Immunology 1983 **49** 183

A vascular endothelial cell antigen with restricted distribution in human foetal, adult and malignant tissues

Y. C. CUI,* P-C. TAI, K. C. GATTER,† D. Y. MASON† & C. J. F. SPRY *Department of Immunology, Royal Postgraduate Medical School, London and †Nuffield Department of Pathology, John Radcliffe Hospital, Oxford*

Accepted for publication 22 November 1982

Summary. Human vascular endothelial cells were isolated by collagenase digestion of umbilical veins. Hybridomas secreting monoclonal antibodies were raised by fusing a mouse myeloma cell line to spleen cells from mice immunized with the isolated endothelial cells. A clone was selected which produced an antibody binding strongly to human umbilical vein endothelial cells. This antibody, EN 3, was shown to be directed against a major antigen on the surface of the cells, and appeared to be distinct from other antigens previously described on vascular tissues. The antibody bound to a lesser extent to umbilical artery endothelial cells and syncytiotrophoblast. Capillary endothelial cells in adult oesophageal tissues and tonsil were also labelled by the antibody, as were capillaries in a seminoma and infiltrating duct carcinoma of the breast. This well defined distribution in some foetal, adult and malignant tissues suggests that there is structural heterogeneity amongst endothelial cells in different sites, which may be linked to differences in differentiation or function.

* Present address: Department of Microbiology, The Fourth Military Medical School, Xian, Shanxi, China.

Abbreviations: PBS, phosphate-buffered saline; HECS, human endothelial culture supernatant; PBS/Tw, phosphate-buffered saline with 0·05% (v/v) Tween 20; FITC, fluorescein isothiocyanate; ELISA, enzyme-linked immunosorbent assay; PAP, peroxidase anti-peroxidase.

Correspondence: Dr Christopher Spry, Department of Immunology, Royal Postgraduate Medical School, 150 DuCane Road, London W12 OHS.

0019-2805/83/0500-0183$02.00

INTRODUCTION

A widely used method for studying endothelial cells *in vitro* involves the isolation of human umbilical-cord vein endothelial cells by collagenase digestion (Striker, Harlan & Schwartz 1980). Cells prepared in this way have been shown to possess blood group antigens (Jaffé *et al.*, 1973), class 1 histocompatibility antigens (Gibofsky *et al.*, 1975), class 2 histocompatibility antigens (Moen, Moen & Thorsby, 1980; Hirschberg, 1981) and an antigen shared with blood monocytes (Moraes & Stastny, 1977). Cultured umbilical cord endothelial cells have also been shown to be able to act as accessory cells in both mitogen-induced (Ashida, Johnson & Lipsky, 1981) and antigen-induced lymphocyte proliferation (Hirschberg, Bergh & Thorsby, 1980). In addition they have binding sites for thrombin (Stein & Hoak, 1981), factor VIII/von Willebrand's factor (Wall *et al.*, 1980), and C1q (Andrews *et al.*, 1981). Factor VIII/von Willebrand's factor may also be an integral membrane protein of umbilical cord endothelial cells (Jones *et al.*, 1981).

The purpose of the present study was to define the antigenic properties of human umbilical cord endothelial cells. For this reason monoclonal antibodies were prepared which bound to membrane constituents of isolated endothelial cells. Monoclonal antibodies have a number of advantages compared to polyspecific heterologous antisera in the study of membrane antigens (Barnstable *et al.*, 1978; McMichael & Bastin, 1980). They can be used to demonstrate small antigenic differences and quantify and purify membrane

components. This report describes the preparation of a mouse monoclonal antibody which recognizes a human umbilical cord endothelial cell antigen, which appears to be distinct from membrane components previously described on vascular endothelium.

MATERIAL AND METHODS

Endothelial cells

Human umbilical-cord vein endothelial cells were isolated by the method of Jaffé (1980), with modifications. Maximal lengths of the umbilical cord were clamped at each end and placed in sterile phosphate-buffered saline (PBS), pH 7·4, containing penicillin (100 u/ml) and streptomycin (100 μg/ml). Then the umbilical vein was cannulated with a butterfly 16 ST tubing connector (Abbot Ireland Limited, Sligo, Eire), which was held in place with a clamp. Digestion was carried out with 0·1% (w/v) collagenase (Worthington) at 37° for 5 min. The cells were collected in medium 199 (Flow Laboratories, Scotland) without serum. After centrifugation, the cell pellet was resuspended in 'endothelial cell culture medium' which contained medium 199 with 20% pooled human serum (which was not heat-inactivated), 2 mM L-glutamine, penicillin 100u/ml and streptomycin 100 μg/ml. Cells were cultured in 50-ml T/25 flasks (Nunc, Gibco Biocult, Uxbridge, Middx). All flasks for culturing endothelial cells were pro-coated with 0·2% (w/v) gelatin (British Drug Houses, Poole, Dorset) which had been sterilized in an autoclave. 2 ml gelatin was added to each 50-ml flask, left at 4° overnight, and emptied prior to use. Flasks containing endothelial cells were incubated at 37° for 7–10 days to obtain confluent cultures. Attached cells were obtained in suspension by digestion with 0·2% (w/v) trypsin(Wellcome, Beckenham, Kent) in PBS containing 0·3 mM EDTA. Culture media were changed twice weekly and sub-cultures, which were produced by a 1 in 3 dilution, were maintained for 2–3 cycles. The purity of endothelial cultures was examined by phase contrast microscopy and staining with fluoroscein isothiocyanate (FITC)-conjugated anti-factor VIII antibody (Boehringer) (Hoyer, de Los Santos & Hoyer, 1973). Over 98% of the cells were endothelial cells, with occasional fibroblasts.

Human endothelial culture supernatants (HECS)

Confluent cultures of human endothelial cells were incubated with endothelial cell culture medium for 24–72 hr at 37° with 5% CO_2 in air. Supernatants were collected, centrifuged, pooled and stored at −20°.

Production of hybridomas

Three million sub-cultured endothelial cells in 1 ml of phosphate-buffered saline were injected intraperitoneally into 8–12-week-old (CBA × Balb/c) F1 mice. Three weeks later they were given an intravenous injection of the same number of cells in 0·3 ml PBS and the spleens were removed 3 days later. Single-cell suspensions were made and fused to the 8-azaguanine resistant murine myeloma cell P3-NS1/1 Ag 4.1 according to the methods of Köhler & Milstein (1975) with modifications (Oi & Herzenberg, 1980). Ten days later, supernatants from culture wells were screened for antibody to endothelial cells, and cells in selected wells were cloned three times by limiting dilution. The antibody from the clone described in this report was named EN 3. This clone has remained stable for over 3 months, both in liquid N_2 and in sub-cultures. Antibody-containing ascites fluid was also obtained from pristane-treated (CBA × Balb/c) F1 mice injected intraperitoneally with the hybridoma cells. Antibody EN 3 was of subclass IgG1, and was not complement-fixing or cytotoxic for endothelial cells.

Hybridoma screening technique

Culture supernatants from fused mouse myeloma and mouse spleen cells were screened for antibodies to human umbilical-cord endothelial cells by the method of Lansdorp *et al.* (1980) with modifications. Rigid microculture plates (Nunc, Gibco Biocult) were precoated with gelatin as described above and seeded with second- and third-generation human umbilical-cord endothelial cells, 3×10^4 cells/well, and cultured in 5% CO_2 in air at 37°. After about 5 days confluent monolayers were fixed with 0·025% (v/v) glutaraldehyde in PBS for 10 min at 20°. The wells were washed three times with PBS and re-filled with 50 μl PBS containing 0·02% (w/v) gelatin and 1 mM sodium azide. These were stored at 4° until used. Endogenous peroxidase in each well was inactivated with 50 μl 0·1% (v/v) phenylhydrazine (Sigma, Poole, Dorset), in PBS, for 1 hr at 37°. The wells were then washed three times with PBS and once with PBS containing 0·05% (v/v) Tween 20 (PBS/Tw) (Sigma). 50 μl of hybridoma culture supernatant was added to each well with a multichannel pipette (Flow Laboratories), incubated for 1 hr at 37°, and washed three times with PBS/Tw as above. Then 50 μl of a 1/1000 dilution of peroxidase-conjugated rabbit anti-mouse IgG (Miles-Yeda

Laboratories, Slough) in 10% (v/v) glycerol in PBS/Tw was added. The plates were incubated for 1 hr at 37°, and washed five times with PBS/Tw. Fifty microlitres of the substrate was then added to each well. This was freshly made by mixing together 25 ml of 0·1 M phosphate–citrate buffer pH 5·0, containing 20 mg o-phenylenediamine hydrochloride (Sigma), with 25 ml water containing 20 μl 30% H_2O_2 (BDH). After incubation in the dark for 30 min at 20°, the extinction of each well at 492nm was measured with a Titertek Multiskan Plate Reader (Flow Laboratories, Scotland).

Clones giving rise to the highest antibody titre to endothelial cells were selected and these were recloned three times. It was found that a higher recovery and yield of hydridomas producing endothelial-cell antibodies could be produced by culturing selected clones in medium containing 20% human endothelial-cell supernatant (Astaldi *et al.*, 1980).

One immunized mouse provided spleen cells which were grown in two 96-well microculture plates. Of the 192 wells, 144 (75%) grew cells which secreted antibody reactive with human umbilical-cord endothelial cells. Half of the cultures contained antibodies which also reacted with fibroblasts. The remaining cultures were recloned in medium containing 20% human endothelial-cell supernatant. About 20% of the antibody-producing cells were lost during the cloning procedure. Cultures retaining specific antibodies for endothelial cells were recloned and stored in liquid nitrogen. From among 100 clones which were initially found to secrete antibodies to human umbilical-cord endothelial cells, one (clone EN 3) was selected for further study.

Tests for antibody reactivity

A wide range of techniques were used to study the specificity of the antibody, and the range of tissues where it could be found in sections from foetal and adult samples. Soluble protein antigens were studied by coating polyvinyl chloride microtitration plates (Dynatech Laboratories Limited, Sussex) by the method of Voller, Bidwell & Bartlett (1979). The following proteins were studied: pooled human serum, pooled human plasma, human endothelial-cell supernatants (containing factors secreted by endothelial cells) and human fibronectin (Sigma). The amount of antibody bound to these antigens was assayed using peroxidase-conjugated rabbit anti-mouse antibody as described above.

Possible binding of EN 3 to the surface of blood cells was studied by attaching either erythrocytes, mononuclear cells, neutrophils or eosinophils to poly-L-lysine (Sigma)-coated polyvinyl chloride microtitration plates by the method of Lansdorp *et al.* (1980). Antibody binding was assayed using peroxidase-conjugated rabbit anti-mouse antibody as described above.

The binding of EN 3 to antigens in tissue sections was studied by indirect immunofluorescence using FITC-conjugated rabbit anti-mouse IgG and by the peroxidase–anti-peroxidase (PAP) labelling procedure (Mason *et al.*, 1982). Frozen sections of the following human tissues were studied by immunofluorescence: (a) foetal tissues (umbilical cord full term placenta and tissues obtained at abortion); (b) adult tissues (saphenous vein, kidney and penis). Acetone-fixed cryostat sections of the following human tissues were studied by immunoperoxidase staining: (i) normal tissues—stomach, brain, bladder, oesophagus, kidney, skin, breast, liver and tonsil; (ii) malignant tissues—seminoma, carcinomas of the breast, colon and kidney.

Assay for EN 3 binding to the membrane of endothelial cells

The binding of EN 3 antibody to the surface of endothelial cells was studied by indirect immunofluorescence. Fifty microlitres of EN 3 culture supernatants was added to 50 μl endothelial cells, 2×10^6 cells/ml for 20 min at 0°. The cells were washed three times and 50 μl FITC-conjugated rabbit anti-mouse IgG was added. After 20 min at 0° the cells were washed three times and resuspended in 0·1 ml, and examined by phase contrast and fluorescence microscopy.

The binding of EN 3 to monolayers of endothelial cells on gelatine-coated glass cover-clips was studied in the same way. Confluent monolayers were fixed with acetone for 10 min, air dried, and stored at −70°. The monolayers were rehydrated with PBS for 10 min. Supernatants were removed and replaced with single drops of EN 3, incubated for 30 min at 37°. After washing the sections three times, they were stained with FITC-conjugated rabbit anti-mouse IgG and studied as described above. Controls were foetal skin-derived fibroblasts grown on cover-slips.

RESULTS

Results of experiments to determine the specificity of binding of antibody EN 3 to human tissues and the density of the antigen on cells were as follows.

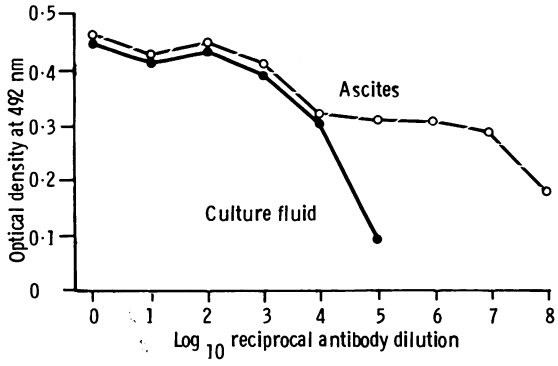

Figure 1. Titration of antibody EN 3. Serial dilutions of the antibody were added to umbilical cord endothelial cells. The extent of binding was assayed with an ELISA peroxidase-linked rabbit anti-mouse IgG assay. (●——●) Hybridoma culture fluid; (O- - -O) hybridoma-induced ascites fluid.

Foetal vascular endothelial cells

The antibody was selected on the basis of its capacity to bind strongly to umbilical-cord endothelial cells in microtitre wells using an ELISA assay. Binding was detected at dilutions of 10^{-4} hybridoma culture supernatants and 10^{-7} ascites fluid (Fig. 1). Single cell suspensions of umbilical-cord endothelial cells which were prepared by trypsin treatment, showed as strong binding (judged by indirect immunofluorescence) as monolayers of the same cells crown on cover slips. This siggested that the membrane antigen was not sensitive to tryptic digestion.

The distribution of the antigen in foetal tissue was studied first by indirect immunofluorescence. Strong staining of umbilical vein endothelial cells was seen and there was lesser staining of umbilical artery endothelium, but there was no staining of placental tissues or other foetal tissues. However when the distribution of the antigen was studied using the PAP labelling procedure, it was seen that the antibody also bound to syncytiotrophoblast (Fig. 2), but not to other placental tissues.

Vascular endothelial cells in normal and malignant adult tissues

Antibody EN 3 bound to capillaries in many different adult normal (Fig. 3 a,b) and malignant (Fig. 3 c,d) tissues.

Specificity of EN 3 antibody

A wide range of studies showed that this antibody recognized an antigen distinct from others already known to be present on vascular endothelium. A large number of proteins were studied to see whether they would bind to antibody EN 3. Serum did not interfere

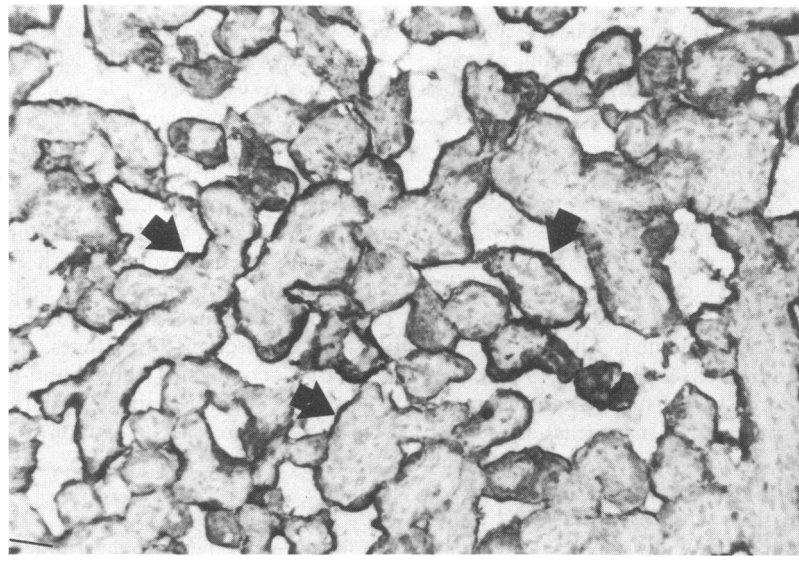

Figure 2. Distribution of the antigen detected with antibody EN 3 in human placenta. Syncytiotrophoblast was strongly stained (arrows). PAP technique × 150.

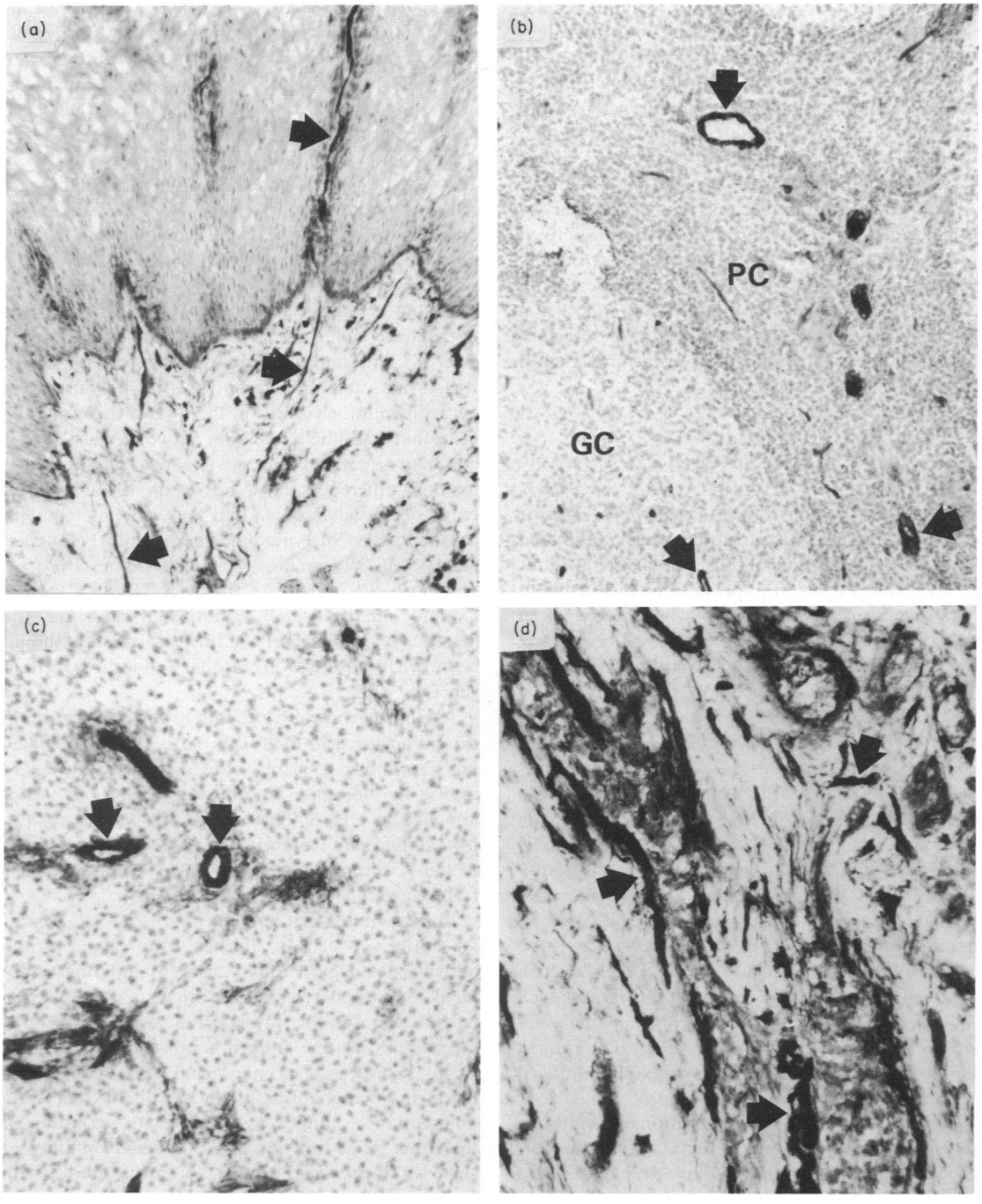

Figure 3. The binding of antibody EN 3 to adult and malignant tissues. (a) Oesophagus; capillaries arising in the connective tissue and ascending into the epithelium where labelled (arrows). (b) Normal human tonsil; there was labelling of capillaries (arrows) in both paracortex (PC) and germinal centres (GC). (c) Seminoma; densely stained capillaries (arrows) were present within and around the tumour. (d) Infiltrating duct carcinoma of the breast; strongly stained capillaries (arrows) were stained. PAP technique $\times 150$.

with the binding of EN 3 antibody to umbilical-cord endothelial cells, and purified fibronectin, which is synthesized and secreted by umbilical-cord endothelial cells, did not inhibit binding. EN 3 also failed to bind to the cytoplasm of endothelial cells which is rich in fibronectin. Microtitre plates coated with human endothelial-cell supernatants containing fibronectin or with purified fibronectin also did not bind the antibody, and the antibody could not be absorbed out with purified fibronectin. This showed that the antigen detected by antibody EN 3 was not actively secreted by endothelial cells and was clearly distinct from fibronectin, serum proteins and other endothelial cell-related proteins. Lack of staining of endothelial-cell cytoplasm also suugested that it was unrelated to factor VIII-related antigen.

DISCUSSION

This study has demonstrated that there is a major membrane antigen with a restricted distribution on human vascular endothelial cells in some foetal, adult and malignant tissues. It appears to be distinct from other membrane components which have been detected on endothelial cells. These include blood group antigens, class I and class II histocompatibility antigens, fibronectin and factor VIII-related antigens. A number of mouse monoclonal antibodies to human leucocytes have been found to cross-react with endothelial cell membrane antigens. These include antibodies to T lymphocytes (T9), monocytes (Mo3, Mo4) and myeloid cells (My7, My8). My7 showed some organ preference for binding to endothelial cells (G. G. Petrányi, 1982, personal communication). Antibody EN 3 did not bind to T lymphocytes, monocytes or myeloid cells. It is therefore likely to be a separate antigen. The antigen did not appear to be secreted by endothelial cells, so it is unlikely to be a component of the vascular basement membrane, such as collagen, microfibrils, elastic fibres, mucopolysaccharides or fibronectin (Jaffé *et al.*, 1976). The antigen was not found in serum, and was probably produced by endothelial cells themselves.

Little is known about the distribution of membrane antigens or endothelial cells in foetal tissues. Sunderland, Redman & Stirrat (1981) have prepared mouse monoclonal antibodies which bind to two antigens on the apical aspects of human syncytiotrophoblast (Gatter *et al.*, 1983). The relationship of these antigens to the antigen detected here remains to be explored, but it is likely that a range of tissue specific antigens will be found in foetal vasculature. The present study suggests that venous endothelial cells have different degrees of expression of at least one of their antigens compared to arterial endothelial cells. A similar antigen has been described recently on murine endothelial cells, using a rat monoclonal antibody (Ghandour *et al.*, 1982). This rat antibody did not bind to human endothelial cells.

A number of vascular sites in adult tissues also possessed the antigen detected with antibody EN 3. The antigen was present on many endothelial cells in small blood vessels, including capillaries, but it was not detected on endothelial cells in large veins or arteries. This suggests that there may be important structural, and possibly functional, differences between endothelial cells in these sites. It is generally held that endothelial cells in blood vessels have a common origin, but there are already suggestions that regional differences exist. For example studies with lymphocytes have shown that they only interact with certain types of vascular endothelium (Butcher, Scollay & Weissman, 1980; Andrews, Ford & Stoddard, 1980). For these reasons, although human umbilical vein endothelial cells are the most easily obtained cells of this type, caution should be shown in extrapolating results to endothelial cells in general.

A useful technical procedure in the preparation of this monoclonal antibody was the use of HECS, which has been shown to increase the frequency and yield of mouse hybridomas (Astaldi *et al.*, 1980). It is not known how HECS affects the survival and growth of hybridomas, but it seems to depend on the absorption of some component to the surface of the tumour cells (Astaldi *et al.*, 1981).

It is concluded that human endothelial cells possess an antigen which is distinct from blood group antigens, histocompatibility antigens and other endothelial cell products which are known to be involved in haemostasis and the formation of vascular basement membranes. As the antigen is not expressed equally strongly on vascular endothelial cells in all adult, foetal and malignant tissues, it is probable that it is a differentiation antigen associated with variations in the structure or function of blood vessels in different sites. If this proves to be the case, further studies with this antibody should provide new insights into the properties of endothelial cells and may provide new approaches to the study of differential susceptibility of blood vessels in diseases.

ACKNOWLEDGMENTS

We thank the midwives of Hammersmith Hospital for supplying fresh umbilical cords, Dr John Davies for advice on isolating umbilical-cord endothelial cells and Dr M. B. Slade for advice on the hybridoma techniques. The work was supported by funds from the World Health Organisation, Celltec Ltd. The Sino-British Trust, the Wellcome Trust, the Leukaemia Research Fund and The Royal Society, to whom we are grateful. We are especially grateful to Professor J. H. Humphrey FRS for making this study possible.

REFERENCES

ANDREWS P., FORD W.L. & STODDART R.W. (1980) Metabolic studies of high-walled endothelium of rat capillary vessels in rat lymph nodes. In: *Blood Cell and Vessel Walls: Functional Interactions* (eds R. Porter, M. O'Connor and J. Whelan), p. 211. Ciba Foundation Symposium 71 (New Series), Exerpta Medica, Amsterdam.

ANDREWS B.S., SHADFORTH M., CUNNINGHAM P. & DAVIS J.S. IV (1981) Demonstration of a C1q receptor on the surface of human endothelial cells. *J. Immunol.* **127,** 1075.

ASHIDA E.R., JOHNSON A.R. & LIPSKY P.E. (1981) Human endothelial cell-lymphocyte interaction. Endothelial cells function as accessory cells necessary for mitogen-induced human T lymphocyte activation *in vitro*. *J. clin. Invest.* **67,** 1490.

ASTALDI G.C.B., JANSSEN M.C., LANSDORP P.M., WILLEMS C., ZEIJLEMAKER W.P. & OLSTERHOF F. (1980) Human endothelial culture supernatant (HECS): a growth factor for hybridomas. *J. Immunol.* **125,** 1411.

ASTALDI G.C.B., JANSSEN M.C., LANSDORP P.M., ZEIJLEMAKER W.P. & WILLEMS C. (1981) Human endothelial culture supernatant (HECS): evidence for a growth-promoting factor binding to hybridoma and myeloma cells. *J. Immunol.* **126,** 1170.

BARNSTABLE C.J., BODMER W.F., BROWN G., GALFRE G., MILSTEIN C. & WILLIAMS A.F. (1978) Production of monoclonal antibodies to Group A erythrocytes, HLA and other human cell surface antigens. New tools for genetic analysis. *Cell,* **14,** 9.

BUTCHER E.C., SCOLLAY R.G. & WEISSMAN I.L. (1980) Organ specificity of lymphocyte migration: mediation by highly selective lymphocyte interaction with organ-specific determination on high endothelial venules. *Eur. J. Immunol.* **10,** 556.

GATTER K.C., SUNDERLAND C.A., SANDERS M.K. & MASON D.Y. (1983) An immuno-electron microscopic study of human placenta using 3 monoclonal antibodies. *Placenta.* (In press.)

GHANDOUR S., LANGLEY K., GOMBOS G., HIRN M., HIRSCH M.R. & GORIDIS C. (1982) A surface marker for murine vascular endothelial cells defined by monoclonal antibody. *J. Histochem. Cytochem.*, **30,** 165.

GIBOFSKY A., JAFFÉ E.A., FOTINO M. & BECKER C.G. (1975) The identification of HL-A antigens on fresh and cultured human endothelial cells. *J. Immunol.* **115,** 730.

HIRSCHBERG H. (1981) Presentation of viral antigens by human vascular endothelial cells *in vitro*. *Human Immunol.* **2,** 235.

HIRSCHBERG H., BERGH O.J. & THORSBY E. (1980) Antigen-presenting properties of human vascular endothelial cells. *J. exp. Med.* **152,** (2 Pt 2) 249s.

HOYER L.W., DE LOS SANTOS R.P. & HOYER J.R. (1973) Antihaemophilic factor antigen. Localization in endothelial cells by immunofluorescent microscopy. *J. clin. Invest.* **52,** 2737.

JAFFÉ E.A. (1980) Culture of human endothelial cells. *Transplant Proc.* **12,** (suppl 3), 49.

JAFFÉ E.A., MINICK R., ADELMAN B., BECKER C.G. & NACHMAN R. (1976) Synthesis of basement membrane collagen by by cultured human endothelial cells. *J. exp. Med.* **144,** 209.

JAFFÉ E.A., NACHMAN R.L., BECKER C.G. & MINICK C.R. (1973) Culture of human endothelial cells derived from umbilical veins. Identification by morphologic and immunologic criteria. *J. clin. Invest.* **52,** 2745.

JONES T.R., KAO K.J., PIZZO S.V. & BIGNER D.D. (1981) Endothelial cell surface expression and binding of Factor VIII/von Willebrand factor. *Am. J. Path.* **103,** 304.

KÖHLER G. & MILSTEIN L. (1975) Continuous cultures of fused cells secreting antibody of predefined specificity. *Nature,* **256,** 495.

LANSDORP P.M., ASTALDI G.C.B., OOSTERHOF F., JANSSEN M.C. & ZEIJLEMAKER W.P. (1980) Immunoperoxidase procedures to detect monoclonal antibodies against cell surface antigens. Quantitation of binding and staining of individual cells. *J. immunol. Meths.* **39,** 393.

MCMICHAEL A.J. & BASTIN J. (1980) Clinical applications of monoclonal antibodies. *Immunology Today,* **1,** 56.

MASON D.Y., CORDELL J.L., ABDULAZIZ Z., NAIEM M. & BORDENAVE G. (1982) Preparation of peroxidase:antiperoxidase (PAP) complexes for immunohistological labelling of monoclonal antibodies. *J. Histochem. Cytochem.* **30,** 1114.

MOEN T., MOEN M. & THORSBY E. (1980) HLA-D region products are expressed in endothelial cells. *Tissue antigens,* **15,** 112.

MORAES J.R. & STASTNY P. (1977) A new antigen system expressed in human endothelial cells. *J. clin. Invest.* **60,** 449.

OI V.T. & HERZENBERG L.A. (1980) Immunoglobulin producing hybrid cell lines. In: *Selected Methods in Cellular Immunology* (eds B. B. Mishell and S. M. Shiigi), p. 351. Freeman, San Francisco.

STEIN M.N. & HOAK J.C. (1981) Thrombin receptors on human endothelial cells: a morphologic study. *J. Ultrastruct. Res.* **74,** 156.

STRIKER G.E., HARLAN J.M. & SCHWARTZ S.M. (1980) Human endothelial cells *in vitro*. *Meths. Cell Biol.* **21a,** 135.

SUNDERLAND C.A., REDMAN C.W.G. & STIRRAT G.M. (1981) Monoclonal antibodies to human syncytiotrophoblast. *Immunology,* **43,** 541.

VOLLER A., BIDWELL D.E. & BARTLETT A. (1979) *The enzyme linked immunosorbent assay (ELISA). A guide with abstracts of microplate applications.* Dynatech Europe, Guernsey.

WALL, R.T., COUNTS R.B., HARKER C.A. & STRIKER G.E. (1980) Binding and release of factor VIII/von Willebrand's factor by human endothelial cells. *Br. J. Haematol.* **46,** 287.