CHARACTERIZATION OF PRIMARY HUMAN ENDONEURIAL ENDOTHELIAL CELLS THAT FORM THE BLOOD-NERVE BARRIER.

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INTRODUCTION

Endoneurial homeostasis is achieved by specialized microvascular endoneurial endothelial cells and perineurial myofibroblasts.

Blood-nerve barrier (BNB) is formed by endoneurial microvessels. Phenotypic and functional characteristics largely unknown.

Differences in vascular endothelium from different tissues and macro- and microvascular endothelium within the same tissue occur.

Understanding physiologic or pathologic processes at the human BNB requires study of endoneurial endothelial cells.

This knowledge may provide insights to how solutes and macromolecules may gain access into the endoneurium from the bloodstream.

OBJECTIVE

To isolate and characterize **p**rimary **h**uman **end**oneurial **e**ndothelial **c**ells (pHEndECs) that form the blood-nerve barrier.

METHODS

Both sciatic nerves were harvested from recently decedent individuals (<24 hours post-mortem) undergoing autopsy at St. Luke's Episcopal Hospital.

Endoneurial stripping (Teased fiber technique) followed by digestion with Type I collagenase and Type I hyaluronidase for 18-20 hours was performed.

Crude microvessels were released from debris, myelin, axons and other cells by density centrifugation in 15% dextran.

Crude microvessels were further digested in collagenase-dispase to release cells from surrounding basement membrane.

Released cells were further purified using a continuous 43% Percoll gradient.

Isolated pHEndECs are cultured on rat tail collagen-coated Corning CellBIND® dishes/ flasks.

Media changed every 48-72 hours. Cells passaged at 80-90% confluence.

Isolated cells were studied at passages 3-10.

Morphometric analysis was performed on digital phase and differential interference contrast photomicrographs.

Matrigel assay was used to study differentiation and angiogenesis *in vitro*.

Transendothelial electrical resistance (TEER), solute permeability to high molecular weight dextran and electron microscopy were used to study barrier function of pHEndECs grown on 6.5 mm diameter, 3.0 µm pore size, rat tail collagen-coated polyester transwell inserts.

Enzyme cytochemistry, indirect immunohistochemistry, flow cytometry and polymerase chain reaction used to study specialized transporters.

RESULTS

Figure 1. pHEndEC morphology and growth *in vitro*

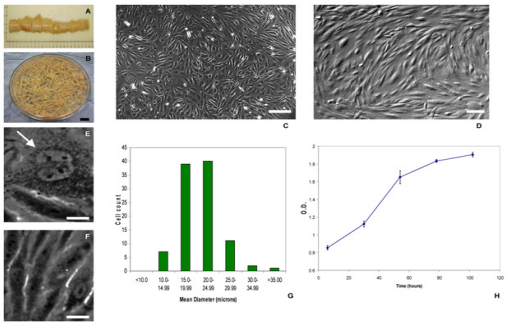


Figure 2. *In vitro* differentiation and angiogenesis

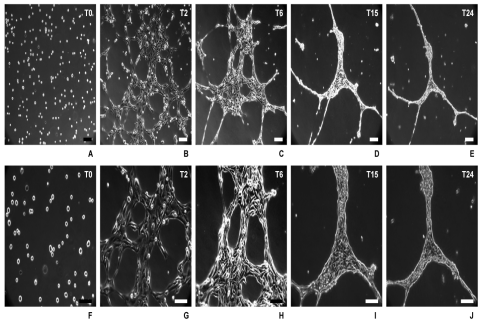


Figure 3. Vascular endothelial cell markers

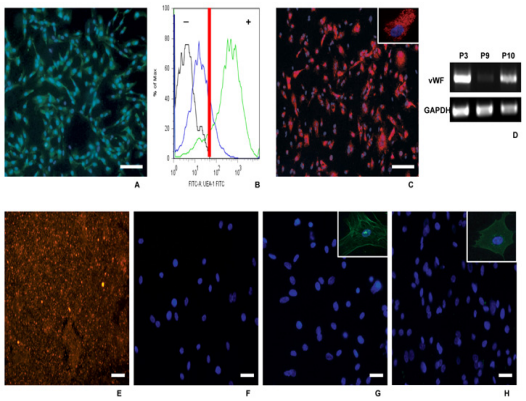


Figure 4. Specialized transporter expression

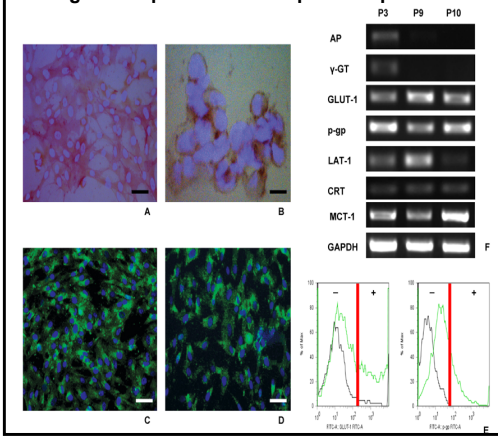
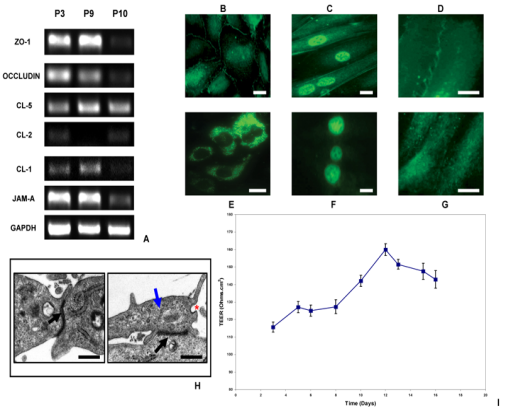


Figure 5. Tight Junction Barrier properties



CONCLUSIONS

pHEndECs are the endothelial cells that form the human blood-nerve barrier *in vivo*.

Specialized transporters are expressed, implying a critical functional role of the blood-nerve barrier in nutrient entry into and toxin removal from the peripheral nerve endoneurium.

Lactate and creatine may be utilized for energy production within the endoneurium at times of starvation.

pHEndECs form a high resistance endothelial barrier *in vitro* with expression of specific tight-junction proteins at sites of cell-to-cell contact.

The *in vitro* blood-nerve barrier model provides an assay to study mechanisms of solute influx and efflux in peripheral nerves.

Methods of enhancing drug transport across the blood-nerve barrier to improve efficacy or limit toxicity may be studied.

Further Reading:

Yosef N, Xia RH, Ubogu EE. Development and characterization of a novel human in vitro blood-nerve barrier model using primary endoneurial endothelial cells. J Neuropathol Exp Neurol 2010; 69:82-97.