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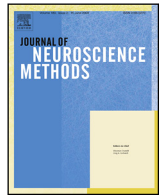

Co-culture model consisting of human brain microvascular endothelial and peripheral blood mononuclear cells

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HIGHLIGHTS

- The immortalized human BMEC cell line, hCMEC/D3, in vitro blood-brain barrier model is useful in long-term functional studies.
- hCMEC/D3 media components EBM-2 and hydrocortisone are cytotoxic to peripheral blood mononuclear cells (PBMCs).
- Co-culture media excluding EBM-2 and hydrocortisone support hCMEC/D3 barrier function as well as continued PBMC viability.
- Co-culture media can be used in assays of PBMC transmigration.

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ABSTRACT

Background: Numerous systems exist to model the blood–brain barrier (BBB) with the goal of understanding the regulation of passage into the central nervous system (CNS) and the potential impact of selected insults on BBB function. These models typically focus on the intrinsic cellular properties of the BBB, yet studies of peripheral cell migration are often excluded due to technical restraints.
New method: This method allows for the study of in vitro cellular transmigration following exposure to any treatment of interest through optimization of co-culture conditions for the human brain microvascular endothelial cells (BMEC) cell line, hCMEC/D3, and primary human peripheral blood mononuclear cells (PBMCs).
Results: hCMEC/D3 cells form functionally confluent monolayers on collagen coated polytetrafluoroethylene (PTFE) transwell inserts, as assessed by microscopy and tracer molecule (FITC-dextran (FITC-D)) exclusion. Two components of complete hCMEC/D3 media, EBM-2 base-media and hydrocortisone (HC), were determined to be cytotoxic to PBMCs. By combining the remaining components of complete hCMEC/D3 media with complete PBMC media a resulting co-culture media was established for use in hCMEC/D3—PBMC co-culture functional assays.
Comparison with existing methods: Through this method, issues of extensive differences in culture media conditions are resolved allowing for treatments and functional assays to be conducted on the two cell populations co-cultured simultaneously.
Conclusion: Described here is an in vitro co-culture model of the BBB, consisting of the hCMEC/D3 cell line and primary human PBMCs. The co-culture media will now allow for the study of exposure to potential insults to BBB function over prolonged time courses.

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1. Introduction

The blood–brain barrier (BBB) regulates passage between peripheral circulation and the central nervous system (CNS). Aside from limiting permeability to circulating proteins, ions, and therapeutic compounds, the BBB also controls cellular transmigration and immune surveillance (Loeffler et al., 2011; Strazza

Abbreviations: BBB, blood–brain barrier; BMEC, brain microvascular endothelial cells; CNS, central nervous system; FITC-D, fluorescein isothiocyanate dextran; PBMC, peripheral blood mononuclear cells; Pe, permeability coefficient; PTFE, polytetrafluoroethylene; TEER, transendothelial electrical resistance.

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et al., 2011). In comparison to other tissues and organs throughout the body, immune surveillance of the CNS is more limited and tightly regulated in order to control the neuroinflammatory response and to minimize the risk of deleterious neuroinflammation within the brain (Miner and Diamond, 2016; Ousman and Kubes, 2012; Ransohoff and Engelhardt, 2012). In this regard, accelerated immune cell extravasation has been linked to a number of disease pathologies (Katsetos et al., 1999; Ryan et al., 2005; Song et al., 2015). Understanding the impact of compounds or proteins that alter proper BBB function is a critical first step in developing appropriate interventions to specific neuropathologic conditions.

In order to study BBB integrity following exposure to experimental conditions, model systems must be utilized. These model systems, either in vivo or in vitro, incorporate, at minimum, brain microvascular endothelial cells (BMEC), which comprise the BBB endothelium into selected co-culture combinations with astrocytes, pericytes, and under certain circumstances, neurons. In vitro models using human cells are generally preferred to animal models when addressing many types of experimental questions, and the culture of primary human BMECs is generally accepted to require or be greatly enhanced by co-culture with primary human astrocytes (Eugenin and Berman, 2003; Hayashi et al., 1997). The in vitro primary co-culture model maintains the BBB characteristics for a window of one to two days beyond establishing confluence, which can limit the duration of experimental exposures. The recent development of the immortalized human BMEC cell line, hCMEC/D3 cells, has led to the development of another human in vitro BBB model; the hCMEC/D3 cell line was established through the in vitro immortalization of BMECs isolated from the temporal lobe (Weksler et al., 2005). Critical to defining the hCMEC/D3 cell line as a BBB model, these cells were shown to express BBB-specific markers, including ZO-1, JAM-A, occludin, and claudin-5 (Eigenmann et al., 2013; Helms et al., 2016; Huang et al., 2009; Pu et al., 2005; Weksler et al., 2005). In addition, hCMEC/D3 cells maintain the functional characteristics of the BBB in monoculture for several days beyond confluence, allowing for extended duration exposures that are critical to drug studies as well as models of chronic inflammatory diseases (Daniels et al., 2013; Jacob et al., 2015; Urich et al., 2012; Weksler et al., 2013a, 2005). Recent studies have been aimed at defining the specific attributes of hCMEC/D3 cells, as well as other endothelial cell lines that are emerging in popularity as BBB models (Helms et al., 2016). These studies confirmed tight junction protein expression and low transendothelial permeability that was not enhanced through co-culture with astrocytes or pericytes, and conclude that the mono-culture of the immortalized human cell line will provide another valuable tool in the in vitro studies of the BBB (Cucullo et al., 2008; Eigenmann et al., 2013; Weksler et al., 2005).

Beyond studying characteristics of the BBB such as transendothelial electrical resistance (TEER), tight junction protein expression and localization, cell adhesion molecule expression, and small molecule permeability, the interaction between immune cells and the BBB during the process of transmigration must also be assessed following an experimental exposure in order to fully understand the nature of the dysfunction induced. Transmigration functional assays require the co-culture of BMECs with immune cell populations, in the absence or presence of astrocytes, pericytes, and cells relevant to a functional BBB. The merging of these cell types into a common culture setup creates the obstacle of also bringing together the culture conditions that were previously defined and optimized for monoculture of each unique cell type. In the case of the hCMEC/D3 cell line, the defined media for cell growth contains a complex combination of media components, each serving distinct roles while collectively promoting the in vitro growth and maintenance of the cells. In contrast, primary human PBMCs are often maintained in a simple media formulation consisting of a few essential supplements to the base media. Presented here is a unique media formulation that maintains viability and function of primary human PBMCs and hCMEC/D3 endothelial cells.

2. Methods

2.1. Materials

Mannitol and FITC-dextran (70 kDa) (FITC-D) were purchased from Sigma–Aldrich. SDF-1α was purchased from eBioscience. IL-2 was obtained through the NIH AIDS Reagent Program, Division of AIDS, NIAID, NIH, from Dr. Maurice Gately, Hoffmann—La Roche Inc. (Lahm and Stein, 1985).

2.2. Cell culture and treatment

The human BMEC line, hCMEC/D3, was obtained from Dr. Babette Weksler (Weill Cornell Medical College) and cultured in EBM-2 (Lonza) supplemented with heat inactivated fetal bovine serum (FBS; 5%, GemBio), penicillin–streptomycin (1%, CellGro), hydrocortisone (1.4 μM, CellGro), ascorbic acid (5 μg/mL, Sigma–Aldrich), chemically defined lipid concentrate (1%, Invitrogen), HEPES (10 mM, CellGro), and basic fibroblast growth factor (1 ng/mL, Sigma–Aldrich) (designated herein as "CMEC media"). All experiments were conducted between cell passages 27–32. Cells were grown on petri dishes or 12-well plates coated with Cultrex rat collagen I (150 μg/mL, Trevigen) in H_2O. hCMEC/D3 cells were seeded on petri dishes and 12-well plates at a density of 37,000 cells/cm^2 or on transwell inserts (details below). At confluence on plates or in transwells, hCMEC/D3 cells were treated where indicated with mannitol (1.4 M, Sigma–Aldrich) for 30 min.

Primary human PBMCs were obtained from the Comprehensive NeuroAIDS Center (Basic Science Core 1, Temple University School of Medicine). Cells were cultured overnight under unstimulated, non-adherent conditions in polypropylene 50 mL conical tubes at a density of 1×10^6 cells/mL in RPMI media supplemented with heat-inactivated FBS (10%), penicillin–streptomycin (1%), and IL-2 (20 U/mL) (designated herein as "PBMC media").

Co-culture studies were performed in "co-culture media." Co-culture media consists of RPMI supplemented with heat-inactivated FBS (5%), penicillin–streptomycin (1%), ascorbic acid (5 μg/mL), chemically defined lipid concentrate (1%), HEPES (10 mM), basic fibroblast growth factor (1 ng/mL), and IL-2 (20 U/mL).

2.3. In vitro blood–brain barrier model

hCMEC/D3 cells were seeded on collagen-coated porous polytetrafluoroethylene (PTFE) transwell inserts (Corning, NY) at a density of 4.5×10^4 cells/cm^2 and grown for 10 days to achieve confluence for use in FITC-D and PBMC transmigration assays. Of note, transwell inserts were purchased pre-coated with collagen, limiting the variability to which coating with collagen prior to each experiment may influence cell growth and membrane permeability. Addition of treatments to the upper chamber of the transwell inserts was used to mimic a peripheral exposure.

2.4. FITC-dextran permeability assay

Any indicated treatments of the hCMEC/D3 monolayers were performed at confluence on porous PTFE transwell inserts (0.4 μm). The monolayers were subsequently washed with 10 mM HEPES in 1X HBSS and the FITC-dextran (FITC-D) permeability assay was performed as previously described (Weksler et al., 2005) with minor modification. The use of the FITC-D assay to monitor confluence and small-molecule permeability is the preferred method

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