Title: Mechanisms of Fatigue in Post-Viral Syndromes: Modeling Blood-Brain Barrier Disruption and Immune-Brain Crosstalk In Vitro

Scientific Background

Fatigue is a complex and debilitating symptom commonly observed in various post-viral syndromes, particularly in Post-COVID Syndrome (PCS) and Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS). Increasing evidence highlights the neuroimmune axis as a central component in the pathogenesis of these conditions. Specifically, dysfunction of the blood-brain barrier (BBB) is thought to contribute to central fatigue by permitting peripheral immune activation to influence the central nervous system (CNS), thereby promoting neuroinflammation and neuronal stress.

Studies have demonstrated that viral proteins, including SARS-CoV-2 spike S1, can directly impair BBB integrity by altering tight junctions and activating endothelial inflammatory responses. Other viruses, particularly members of the herpesvirus family such as Cytomegalovirus (CMV) and Epstein-Barr Virus (EBV), are known to reactivate in ME/CFS and PCS patients and express proteins that trigger inflammatory cascades in brain endothelial and glial cells. Additionally, neurodegenerative peptides like amyloid-β (Aβ1-42) and α-synuclein are known to interact with viral components, potentially amplifying CNS inflammation and cellular stress via innate immune mechanisms such as the NLRP3 inflammasome.

When BBB integrity is compromised, immune cells, including monocytes, macrophages, neutrophils, and natural killer (NK) cells, can infiltrate the CNS. These infiltrating immune cells contribute to neuroinflammation by releasing pro-inflammatory cytokines, reactive oxygen species, and other mediators that exacerbate glial activation and neuronal stress. Immune cell trafficking across the BBB is increasingly recognized as a critical factor in neuroimmune interactions during post-viral syndromes. Despite growing clinical recognition of these processes, there remains a critical need for mechanistic in vitro models to dissect the molecular interactions underlying BBB disruption and immune-brain crosstalk in the context of post-viral fatigue.

Hypotheses

- Viral antigens (e.g., SARS-CoV-2 spike S1, EBV gp350) disrupt BBB integrity and induce inflammatory responses in endothelial and glial cells.
- BBB breakdown allows immune cells (e.g., monocytes, macrophages) to infiltrate CNS-like environments.
- Neurodegenerative peptides (e.g., Aβ1-42, α-synuclein) synergize with viral components to exacerbate inflammation and cellular stress.

Objectives

- Model BBB dysfunction using human brain endothelial and glial cells under stimulation with viral antigens.
- Characterize molecular and cellular responses using advanced molecular biology techniques.
- Evaluate immune cell transmigration and synergy with neurodegenerative peptides.

Methodology

1. Cell Models

- Human brain microvascular endothelial cells (e.g., hCMEC/D3 or hBMVEC)
- Human astrocytes and microglia (e.g., primary or immortalized lines)
- Immune cells: Monocytes/macrophages (e.g., THP-1 cells or primary PBMCs); neutrophils (primary or differentiated cell lines), and NK cells (isolated from PBMCs or NK cell lines)

2. Stimulation Conditions

- Recombinant viral antigens: SARS-CoV-2 spike S1, EBV gp350, CMV glycoproteins, and other herpesvirus antigens
- Neurodegenerative peptides: Amyloid-β (Aβ1-42), α-synuclein
- Combined stimulation to evaluate synergistic effects

3. Readouts and Techniques

- Gene Expression: RT-qPCR for inflammatory and neurodegenerative markers: IL-6, IL-1β, TNF-α, CCL2, CXCL10, GFAP (astrocyte marker), IBA1/AIF1 (microglial activation), APP, MAPT (tau), SNCA (α-synuclein), NEFL (neurofilament light chain, if neuronal cells included), NLRP3, TLR2/4.
- Protein Analysis: ELISA, Western blot or Luminex assay for cytokines and neurodegenerative proteins (APP, Aβ, α-synuclein, NFL).
- Immunofluorescence: To assess BBB integrity, junctional protein localization, and glial activation.
- FACS (Flow Cytometry): For immune cell activation markers and transmigration assays.

4. Functional Assays:

- TEER (Transendothelial Electrical Resistance) measurements for BBB integrity.
- Permeability assays using fluorescent tracers.
- Cellular and Mitochondrial ROS assay
- Cytotoxic and cell death assays

5. Co-culture and Immune Cell Transmigration Models

- Establish co-cultures of endothelial cells with astrocytes and microglia to mimic the neurovascular unit, thereby recapitulating key cellular interactions that maintain BBB integrity and regulate neuroinflammation.
- Incorporate immune cells (monocytes, macrophages, neutrophils, NK cells) in transmigration assays using Transwell systems. Immune cells will be added to the

vascular (apical) side and their migration across the endothelial barrier will be assessed under stimulation with viral antigens and neurodegenerative peptides.
- This setup allows evaluation of how viral and neurodegenerative stimuli alter BBB permeability and promote immune cell infiltration, as well as the subsequent impact of immune cells on endothelial and glial activation.
- Co-stimulation experiments will explore synergistic effects on barrier function, cytokine production, and cell viability.
- TEER and permeability assays will be performed dynamically during immune cell transmigration to monitor barrier integrity in real-time.

Expected Outcomes

This study aims to delineate how viral antigens and neurodegenerative peptides contribute to BBB disruption and neuroinflammation. It is anticipated that:

- Viral proteins will compromise barrier integrity and trigger pro-inflammatory responses.
- Immune cells will transmigrate more effectively when the BBB is pre-exposed to viral antigens.
- Co-stimulation with Aβ or α-synuclein will exacerbate inflammatory and stress responses, mimicking a neurodegenerative environment.
- Co-culture and transmigration models will provide physiologically relevant insights into the cellular mechanisms underlying post-viral fatigue, informing potential therapeutic targets in PCS and ME/CFS.

This work will provide mechanistic insights into post-viral fatigue and may identify targets for therapeutic intervention in PCS and ME/CFS.

Student Research Roles

The student will be actively involved in all phases of the project, including:

- Reading and reviewing relevant scientific literature to support experimental design and interpretation.
- Performing laboratory experiments
- Collecting and analyzing data
- Evaluating experimental results critically and participating in troubleshooting.
- Contributing to the preparation of reports, presentations, and potentially manuscripts based on the findings.