. QBEND30, QBEND40, and QBEND41 are anti-endothelial MoAbs[30] used as additional controls.

thelium lining the maternal lacuna or sinuses. The placental capillaries were CD34+ but only weakly stained for vWF:Ag, whereas the endothelium of the maternal sinuses were CD34− (or very weakly positive) but vWF:Ag+ (Fig 2C and D). The endothelial cells of hepatic sinuses in the fetal liver were also CD34−.

In the fetal skin, CD34+ cells form a nest around the base of developing follicles (Fig 2G); the mesenchymal origin of these cells is suggested by their reactivity with the anti-vimentin MoAb, the absence of hematopoietic, muscular, neural, and melanocytic markers (data not shown), and lack of pan-endothelial, vWF:Ag staining (Fig 2H). In addition, these cells appear in a resting state, since they are negative with the proliferation marker Ki67,[19] contrary to the nearby keratinocytes that are strongly positive. In adult skin, reticular fibers around hair follicles appeared to be CD34+. Also, the basal membranes of the mammary, sebaceous, and apocrine sweat glands appeared to be CD34+. This possibility was investigated in detail with methacarne fixed, paraffin-embedded breast sections using other antibodies, including 24.128 reactive with basement membrane (collagen IV) for comparison. In these sections where morphologic integrity was well preserved, it appeared that the CD34 staining around ducts with QBEND10 was associated not with basal membranes but with a closely associated network of capillary endothelial cells (Fig 2E). The pattern of reactivity in breast and skin sections, as well as other tissues (Table 1), also suggested some staining of stromal matrix (Fig 2E, arrow).

Ultrastructural localization of CD34 antigen. The distribution of CD34 molecules on umbilical cord vessel, kidney, and breast capillary endothelium was studied by an immunogold/electron microscopy method. Using this technique, QBEND10 but not ICH3 was able to recognize CD34 antigen. Label was associated with cell surfaces, particularly lumenal membranes (Fig 3A). There was a striking concentration of CD34 molecules in regions of cell surface activity characterized by extended processes or villi (Fig 3B through D). These were usually close to the lumenal side and often appeared to form interdigitating surfaces between adjacent endothelial cells (Fig 3A, B, and C). In some instances, the plane of section makes it difficult to assess the positioning of CD34 membrane processes relative to the symmetry of endothelial cells. Regions of established contact between endothelial cells involving tight junctions (Fig 3B, arrow) were free of label.

Detection of CD34 mRNA. CD34 mRNA isolated from cultured umbilical cord vessel endothelial cells, breast tissue, and placental tissue can be detected on Northern blots and is the same size (2.3 kb) as CD34 mRNA in KG1 hematopoietic cells (Fig 4), showing that endothelial cells do express the CD34 gene.

Immunoblotting. QBEND10 identified a band of ≈100 to 110 Kd in Western blots of whole cell lysates of KG1a cells and freshly isolated endothelial cells (Fig 5, lanes A and B). Similar bands were detected on Western blots of immune complexes made from KG1a or endothelial cells using TUK3 (Fig 5, lanes C and E). When these immune complexes were subjected to cleavage with neuraminidase before SDS-PAGE and Western blotting analysis, the CD34 antigen was resolved at ≈150 Kd in both cases (Fig 5, lanes D and F). Considerably less CD34 antigen was detected in solubilized extracts of endothelial cells compared with KG1a cells (Fig 5, lane B, compared with lanes A and E, compared with lane C). There was also a suggestion that the antigen might be of slightly higher molecular weight in endothelial cells compared with KG1a (Fig 5, lane E, compared with lane C). This small difference was not observed in immunoblotted whole cell lysates (Fig 5, lanes A and B); in this situation, other solubilized material and detergent may influence relative mobilities. Further studies are necessary to determine if

Table 1. CD34 and vWF:Ag Expression in Various Fetal and Adult (Normal and Neoplastic) Tissues

Tissue	CD34		Other Reactivities	vWF:Ag	
	Cap	Vess		Cap	Vess
Fetal					
Foot	+++	++		++	++
Kidney	+++	++	Capsule and perivascular stroma	−	+
Heart	+++	++		−	+
Cerebral cortex	+/−	+	Rare large cells (nests)	+	++
Liver	+/−	+	Rare cells surrounding vessels	+/−	+
Skin	+++		Mesenchymal perifollicular cells; subcut. matrix (?)	++	++
Adult					
Kidney	+++	+/−		++	+++
Skin	++		(see text)	++	
Breast	++		(see text)	++	
Spinal cord*	+†	+		+†	+++
Cerebellum*	+†	+	Stromal matrix (?)	+†	+++
Olivary nucleus*	++	+		++	+++
Striatum*	+†	+		+†	+++
Hypophysis*	++	+ (artery ++; vein −)		+	+++
Pathologic					
Granulation tissue	++			++	
Angiomyosarcoma	+		Tumor cells +++	+	
Leiomyosarcoma	+			+	
Hypernephroma	+	+		+	+

Degree of reactivity indicated by +; +/− to +++, weak to strong staining with antibodies; −, no staining.

Abbreviations: CAP, capillary; VESS, large vessel.

*Postmortem specimens.

†vWF:Ag and CD34 reacted with a minority of capillaries.

endothelial cell CD34 protein is consistently of a higher molecular weight and perhaps processed differently from KG1a CD34 protein. The endothelial cell preparations used in these experiments contained less than 5% CD45/T200 positive cells (ie, leukocytes), and the few that were present were mainly granulocytes. Therefore, CD34+ cord blood progenitor cells[28] are unlikely to contribute significantly to the observations made. These data indicate that freshly isolated endothelial cells express bona fide CD34 antigen.

Cultured HUVE cells. We found that freshly isolated HUVE cells are CD34+ (greater than 90%), but by passage 10 to 15 are CD34−, despite having detectable amounts of CD34 mRNA. Binding of vWF:Ag antibody, PAL-E,[29] and other endothelial cell MoAbs[30] verify that the cultured cells are indeed derived from vascular endothelium.

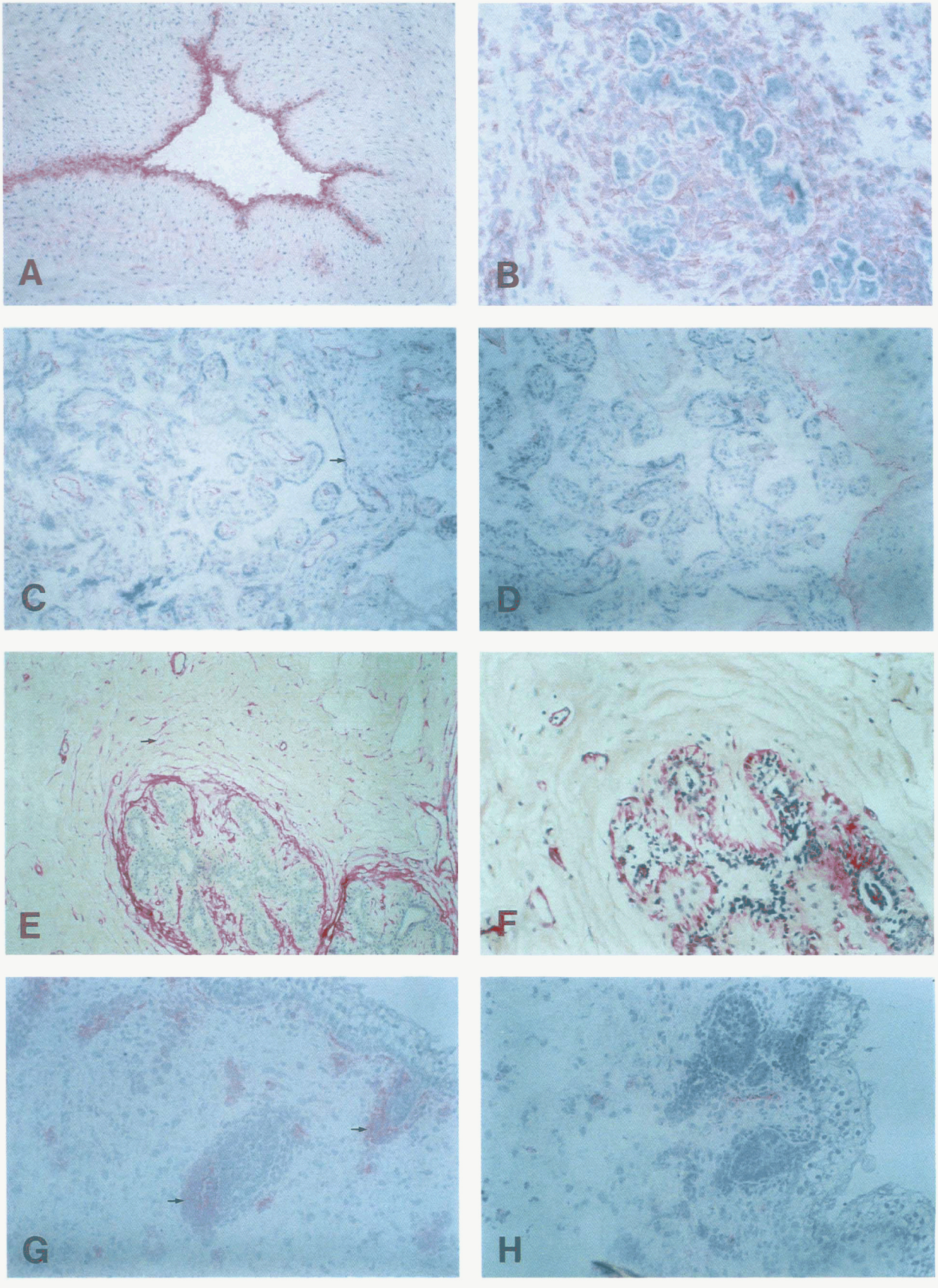
We investigated whether different culture conditions might maintain or induce the expression of the CD34 molecule or its epitopes. HUVE cells were grown for 48 hours on 24-well plastic plates (Costar, Cambridge, MA) and precoated with either 1% gelatin or Matrigel (Flow; coating with the latter was carried out at 4°C as specified by the suppliers), in the presence of the following cytokines used alone or in cross combinations: recombinant human interleukin-1α (IL-1α) and IL-1β (Genzyme, Boston, MA), used at 5 U/mL; purified human transforming growth factor-β (TGF-β) and bovine beta-fibroblast growth factor (FGF; British Biotechnology, Oxford, UK), used at 1 ng/mL.

In these experiments, other media supplements in addition to the standard HUVE culture medium were also assayed: medium 199 with 10% human serum or human plasma, with or without EGS or heparin. The cells were immunostained in situ by APAAP and, after fixation for 5 minutes with 2% paraformaldehyde, with MoAbs specific for vWF:Ag, CD34, and ICAM-1. The latter was included as positive control, since it is known that IL-1 upregulates the expression of adhesion molecules on cultured endothelial cells.[31] Under these culture conditions, no expression of CD34 antigen was detected; however, vWF:AG was always strongly positive, and ICAM-1 was upregulated by IL-1 (ie, considerably higher intensity of staining).

DISCUSSION

CD34 MoAbs bind selectively to hematopoietic progenitor cells[1-7] and have all been raised against the leukemic progen-

Fig 2. Immunohistochemical staining of tissues with CD34 antibodies. Sections prepared as detailed in Materials and Methods and stained by the APAAP technique. (A) Umbilical artery stained with ICH3. (B) Angioblastoma stained with IC3. (C) Placenta stained with ICH3 showing reactivity of fetal capillaries and lack of staining on maternal sinusoidal endothelial cells (arrow). (D) Placenta stained with anti-vWF:Ag showing weak reactivity of fetal vessels and strong staining of maternal sinusoidal endothelial cells. (E) Breast section stained with QBEND10 showing staining of periductal capillary endothelial cells and stromal matrix (arrow). (F) Breast section stained with a MoAb 24.128 to basement membrane collagen IV.[48] (G) Fetal skin section stained with ICH3 showing CD34+ perifollicular mesenchymal cells (arrow). (H) Fetal skin section stained with anti-vWF:Ag. CD34+ perifollicular cells are vWF:Ag−.



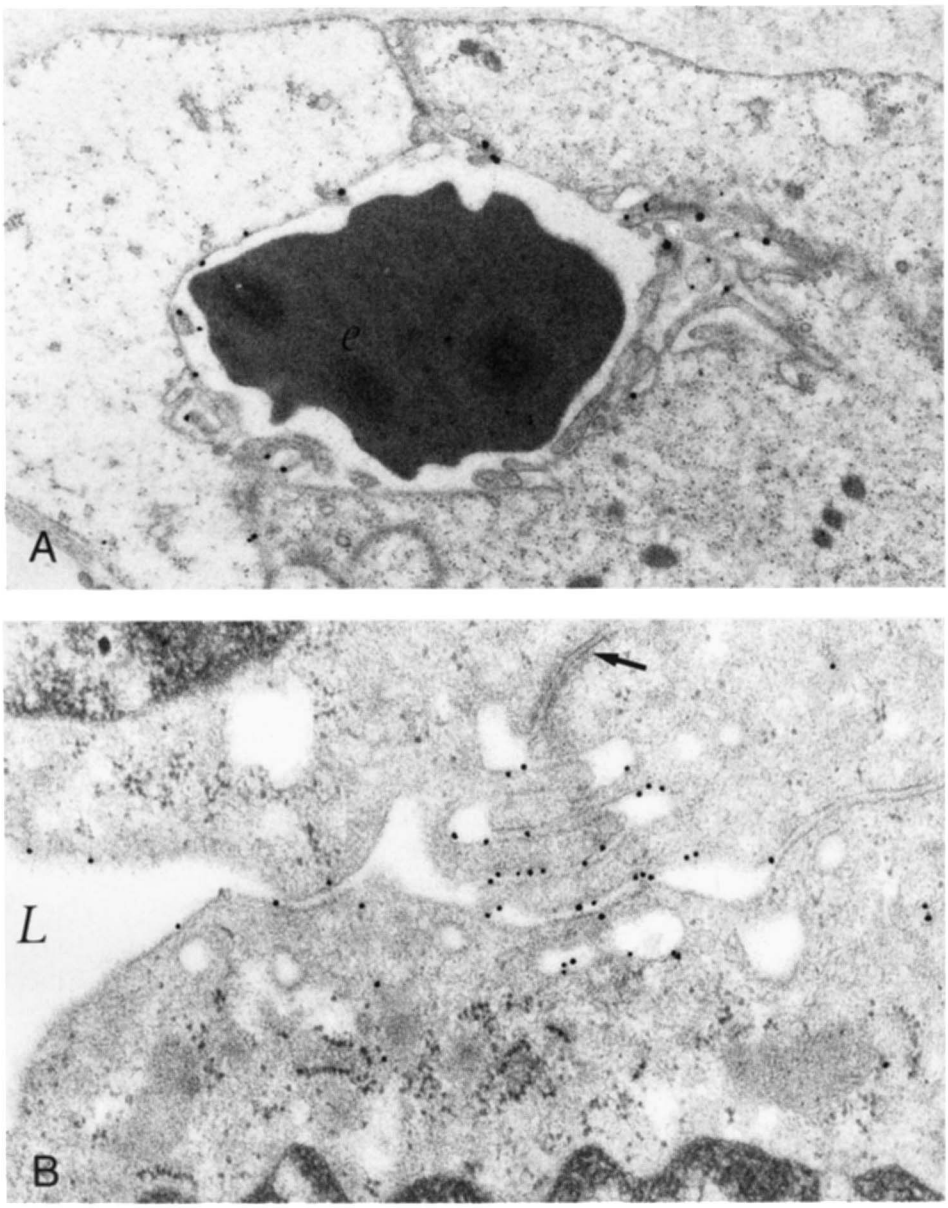


Fig 3. Ultrastructural localization of CD34 antigen. Panels A through D are electron micrographs of endothelial cells. The sections have been reacted with QBEND10 antibody localized with colloidal gold markers as described in Materials and Methods. (A) Capillary endothelial cells from human breast. The majority of the gold marker is located on the lumenal surface of the endothelial cells. Original magnification, ×29,000; e, erythrocyte. (B) A region of opposing membranes of two endothelial cells from human umbilical artery. Some colloidal gold is present on the lumenal membrane (L) of the cells, but the majority of the gold marker is located on the lateral membranes of the cells, where they form complex interdigitations. Colloidal gold is not present on the membrane in areas of cell junctions, such as tight junctions (arrow). Original magnification, ×77,500. (C) Human breast endothelial cells. The colloidal gold marker is localized predominantly on the endothelial cell membrane where adjacent cells form complex interdigitations. Original magnification, ×27,000; e, erythrocyte. (D) Endothelial cell from a different sample of human breast. The gold marker is located on structures similar to those seen in the umbilical artery shown in B. Original magnification, ×39,000; e, erythrocyte. Note that the uneven size of gold particles in A through D is an effect of the silver enhancement procedure.

itor cell lines KG1 and KG1a[32,33] with the exception of QBEND10, for which a placental perfusion preparation was used as an immunogen. As reported here, all seven antibodies bind to the same monomeric glycoprotein of ≈110 Kd extracted from KG1/KG1a cells, and binding of all seven antibodies to COS cells is dependent upon the activity of a transfected CD34 cDNA. Therefore, there is concordance of reactivity with the product of a single CD34 gene, recently mapped to chromosome 1q.[10] The full length CD34 cDNA sequence predicts a type I integral membrane protein of ≈40 Kd with a maximum of nine potential N-glycosylation sites (DL Simmons, personal communication, December 1988). Since the de–N-glycosylated and desialylated forms of cell surface derived CD34 antigen are ≈90 Kd and ≈150 Kd, respectively,[9] the native molecule must contain a considerable amount of O-linked carbohydrate. Indeed, several CD34 antibodies recognize sialic acid-dependent epitopes, and most, if not all, may detect carbohydrate-dependent epitopes rather than the protein backbone.[5,6,9]

Previous studies have noted the reactivity of the CD34 antibodies MY10[11] and BI3C5[5] with capillaries in several tissues, as well as tumors. The experiments reported here demonstrate that all seven CD34 antibodies bind to vascular endothelium, that an ≈110 Kd protein extracted from freshly isolated umbilical vessel endothelium can be identified with CD34 antibodies in Western blots, and that cultured umbilical vein endothelial cells, as well as other tissues rich in vascular endothelium (breast, placenta), express a CD34 cDNA mRNA of the same size (2.3 kb) as that found in KG1 cells. These data establish unequivocally that the reactivity of CD34 antibodies with vascular endothelium is to the CD34 gene product and its associated epitopes, and not to some other crossreactive structure.

Not all endothelial cells in vivo are CD34+. Although at

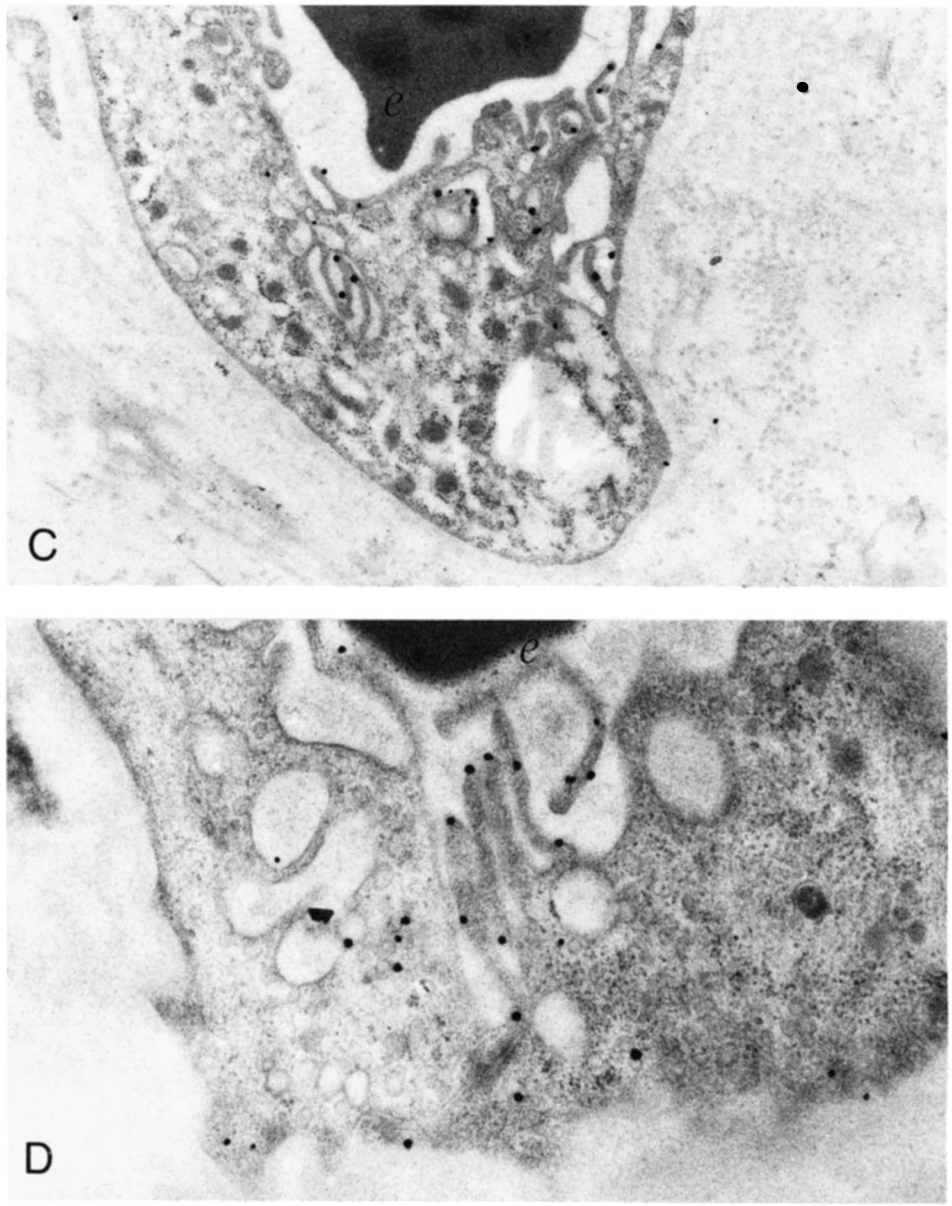


Fig 3. (cont'd).

least some capillaries in all tissues examined were positive, the degree of staining varied greatly. The endothelial cells of many large veins and arteries were CD34−, as was the endothelial lining of placental and fetal liver sinuses. Lymphatic endothelium is also CD34−.[34] In these respects, CD34 antibody binding is discordant with other markers of vascular endothelium, including Factor VIII and *Ulex europaeus* lectin, but is similar to a previously described anti-endothelial MoAb PAL-E.[29] PAL-E differs from CD34 antibodies in its lack of reactivity with the capillaries of the kidney glomerulus, in the ultrastructural localization of antigen,[29] and its lack of reactivity with the KG1 cell line or CD34 cDNA transfected COS cells.[35] Several other antibodies with selective reactivity to vascular endothelium have been described,[36-39] but from the reported biochemical characteristics of the antigens involved, these antibodies do not appear to be identifying CD34.

CD34 is also present on an unusual population of mesenchymal perifollicular cells in fetal skin. These structures are very similar to organ specific mesenchyme previously described in developing hair follicles and teeth, and, in this context, the distribution of CD34 staining parallels that of syndecan, a heparan sulphate binding matrix receptor, and tenascin, a matrix glycoprotein.[40-42] These molecules are believed to be involved in matrix mediated epithelial-mesenchymal cell interactions.[40] CD34 and syndecan have weak N-terminal sequence homology (references 42, 43, and DL Simmons, personal communication, December 1988). CD34 antibodies also bind to stromal matrix components of skin, breast, and other tissues. The structural basis of this reactivity was difficult to determine at the light microscopic level and had a variable appearance, including cellular structures that could have been small collapsed capillaries (Fig 2E, arrow) and in other situations of matrix material or reticular fibers (eg, in skin). Electron microscopic evidence suggests that at least some of this CD34 positivity (in breast

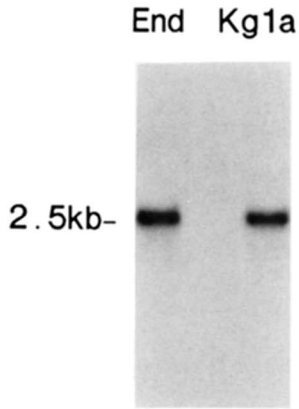


Fig 4. Detection of CD34 mRNA in vascular endothelial cells. Northern blot analysis of mRNA extracted from cultured umbilical cord vein cells (End) and KG1a cells.

tissue) is on extended processes of fibroblasts.[44] We previously reported that CD34 antibodies react with basement membranes in testis, thymus, and other tissues.[5] Although we cannot rule out CD34 positivity of basement membranes at some sites, our data now suggest that most of this apparent reactivity is due to the endothelial cells of closely associated capillaries. We previously reported that a wide variety of tumor cell types were all CD34−, although capillaries within these tumors were usually CD34+.[5] In this study, we found angioblastoma cells to be strikingly CD34+, which presumably reflects their origin from capillary endothelial cells. Karposi sarcoma cells are also CD34 positive.[34]

We presume that the expression of the CD34 glycoprotein by hematopoietic progenitor cells, vascular endothelium and some mesenchymal cells in the fetus reflects a common functional requirement. The structural and biochemical features of the proteoglycan-like CD34 protein,[5,9] its lack of tyrosine kinase activity,[9] its relatively high density[45] compared with most growth factor receptors,[46] and its slow rate of synthesis and turnover[9] suggest that it is not a growth factor receptor but is more likely to be involved in cell interactions and cell adhesion. We sought to further investigate this possibility by analyzing CD34 expression on cultured endothelial cells and by ultrastructural localization of CD34 molecules in situ on vascular endothelium. Although freshly isolated, umbilical vein cells are CD34+ after a few passages in vitro, and under a variety of culture conditions promoting proliferation or differentiation, no CD34 antigen is detectable on the cell surface or intracellularly. However, CD34 mRNA is present (passages 10 to 15), suggesting that CD34 protein may be downregulated or processed into another form that is not recognized by CD34 antibodies. This observation suggests that the expression of CD34 antigen by vascular endothelial cells is not constitutive and requires a more regulated or structured environment than that provided by the in vitro culture methods used. In vivo, CD34 molecules have a striking ultrastructural localization on endothelial cells in all three tissues examined, being concentrated primarily on the lumenal side and, in particular, on membrane processes, many of which interdigitate between adjacent endothelial cells. This distribution accords with a possible role in cell interaction, but it is significant that very little or no label was present in regions of intercellular adherence characterized by tight junctions. In contrast, the recently described antibody Hec7[47] binds selectively to areas of inter-endothelial membrane contact and tight junctions. The antigen identified by Hec7 antibody has a molecular weight of ≈135 Kd with a precursor form (present on cell surfaces) of ≈110 Kd. The relationship, if any, between the Hec7 antigen and CD34 is being further investigated. Additional studies being reported elsewhere[48] have revealed that in tumor stroma with angiogenesis, CD34 molecules are found in association with abluminal endothelial microprocesses occurring at the tips of vascular sprouts, further suggesting a role in adhesion and/or migration.[49]

It is unlikely that the CD34 antigen alone could directly mediate homotypic intercellular interactions due to the highly negatively charged nature of the fully glycosylated molecule. However, it is possible that the CD34 antigen is adhering to a component of the extracellular matrix laid down between the endothelial cells. Alternatively, the CD34 antigen may be serving as a ligand for a lectin-like component of the extracellular matrix. Modification of the molecule by changes in glycosylation could then modulate these adhesion interactions, as well as changing the availability of carbohydrate epitopes recognized by some CD34 antibodies. CD34 molecules on hematopoietic cells could play a similar

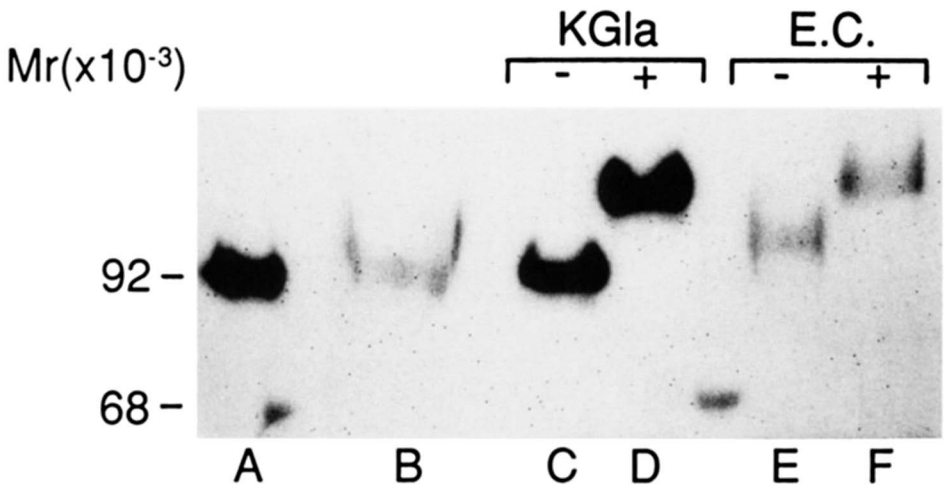


Fig 5. Western blotting of CD34 antigen on KG1a cells and fresh endothelial cells. Whole cell lysates (lanes A and B) or immune complexes made with TUK3 (lanes C through F) were separated by SDS-PAGE and transblotted, and the blots were developed with QBEND10, rabbit anti-mouse immunoglobulin, and ^{125}I-Protein A. Lanes A, C, and D, KG1a cell lysate; lanes B, E, and F, endothelial cell lysates. Immune complexes digested with neuraminidase (25 μg/mL) before SDS-PAGE (lanes D and F).

role in interaction with components of the bone marrow stromal microenvironment. These ideas are now open to experimental investigation.

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