Establishment and characterization of a clonal line of parathyroid endothelial cells

MARIA LUISA BRANDI,*[,1] RICHARD L. ORNBERG,[†] KAZUSHIGE SAKAGUCHI,* FRANCESCO CURCIO,[‡] ANDREA FATTOROSSI,* PETER I. LELKES,[†] TOSHIMITSU MATSUI,[§] MARK ZIMERING,* AND GERALD D. AURBACH*

*Section on Endocrine Regulation, Metabolic Diseases Branch, and [†]Laboratory of Cell Biology and Genetics, National Institute of Diabetes and Digestive and Kidney Diseases, [‡]Laboratory of Genetics and [§]Laboratory of Cellular and Molecular Biology, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20892, USA

ABSTRACT

We have isolated endothelial cells derived from bovine parathyroid tissue. These cells have been cloned and maintained by serial passage for more than 40 months without showing signs of senescence. Prolonged culture was accomplished by using a medium favoring endothelial cell growth and methods for enriching endothelial cells in primary culture. The cloned parathyroid endothelial cells contained factor VIII-related antigen, took up acetylated low-density lipoproteins and parathyroid hormone, and showed morphological features comparable to other endothelial cells. Bovine parathyroid endothelial cells replicated with a mean doubling time of 65 h. Fibroblast growth factors, platelet-derived growth factor, and calcium acted as mitogens for parathyroid endothelial cells, whereas transforming growth factor β inhibited proliferation.—BRANDI, M. L.; ORNBERG, R. L.; SAKAGUCHI, K.; CURCIO, F.; FATTOROSSI, A.; LELKES, P. I.; MATSUI, T.; ZIMERING, M.; AURBACH, G. D. Establishment and characterization of a clonal line of parathyroid endothelial cells. *FASEB J.* 4: 3152–3158; 1990.

Key Words: parathyroid • endothelium • cell culture • platelet-derived growth factor • fibroblast growth factors

EARLIER STUDIES SUGGEST THAT there are nearly as many varieties of endothelial cells as there are organs and tissues. The most striking feature of endothelial variability is the apparent adaptation of structure and function to specific tissues. The capillaries of endocrine glands characteristically show unique ultrastructural features called fenestrae, which are important in transport function. Specific interactions between endocrine cells and adjacent endothelial cells have been proposed for the adrenal medulla, where chromaffin cells and neighboring endothelial cells form a functional unit (1, 2). Cultures of endothelial cells from diverse endocrine organs would make it possible to test for similar functional specialization in each gland. Because endocrine tissues are highly vascularized, they are ideal sources for isolation of endothelial cells.

To learn more about parathyroid gland physiology and to study interrelationships between parathyroid secretory and endothelial cells, we have isolated and cultured the latter. We report the cloning and culture of such cells derived from bovine parathyroid tissue. These cells grow on plastic surfaces in media containing serum substitutes, show morphological characteristics typical of endothelial cells, exhibit positive reaction for factor VIII-related antigen (factor VIII R:Ag)[2], and take up acetylated low-density lipoprotein as well as parathyroid hormone (PTH) itself.

MATERIAL AND METHODS

Preparation and maintenance of primary culture of bovine capillary endothelial cells

Bovine parathyroid glands were brought to the laboratory under sterile conditions from the slaughterhouse. One gram of tissue was trimmed, minced into small fragments, washed twice in 8-mM sodium phosphate/138 mM NaCl, pH 7.2 (PBS), and incubated for 1 h at 37°C in 10 ml of PBS with 0.3% collagenase type II (Worthington, N.J.), 0.1% glucose, and 0.3% BSA fraction V. The suspension was centrifuged and the pellet incubated for 1 h at 37°C in dissociating medium containing neutral dispase (Collaborative Research, Mass.) at a concentration of 50 caseinolytic units per milliliter in Hank's balanced salt solution, pH 7.4. The aggregates were mechanically dispersed and the sus-

[1]To whom correspondence and proofs should be sent, at present address: Department of Physiopathology, Endocrine Unit, Viale Pieraccini 6, 50139 Firenze, Italy

[2]Abbreviations: PTH, parathyroid hormone; PT-b, bovine parathyroid; FCS, fetal calf serum; HSF, human skin fibroblasts, BAME, bovine adrenal medullary endothelial; aFGF, acidic fibroblast growth factor; PDGF, platelet-derived growth factor; TGFβ, transforming growth factor β; factor VIII R:Ag, factor VIII-related antigen.

0892-6638/90/0004-3152/$01.50.

pension was then passed through a nylon-covered funnel (86-μm NITEX mesh screens, Tetko, N.J.). The material that passed through the filter was collected and passed through a 15-μm screen to remove single cells. The material retained on the second screen was collected by retrograde flushing with a stream of culture medium. The suspension was centrifuged and the pellet was resuspended in growth medium: Coon's modified Ham's F12 containing 1 mM ionized calcium, 10% NU-SERUM (Collaborative Research), 2% ULTROSER-G (IBF, Md.), 200 μg/ml of D-galactose, 100 units/ml of penicillin, and 100 μg/ml of streptomycin. Both NU-SERUM and ULTROSER-G are serum-substitutes that contain several growth factors and nutrients, including fibroblast growth factors that favor endothelial cell growth. Cells were then distributed into gelatinized 60-mm dishes and incubated at 37°C. The ability of endothelial cells to adhere to gelatin (3) has been exploited as a way to remove parenchymal cells from the culture by simply rinsing the culture dishes with growth medium 1 and 3 h after plating. After aspiration of supernatant, a mixture of (1:1) growth medium and 24-h conditioned medium from long-term cultures (4) of bovine parathyroid (PT-b) cells was added.

Cloning procedure

After 72 h incubation the medium was removed and replaced for 5 days with conditioned medium from PT-b cells containing 1 μg/ml of sodium ethyl mercurithiosalicylate (thimerosal) to specifically remove fibroblastoid cells (5). The cells were then cultured in thimerosal-free conditioned medium from PT-b cells for 2 more days. Colonies of 150–200 cells were isolated by cloning cylinders using 0.05% trypsin in PBS/2 mM EDTA. The cells derived from a single colony were resuspended in conditioned medium from PT-b cells and plated at high dilution onto 100-mm plastic Petri dishes coated with confluent PT-b feeder cells previously treated for 2 h with 10 μg/ml of mitomycin. Colonies of 150–200 cells derived from a single cell were removed by cloning cylinders with trypsin/EDTA and plated in a well of a gelatinized 24-well plate, using conditioned medium. Only the cells of the colony, not the original feeder cells, survived this transfer. Upon reaching confluence, cells in flat close-packed monolayers were detached from wells with trypsin/EDTA and cloned by limiting dilution using conditioned medium from PT-b cells. Cloning efficiencies were about 30% under these conditions. After 2 to 3 wk, several clones exhibited typical cobblestone endothelial morphology. Subsequently the cloned endothelial cells were passaged to uncoated dishes using growth medium. One clone, designated BPE-1 and established on May 23, 1986, was used for characterization studies.

Phenotypic characterization of BPE-1 cell cultures

Cell morphology was monitored using phase-contrast microscopy. Stocks of BPE-1 cells were frozen in liquid nitrogen from selected passages in a mixture of 90% growth medium and 10% dimethylsulfoxide. The plating efficiency of thawed cells was about 60%. Mycoplasma contamination was tested by a direct method and DNA binding fluorochrome stain using bisbenzimidazole (6). Chromosome counts were made on actively growing cells treated with colchicine (10 μg/ml). One hundred spread metaphases were counted. The growth assay in soft-agar was carried out using 10^4 cells per milliliter of 0.3% agar in growth medium. Two milliliters of cell suspension was pipetted onto a 2-ml base layer of 0.5% agar in 60-mm tissue culture dishes. Cultures were incubated at 37°C for 2 wk without further refeeding (7).

Other cell cultures

PT-b cells were cultured as previously described in detail (4) and used between the 2nd and 15th passages. Pulmonary artery endothelial (CPAE) cells (ATCC no., CCL 209) were maintained in minimum essential medium with antibiotics and 20% fetal calf serum (FCS). Normal human skin fibroblasts (HSF) (ATCC no., CRL 1229) were maintained in DME medium supplemented with antibiotics and 20% FCS. Bovine adrenal medullary endothelial (BAME) cells were grown in minimal essential medium supplemented with antibiotics and 10% heat-inactivated FCS as described (1).

Measurement of cell growth

Cells were plated at the indicated density on Petri dishes and incubated in growth medium for 24 h at 37°C to allow complete attachment. The growth medium was replaced with a medium with no growth factors and nutrients for 48 h. Then the growth experiments were carried out in the indicated conditions and for the noted times. The cells were released with trypsin/EDTA and counted using a hemocytometer.

Fluorescence labeling of cells

Endothelial or control cells were cultured on glass LAB-TEK chamber-slides (Miles Scientific, Ill). The cells were fixed for 1 min in cold methanol/acetone (1:1, vol:vol), treated for 1 min in cold methanol/water (1:1, vol:vol), and then with 10 mM Tris-HCl buffer, pH 7.4, containing 0.5% BSA, 160 mM NaCl, and 2% FCS, for 10 min. They were then incubated overnight at 4°C with a rabbit antiserum to human factor VIII R:Ag (Behring, Calif.) diluted 1:800 in PBS or with normal rabbit serum. The preparations were then washed with PBS and incubated 1 h in 6% normal goat serum. The second antibody used was an FITC affinity-purified goat anti-rabbit IgG (both heavy and light chains, Kirkegaard and Perry Laboratories, Gaithersburg, Md.) diluted 1:50 in PBS containing 0.5% BSA. The incubation was carried out overnight at 4°C. Interaction of acetylated low-density lipoprotein with endothelial cells

was visualized by incubating the cultures at 37°C for 3 h with 10 μg/ml of 1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate-acetylated low-density lipoprotein (Dil-Ac-LDL, Biochemical Technologies Inc., Mass.). After washing with DiI-Ac-LDL-free media for 10 min, cells were fixed for 10 min with 4% Formalin in PBS. PTH was analyzed as described earlier (4) by indirect immunofluorescence in cells exposed for 48 h to 1 nM bovine PTH (1-84) (Peninsula Laboratories, Calif.) using 1:100 dilutions of goat NG5 antiserum or with comparable dilutions of preimmune serum. Appropriate positive and negative controls were used throughout. Sections were examined with a Zeiss Photo microscope.

RESULTS

Culture characteristics

BPE-1 cells have maintained a homogeneous morphology without signs of senescence, such as a reduction in the rate of cell growth and the appearance of large, dendritic, multinucleated cells. They have been cultured for many population doublings, well beyond the stage that nonpermanent cell lines usually succumb to senescence. BPE-1 cells grew in small groups (**Fig. 1*a***), and at confluence displayed characteristic cobblestone morphology (Fig. 1*b*), when overconfluent cells showed occasional sprouting. BPE-1 cells maintained in growth medium grew vigorously with a generation time of about 65 h. Population doubling times have not varied over 40 months in culture. BPE-1 cells grew rapidly toward confluence in growth medium with four cumulative population doublings over the first 15-day period (**Fig. 2**). The growth rate of BPE-1 decreased markedly as the population density approached 1×10^6 cells per 35-mm dish (Fig. 2). Cells maintained in medium depleted of growth factors slowly increased in number, doubling within 30 days (Fig. 2); such growth may be dependent on the ability of these cells to condition their own medium. Cell proliferation in growth medium was stimulated by increasing calcium concentrations, with maximal effect at 1.0 mM ionized calcium (Fig. 2). In the presence of 1 mM ionized calcium acidic fibroblast growth factor (aFGF) and basic fibroblast growth factor (bFGF) at a concentration of 100 ng/ml, growth of BPE-1 and CPAE cells was stimulated (**Fig. 3**). Under the same experimental conditions platelet-derived growth factor (PDGF) increased BPE-1 cell proliferation without affecting CPAE cell growth, and transforming growth factor β (TGFβ) inhibited BPE-1 and CPAE cell replication (Fig. 3). No significant mitogenic effect was observed with epidermal growth factor (100 ng/ml), insulin-like growth factor type I (100 ng/ml) or II (100 ng/ml), insulin (10 μg/ml), or transferrin (10 μg/ml) (not shown). BPE-1 cell cultures remained free of detectable mycoplasma infection. Ninety-two percent of BPE-1 cells showed a diploid chromosome number (2 n = 60). BPE-1 cells did not grow in soft agar even when inoculated at high concentrations.

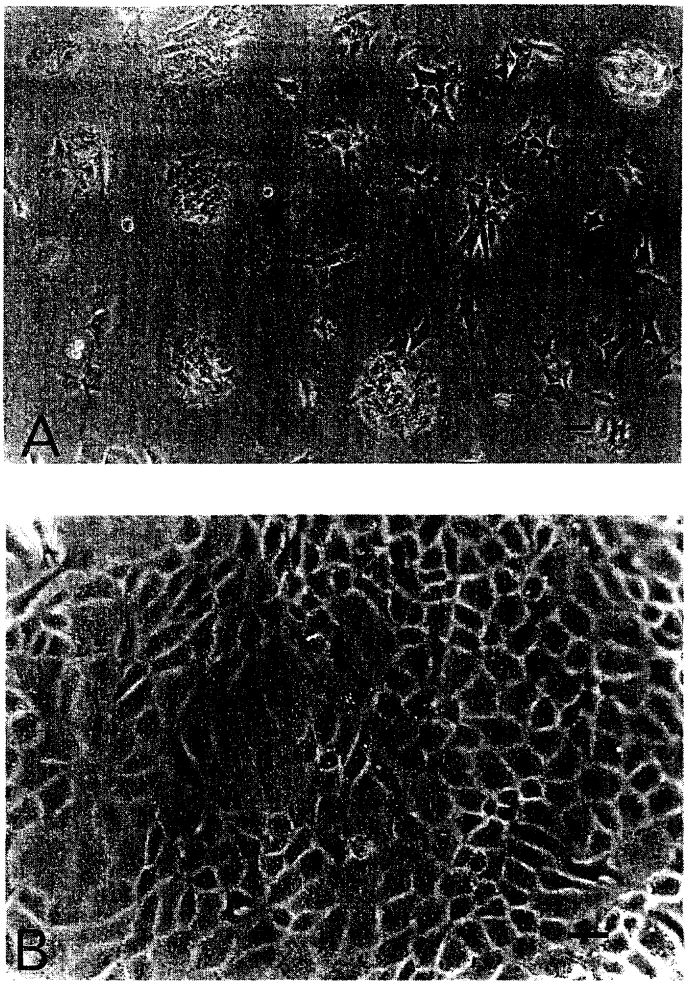

Figure 1. BPE-1 cells viewed by phase contrast microscopy. *A*) BPE-1 cells typically grow in clusters of closely apposed cells. *B*) Monolayer of confluent BPE-1 cells with the typical cobblestone morphology (bars = 20 μm).

Fluorescence microscopy

Indirect immunofluorescence staining with antiserum to factor VIII R:Ag revealed the antigen in nearly every BPE-1 cell after 50 population doublings (**Fig. 4*a***). The antigen was localized in cytoplasmic granules concentrated in the perinuclear space. After 20-30 passages the granular pattern became more diffuse (not shown). No reactivity was found with normal rabbit serum used instead of specific anti-factor VIII R:Ag antiserum. HSF cells showed no detectable factor VIII R:Ag, whereas CPAE and BAME cells showed a strongly positive reaction (not shown). BPE-1, like CPAE and BAME cells, exhibited a profound ability to take up and package exogenous DiI-Ac-LDL in punctuate storage sites in the perinuclear cytosol (Fig. 4*b*). Cells from replicate cultures not exposed to DiI-Ac-LDL showed no fluorescence (not shown). Nonendothelial parathyroid cells exposed to DiI-Ac-LDL showed a different type and distribution of fluorescence, with low background reactivity in HSF and with PT-b cells containing only brightly fluorescent colonies. BPE-1 cells exposed to bovine PTH (1-84) showed a bright fluorescence localized in the cytosol, to the sur-

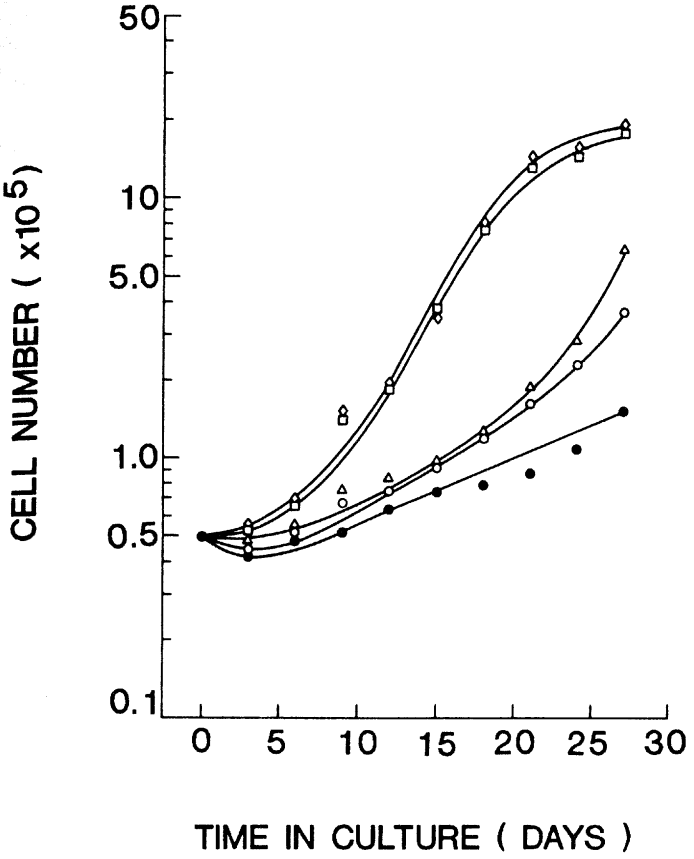

Figure 2. Characteristics of BPE-1 cell proliferation. BPE-1 cells were plated at a concentration of 5 × 10^4 cells per 35-mm plastic Petri dish and allowed to grow for 27 days. The medium was changed every 2 days. The conditions tested were: medium with no growth factors (●) and growth medium with the following ionized calcium concentrations: 0.03 (○), 0.3 (△), 1.0 (□), and 1.5 (◇) mM. Cells were counted in triplicate. The coefficient of variation was 10% or less.

face of the cell, and in many filiform pseudopodia at the perimeter of the cell (Fig. 4*c*). HSF, CPAE, and BAME cells uniformly lack detectable fluorescence after addition of exogenous PTH, therefore the phenomenon appears to be tissue-specific. Normal goat serum instead of specific PTH antibody yielded no reactivity. PT-b cells showed a strongly positive reaction for PTH as reported previously (4).

Electron microscopy

The ultrastructure of the BPE-1 cells was consistent with that of other endothelial cells (**Fig. 5**). The BPE-1 cells showed a flattened appearance in cross section with numerous, long extended processes. Confluent cells had become overgrown to form multilayer clusters with occasional interspersed patches of extracellular matrix (Fig. 5*a*). In en face sections, i.e., parallel to the culture dish substrate, BPE-1 cells showed an elongated shape with a centrally located nucleus. The perinuclear cytoplasm contained two distinct granule organelles, one that was clear with virtually no matrix present and the other that contained a very dense matrix with membranous myelin-like elements (Fig. 5*b* and Fig. 5*c*). Dense granules were also found in the thin flattened processes. In addition, larger aggregates of glycogen particles were restricted to the perinuclear region (not shown). Mitochondria profiles of the interconnected tubular type were also concentrated in the perinuclear space (Fig. 5*c*). The overall cell shape conformed to the arrangement of extensive bundles of actin filaments that lay beneath the plasmalemma. Microtubules were segregated from the actin bundles and were concentrated into distinct domains in thin extended processes of the cell. An extensive network of both rough and smooth endoplasmic reticulum, each containing dense material, was found throughout the cytoplasm usually aligned parallel to the axis of actin bundles (not shown). Few specific intercellular interactions were observed in cultures of 1 wk or older. The most apparent structure suggesting cell-cell interaction appeared as a close, tight contact between thin extended processes cells (Fig. 5*d*). Numerous smooth caveoli were seen underlying and studding the plasmalemma. In en face sections, these structures appeared as cytoplasmic vesicles interacting with actin bundles, although it was clear from cross sections that the majority were true invaginations of the plasmalemma. In thin cell processes, these vesicles appear to be part of a vesicular shuttle (Fig. 5*e*), as found in other capillary endothelial cells (8).

Northern blot analysis

Northern blot hybridization (9, 10) using the cDNA insert representing the PDGF receptor in pGR102, a gift of Drs. L. T. Williams and J. A. Escobedo, showed a single mRNA band at approximately 5.3 kb from bovine parathyroid endothelial cells; this corresponds to results obtained in other cell types (11) (**Fig. 6**). There was, however, no mRNA detectable with this probe in CPAE cells (Fig. 6).

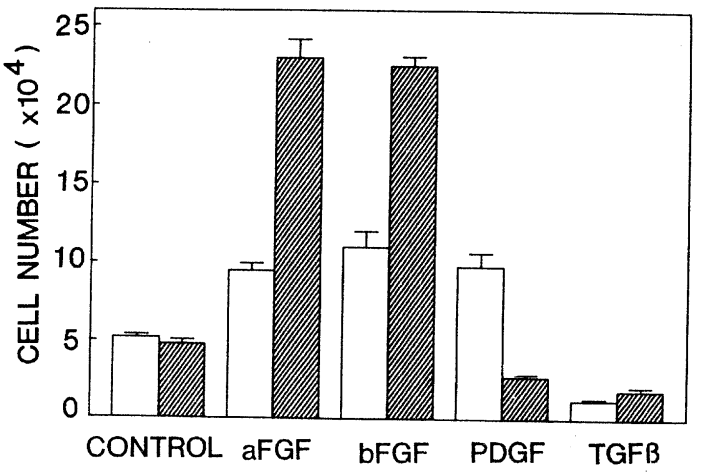

Figure 3. Effect of 100 ng/ml aFGF, bFGF, PDGF, and TGF-β on growth of BPE-1 (open bars) and CPAE (hatched bars) cells. Cells were seeded at 5 × 10^4 per 35-mm plastic Petri dish. The experiments were carried out for 8 days in medium with no growth factors and nutrients, containing 1 mM ionized calcium. The medium was changed every 2 days. The error bars represent SD of mean values of triplicate experimental points.

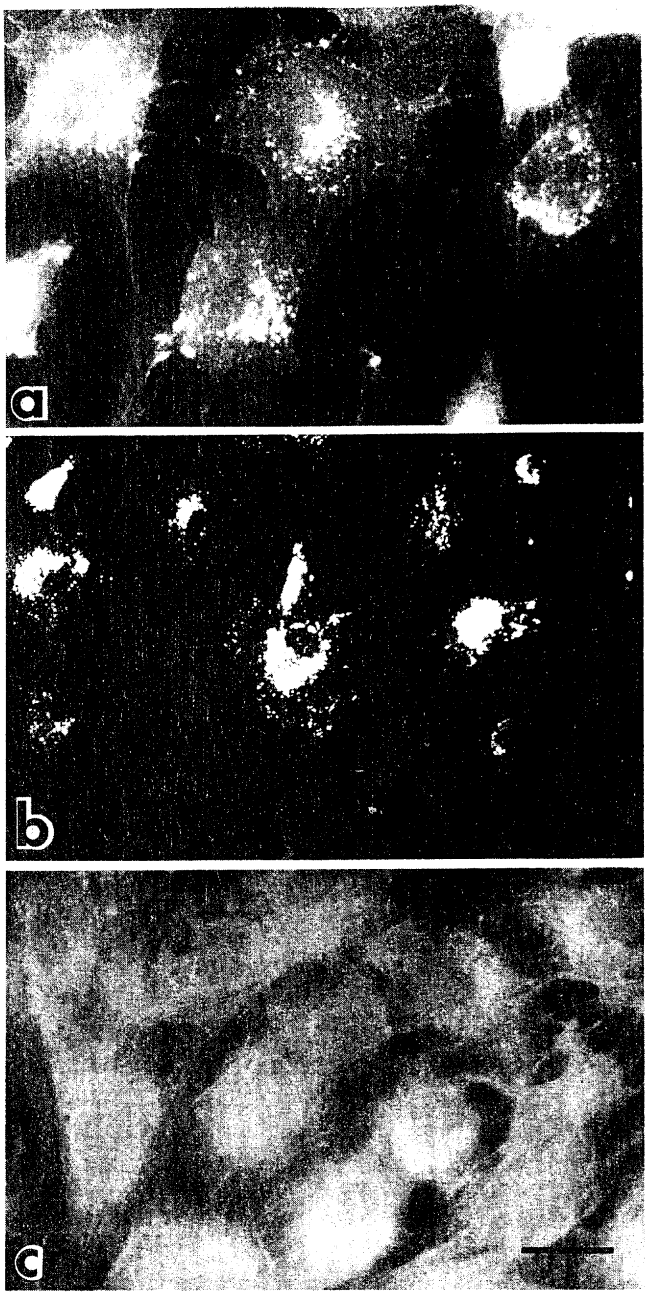

Figure 4. Immunofluorescent staining of BPE-1 cells with anti-human factor VIII R:Ag (*a*) and DiI-Ac-LDL (*b*). Photomicropgraph *c* shows uptake of bovine PTH (1-84) by BPE-1 cells (bar = 10 μm).

DISCUSSION

The vascular endothelium in endocrine tissues is histologically unique. The most prominent feature is the microvasculature consisting of capillaries, small venules, and arterioles. Culture of fully characterized and differentiated endothelial cells is the most direct means of studying specific metabolic properties of such cells, which, as it is now becoming apparent, interact abundantly with secretory cells (1, 2). We have isolated and cloned bovine parathyroid endothelial cells.

Success with this system is probably attributable to several factors: *1*) Choice of a tissue enriched in endothelial cells; *2*) Relative ease of culturing endothelial cells from the bovine compared with other species; *3*) Use of media conditioned by long-term cultures of bovine parathyroid cells. This approach stems from previous observations on growth of thyroid perifollicular blood vessels stimulated by angiogenic factors secreted by neighboring epithelial cells (12, 13); *4*) Use of feeder cells, critical in transfer of primary isolated colonies of endothelial cells; *5*) Use of thimerosal, which encourages growth of endothelial cells by virtue of killing pericytes (5); *6*) Addition of calcium to 1 mM , a concentration that inhibits proliferation of parathyroid epithelial cells (4); *7*) Use of a medium rich in growth factors favoring endothelial cell growth. The medium we used seemed critical for cloning and growth of BPE-1 cells; neither fetal nor adult calf serum were suitable for this purpose. The medium included factors such as aFGF and bFGF that favor endothelial cell growth and excluded concentrations of serum that im-

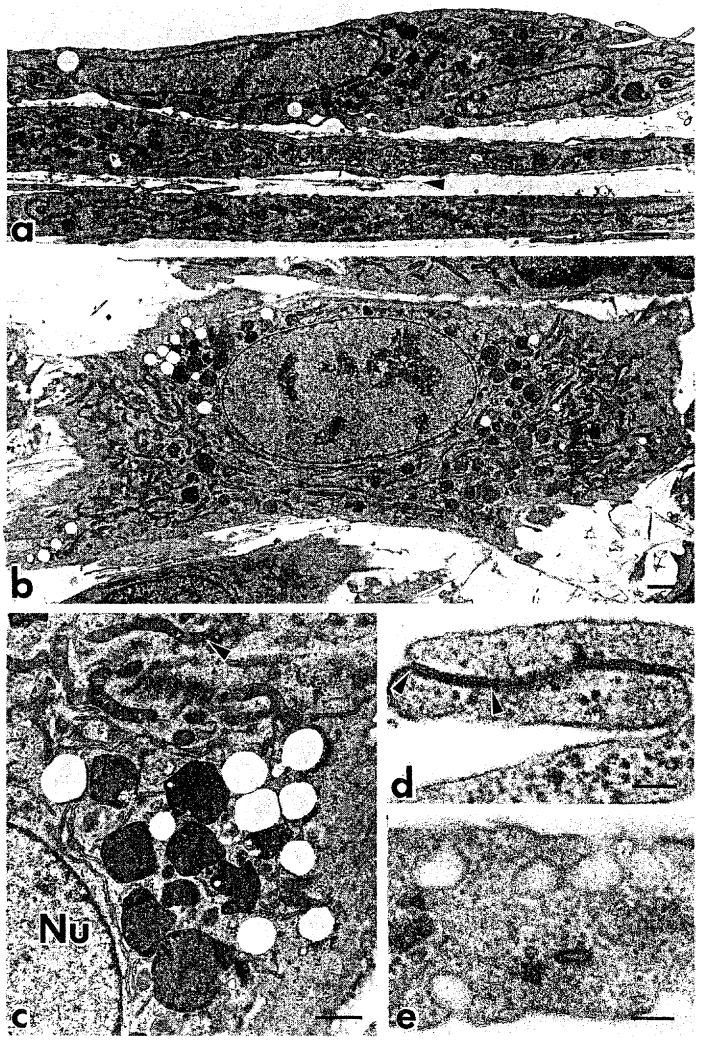

Figure 5. Ultrastructure of 7-day-old parathyroid endothelial cells. *A*) Low magnification view of flattened, multilayered cells with interspersed extracellular matrix (arrowhead). *B*) En face view of perinuclear organelles from section shown in *A*. *C*) Clear and dense cored granules and tubular mitochondria (arrowhead) typically found in perinuclear region of endothelial cells. *D*) Typical plasmalemmal contact (between arrowheads) routinely observed between extended processes of juxtaposed endothelial cells. *E*) Vesicle shuttling or transcytosis seen in thin extended process of endothelial cells. (A, B, and C bar = 1 μm; D and E bar = 0.1 μm).

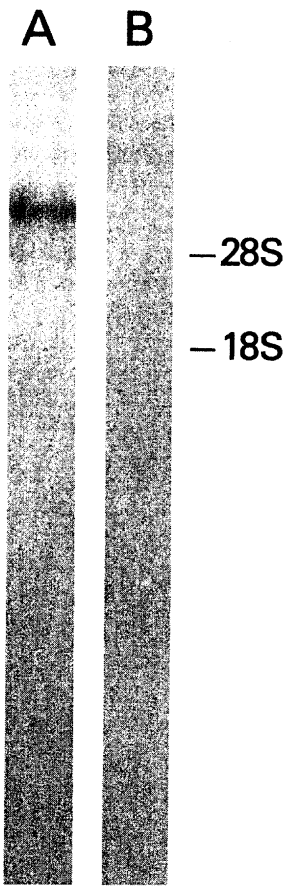

Figure 6. Identification of PDGF receptor mRNA by Northern blot hybridization analysis. Total RNA (20 μg) from BPE-1 (*A*) and CPAE (*B*) cells was isolated, submitted to electrophoresis, transferred to nitrocellulose, and hybridized with the cDNA probe for PDGF receptor.

pair fibroblast growth. The mixtures of serum substitutes are proprietary; hence we cannot define in detail all the factors that contribute to the optimal growth of the BPE-1 cells. Nevertheless, this type of medium has also been used to obtain a clonal population of bovine bone endothelial cells (14).

Throughout their in vitro life BPE-1 cells retained morphological features of endothelial cells with a cobblestone appearance, characteristic of endothelial cells from diverse sources. However, BPE-1 cells did not organize into tubular structures under our standard culture conditions, as described for other types of endothelial cells (1, 15). This might be due to the reciprocal relationship between growth and organization of endothelial cells, with three-dimensional tubular structures appearing under conditions that minimize cell growth (15). Production of factor VIII R:Ag and uptake of DiI-Ac-LDL are markers of endothelidal cells, and BPE-1 cells reacted positively for both. The diffuse distribution of factor VIII R:Ag fluorescence seen in long-term cultures of BPE-1 cells might represent antigen bound in endothelial matrix and/or membranes, perhaps derived from product elaborated from the cells and released into the medium.

BPE-1 cells display ultrastructural features clearly characteristic of endothelial cells. Most notable are the numerous caveoli lining the plasmalemma. Moreover, dense core and clear granule organelles found here are properties described for other endothelial cells (1, 14). Weibel-Palade bodies, characteristic of rodent and human endothelial cells and known to contain factor VIII R:Ag (16), were not found in BPE-1 cells. Although dense granules in BPE-1 cells do not show the characteristic rod-like shape, their size and cellular distribution correlate well with the fluorescent images or factor VIII R:Ag label. Therefore the dense core granules described here, seen in BAME cells (1) and bone-derived endothelium (14), may be the ubiquitous storage site for factor VIII R:Ag in the bovine species. Similarly, the size and distribution of the clear granules and the DiI-Ac-LDL fluorescent images suggest that these clear structures may be storage sites for low-density lipoprotein. Apparent translucence of these clear granules should not be interpreted as a lack of content, as the routine processing of the BPE-1 cells used here could easily extract lipid from these sites. Intercellular junctions (both tight and gap junctions) were rarely observed in BPE-1 cultures. Indeed, only tight junctions of the very leaky type without gap junctions and with few long interconnecting strands are typically found in freeze-fracture views of parathyroid tissue (17).

Several properties of BPE-1 cells deserve comment. The ability to take up PTH is a unique feature of BPE-1 cells. The apparent distribution of hormone in the cells likely represents diffusion of PTH into or absorption onto parathyroid endothelial cells. The physiological significance of PTH uptake into BPE-1 cells cannot be ascertained from the experiments reported currently. Perhaps there is a storage or metabolic function of the endothelium analogous to that found with BAME cells for catecholamines (1). Another distinctive characteristic of BPE-1 cells is the response to fibroblast growth factors. Acidic FGF and bFGF are only weak mitogens for BPE-1 cells, whereas they are usually potent stimulators of other endothelial cells, i.e, CPAE cells. This is one phenomenon that underscores differences among endothelial cells of diverse origin. This lack of marked response to fibroblast growth factors might reflect elaboration of such substances by BPE-1 cells themselves. Such a possibility is supported by the recent finding that BPE-1 cells express the gene for bFGF (18). The BPE-1 cells also responded to PDGF and expressed the gene for the PDGF receptor, although CPAE neither responded to PDGF nor expressed the PDGF receptor gene. These data, together with previous reports that the cerebral microvasculature responds uniquely to PDGF (19, 20), support the theory that the vascular endothelium is heterogeneous in its function and growth regulation. On the other hand, growth inhibition by TGFβ was a characteristic of BPE-1 and CPAE cells as well as other endothelial cells in culture (21). Calcium modulated BPE-1 cell growth in a fashion opposite to that characteristic of parathyroid endocrine cells (4). Finally, the cellular senescence phenomenon reported for endothelial cells of other origins (1, 14, 15) did not apply to BPE-1 cells.

Previously we reported that use of PT-b cells allowed detection of a parathyroid growth factor in multiple endocrine neoplasia type I (22) as well as antibodies to such cells in autoimmune hypoparathyroidism (23). We have found that the antibodies are directed against a component of parathyroid endothelial cells (24) and that it is the parathyroid endothelial cell that is activated by the mitogenic factor in multiple endocrine neoplasia type I (25). The cloning of a pure cell line of parathyroid endothelial cells described herein should allow further detailed studies of the mitogenic factor in multiple endocrine neoplasia type I and a more definitive characterization of the antibody in autoimmune hypoparathyroidism. Such cultures, moreover, may facilitate studies of paracrine interactions among epithelial and endothelial cells, uncover influences of local anatomy on endocrine function, and lead to new knowledge of diseases that may represent perturbations of endothelial cell biology. FJ

We are indebted to the following people for gifts: Dr. T. Maciag for aFGF; Dr. A. Baird for bFGF; Dr. L. E. Mallette for NG5 antiserum; and Drs. L. T. Williams and J. A. Escobedo for the PDGF receptor cDNA. We are grateful to Mrs. G. Goping for expert technical assistance.

REFERENCES

1. Banerjee, D. K., Ornberg, R. L., Youdim, M. B. H., Heldman, E., and Pollard, H. B. (1985) Endothelial cells from bovine adrenal medulla develop capillary-like growth patterns in culture. *Proc. Natl. Acad. Sci. USA* **82**, 4702-4706
2. Mizrachi, Y., Lelkes, P. I., Ornberg, R. L., Goping, G., and Pollard, H. B. (1989) Specific adhesion between pheocromocytoma (PC12) cells and adrenal medullary endothelial cells in coculture. *Cell Tissue Res.* **256**, 356-372
3. Folkman, J., Haudenschild, C. C., and Zetter, B. R. (1979) Long-term culture of capillary endothelial cells. *Proc. Natl. Acad. Sci. USA* **76**, 5217-5221
4. Brandi, M. L., Fitzpatrick, L. A., Coon, H. G., and Aurbach, G. D. (1986) Bovine parathyroid cells: cultures maintained for more than 140 population doublings. *Proc. Natl. Acad. Sci. USA* **83**, 1709-1713
5. Wagner, R. C., and Matthews, M. A. (1975) The isolation and culture of capillary endothelium from epididymal fat. *Microvasc. Res.* **10**, 286-297
6. Barile, M. F. (1978) In *Human and Animal Mycoplasma* (Tully, J. G., and Whitcomb, R. F., eds) pp. 425-474, Academic, New York
7. Todaro, G. Y., Fryling, C., and De-Larco, J. E. (1980) Transforming growth factors produced by certain human tumor cells: polypeptides that interact with epidermal growth factor receptors. *Proc. Natl. Acad. Sci. USA* **77**, 5258-5262
8. Bruns, R. R., and Palade, G. E. (1968) Studies on blood capillaries. II. Transport of ferritin molecules across the wall of muscle capillaries. *J. Cell Biol.* **37**, 277-299
9. Chirgurin, J. M., Przybyla, A. E., MacDonald, R. J., and Rutter, W. J. (1979) Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. *Biochemistry* **18**, 5294-5299
10. Maniatis, T., Fritsch, E. F., and Sambrook, J. (1982) In *Molecular Cloning a Laboratory Manual* (Maniatis, T., Fritsch, E. F., and Sambrook, J. eds) pp. 187-209, Cold Spring Harbor Laboratory, New York
11. Yarden, J., Escobedo, J. A., Kuang, W. J., Yang-Feng, T. L., Daniel, T. O., Tremble, P. M., Chen, E. Y., Ando, M. E., Harvins, R. N., Francke, V., Fried, V. A., Ullrich, A., and Williams, L. T. (1986) Structure of the receptor for platelet-derived growth factor helps define a family of closely related growth factor receptors. *Nature (London)* **323**, 226-232
12. Goodman, A. L., and Rone, J. D. (1987) Thyroid angiogenesis: endotheliotropic chemoattractant activity from rat thyroid cells in culture. *Endocrinology* **121**, 2131-2140
13. Wollman, S. H., Herveg, J. P., Zeligs, J. D., and Ericson, L. E. (1978) Blood capillary enlargement during the development of thyroid hyperplasia in the rat. *Endocrinology* **103**, 2306-2314
14. Streeten, E. A., Curcio, F., Ornberg, R. L., Sakaguchi, K., Marx, S. J., Aurbach, G. D., and Brandi, M. L. (1988) Endothelial cell line cloned from fetal bovine bone. *Proc. Natl. Acad. Sci. USA* **86**, 916-920
15. Maciag, T., Kadish, J., Wilkins, L., Stemerman, M. B., and Weinstein, R. (1982) Organizational behavior of human umbilical vein endothelial cells. *J. Cell Biol.* **94**, 511-520
16. Wagner, D. D., Olmsted, J. B., and Marder, V. J. (1982) Immunolocalization of von Willebrand protein in Weibel-Palade bodies of human endothelial cells. *J. Cell Biol.* **95**, 355-360
17. Thiele, J. (1984) In *Ultrastructure of Endocrine Cells and Tissues* (Motta, P. M., ed) pp. 302-312, Martinus Nijhoff Publishers, Boston
18. Sakaguchi, K., Brandi, M. L., and Aurbach, G. D. (1989) Expression of mRNAs for growth factors and their receptors in parathyroid epithelial and capillary endothelial cells. The Endocrine Society 71st Annual Meeting Program & Abstracts, p. 471
19. Robinson, R. A., Teneyck, C. J., and Hart, M. N. (1986) Methods in laboratory investigation. Establishment and preliminary growth characteristics of a transformed mouse cerebral microvessel endothelial cell line. *Lab. Invest.* **56**, 579-588
20. Robinson, R. A., Teneyck, C. J., and Hart, M. N. (1986) Growth control in cerebral microvessel-derived endothelial cells. *Brain Res.* **384**, 114-120
21. Heimark, R. L., Twardzik, D. R., and Schwartz, S. M. (1986) Inhibition of endothelial regeneration by type-beta transforming factor from platelets. *Science* **233**, 1078-1089
22. Brandi, M. L., Aurbach, G. D., Fitzpatrick, L. A., Quarto, R., Spiegel, A. M., Bliziotes, M. M., Norton, J. A., Doppman, J. L., and Marx, S. J. (1986) Parathyroid mitogenic activity in plasma from patients with familial multiple endocrine neoplasia type I. *N. Engl. J. Med.* **314**, 1287-1293
23. Brandi, M. L., Aurbach, G. D., Fattorossi, A., Quarto, R., Marx, S. J., and Fitzpatrick, L. A. (1986) Antibody cytotoxic to bovine parathyroid cells in autoimmune hypoparathyroidism. *Proc. Natl. Acad. Sci. USA* **83**, 8366-8369
24. Fattorossi, A., Aurbach, G. D., Sakaguchi, K., Cama, A., Marx, S. J., Streeten, E. A., Fitzpatrick, L. A., and Brandi, M. L. (1988) Anti-endothelial cells antibodies: detection and characterization in sera from patients with autoimmune hypoparathyroidism. *Proc. Natl. Acad. Sci. USA* **85**, 4015-4019
25. Brandi, M. L., Zimering, M. B., Marx, S. J., DeGrange, D., Goldsmith, P., Sakaguchi, K., and Aurbach, G. D. (1989) In *Clinical Disorders of Bone and Mineral Metabolism* (Kleerekoper, M., and Krane, S. M., eds) pp. 323-328, Mary Ann Liebert Inc., New York

Received for publication January 31, 1990.
Accepted for publication June 13, 1990.