MECHANISMS OF LEUKOCYTE TRAFFICKING AT THE HUMAN BLOOD-NERVE BARRIER DURING INFLAMMATION: SELECTIVE EXPRESSION AND FUNCTION OF CHEMOKINES AND CELL ADHESION MOLECULES *IN VITRO*.

Nejla Yosef, M.S., Robin H. Xia, M.D., Ph.D., Eroboghene E. Ubogu, M.D.
Neuromuscular Immunopathology Research Laboratory, Department of Neurology,
Baylor College of Medicine, Houston, Texas, United States of America.

INTRODUCTION

Hematogenous leukocyte migration into peripheral nerves and nerve roots is a pathologic hallmark of immune-mediated demyelinating polyradiculopathies (IDP) such as GBS and CIDP.

Leukocyte trafficking across vascular endothelium is a sequential coordinated process dependent on selectins and their glycosylated counter-ligands, chemokines and their receptors, cell adhesion molecules and leukocyte integrins and matrix metalloproteases.

Chemokines are the initial mediators of leukocyte trafficking across chemotactic and haptotactic gradients *in vitro* and *in vivo*.

Specific chemokines (e.g. CCL2 [MCP-1], CXCL10 [IP-10]) and their receptors (CCR2, CXCR3) are highly expressed in the peripheral nerves or cerebrospinal fluid or both of patients with IDP compared to patients with non-inflammatory neuropathies.

Expression of endothelial cell adhesion molecules (CAMs) such as vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1) have been described in peripheral nerves of IDP patients and their animal models.

Mechanistic studies are required to determine the mediators of leukocyte trafficking at the blood-nerve barrier.

OBJECTIVE

To determine the roles of selective proinflammatory molecules in leukocyte trafficking at the blood-nerve barrier *ex vivo*.

METHODS

Primary human endoneurial endothelial cells (pHEndECs, passage 7-8) derived from sciatic nerves were grown to near confluence on 6-well (9.6 cm^2 surface area) rat tail collagen-coated plates, 6.5 mm diameter, 3.0 µm pore polyester transwell insert membranes or glass coverslips placed in 24-well culture plates.

Cells were treated with 10 U/mL tissue necrosis factor-α (TNF-α) and 20 U/mL interferon-γ (IFN-γ) for 24 hours to mimic early inflammatory events at the blood-nerve barrier. Cells retained in culture media were used to represent basal, resting conditions.

Chemokine antibody array (Raybiotech, Norcross, Georgia, USA) was used to semi-quantitatively determine chemokine expression relative to basal conditions.

Indirect fluorescent immunohistochemistry was used to visualize the effect of cytokine stimulus on surface CAM expression by pHEndECs.

Transendothelial electrical resistance (TEER) was measured using a EVOM voltohmmeter with STX2 electrodes.

Peripheral blood mononuclear leukocytes (PBMLs; 55-70% CD3+ T-cells, 10-20% CD14+ monocytes and 5-10% CD19+ B-cells) were isolated from an untreated patient with clinical, cerebrospinal fluid and electrophysiological evidence for GBS by Ficoll density centrifugation.

The dynamic *in vitro* blood-nerve barrier (IVBNB) was formed by plating pHEndECs on 35 mm rat tail collagen-coated Corning CellBIND® dishes attached to a Glyotech parallel plate flow chamber.

200,000 PBMLs/ mL were infused over the IVBNB at a flow velocity of 1-2 mm/s, mimicking estimated capillary flow rates *in vivo*.

The numbers of firmly adherent or transmigrated PBMLs were quantified by video microscopy.

The effect of CCL2-CCR2, CXCL10-CXCR3, ICAM-1 and the alternatively spliced variant of fibronectin called connecting segment-1 (FN CS1) on PBML trafficking was studied using function-neutralizing antibodies or antagonists.

RESULTS

Figure 1. pHEndEC chemokine antibody array

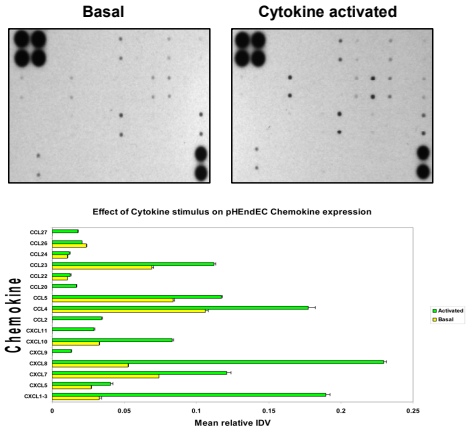


Figure 2. *In vitro* blood-nerve barrier adhesion molecule expression

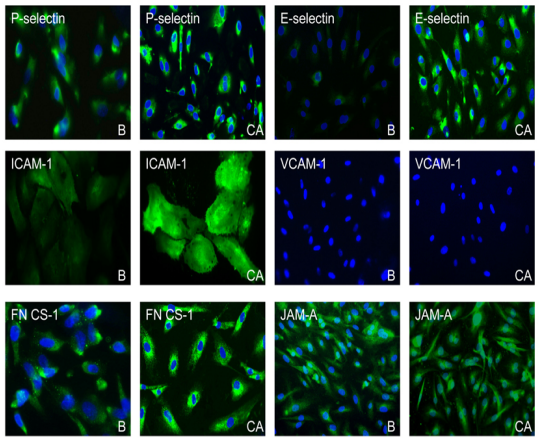
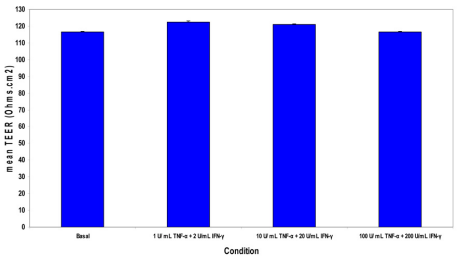


Figure 3. Effect of cytokines on IVBNB TEER



CONCLUSIONS

pHEndECs express specific chemokines known to participate in the innate and adaptive immune responses following low dose cytokine stimulation.

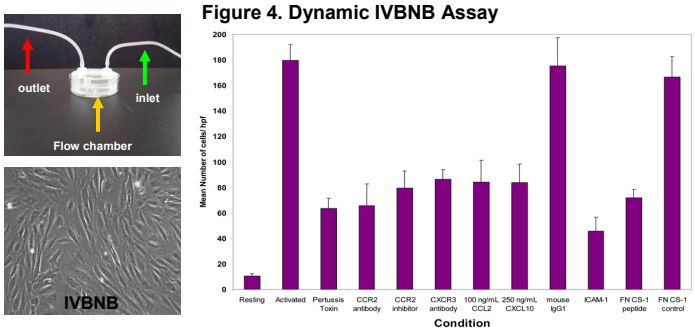
There is increased or induced expression of chemokines implicated in the pathogenesis of GBS and CIDP at the IVBNB following cytokine stimulus.

There is increased expression of P-selectin, ICAM-1 and FN CS-1 with induced E-selectin expression. JAM-A expression was not affected by cytokine stimulus and VCAM-1 expression was not detected or induced on pHEndECs at these passages *in vitro*.

Cytokine stimulation induces GBS patient PBML trafficking at the IVBNB, dependent on chemokine signaling.

Inhibition of CCL2-CCR2, CXCL10-CXCR3, ICAM-1 and FN CS-1signaling results in reduced PBML trafficking at the IVBNB under flow conditions.

Figure 4. Dynamic IVBNB Assay



FUTURE DIRECTIONS

Confirm proinflammatory molecular expression by PCR and western blot and determine time course.

Confirm mechanisms of GBS/CIDP PBML trafficking at the blood-nerve barrier *in vitro*.

Microarrays of cytokine-activated pHEndECs may provide insights to other relevant pro- or anti-inflammatory molecules.

Study effect of inhibition/ gene knockout of selected proinflammatory molecules in mouse models of GBS/ CIDP.