Research Proposal on Cytokine-Mediated Pro-Inflammatory Response in Leber's Hereditary Optic Neuropathy (LHON)

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Project Title

Elucidating Cytokine-Mediated Pro-Inflammatory Response in the Pathogenesis of Optic Nerve Head Injury in Leber's Hereditary Optic Neuropathy (LHON)

Background and Significance

Leber's Hereditary Optic Neuropathy (LHON) is a maternally inherited mitochondrial disorder that results in acute, bilateral, and irreversible vision loss, primarily affecting the retinal ganglion cells (RGCs) and the optic nerve. The visual prognosis is typically unfavorable and can lead to blindness (1). In LHON, dysfunctional mitochondrial respiratory chain complex I generates excessive reactive oxygen species (ROS) (2). This accumulation of ROS is responsible for the development of LHON and other neurodegenerative diseases (3). While the underlying genetic defects in complex I of the electron transport chain are well-established, the specific mechanisms that drive the selective progressive degeneration of the optic nerve in LHON remain elusive (4).

Extensive research has established that the inflammatory response is a critical factor in most neurodegenerative disorders (5, 6). In contrast, reports on the involvement of inflammation in the pathophysiology of LHON are limited (7-10). Patients with primary mitochondrial diseases (PMDs) show neuro-inflammatory signatures besides neurodegeneration (11). The main mitigators of neuro-inflammation are microglia and astrocytes. Microglia express various pattern recognition receptors (PRRs) that sense potential pathogens and tissue disruptions. Astrocytes possess the ability to exhibit profound response to insults, presenting with morphological, molecular and functional changes (12, 13). In addition, mitochondria contain molecules such as mtDNA, mtRNA and cardiolipin with immunogenic characteristics that could activate PRRs (14). During severe injury induced by stress or infection, damage-associated molecular patterns (DAMPs) are released from damaged cells which cause local inflammatory response (15). Mitochondria are a major source of these DAMPs and mitochondrial dysfunction can result in the release of DAMPs, into the cytosol or extracellular environment, thereby result in the activation of the innate immune system (16, 17). Energetic deficiency, reactive oxygen species (ROS) and redox imbalance caused by mitochondrial dysfunction increases the permeability of the mitochondrial membrane, inducing the release of these inflammatory mitochondrial components such as mtDNA and cardiolipin (18). This could help explain the inflammatory response observed in models of LHON. Zhou et al. found that in

LHON-derived fibroblasts (LHON-iFBs), the ratio of phosphorylated IκBα (p-IκBα) to total IκBα protein was increased, along with elevated levels of the inflammatory cytokine IL-6. These results suggest that activation of the NF-κB signaling pathway, which regulates inflammatory gene expression, may be involved in the pathogenesis of LHON (7). Furthermore, Yu et al. investigated the potential of repurposing the drugs papaverine and zolpidem to mitigate vision loss in a mouse model of mitochondrial optic neuropathy. All drugs significantly suppressed innate immune markers and markers of cytokine signaling, with papaverine being the most potent inhibitor of microglial activation (10). None-the-less, LHON is a disease traditionally considered as non-inflammatory and as there is no leakage at the optic dic on fluorescein angiography, this is the clinical maxim. Adaptive Optics Scanning Laser Ophthalmoscope (AOSLO) has the potential to enable the detection of cellular biomarkers, such as inflammatory cells, in healthy LHON carriers before the onset of clinical symptoms, which could improve our understanding of the early disease mechanisms (8). However, Lam et al. explored potential biomarkers and indicators of retinal and optic nerve damage in patients with LHON. The researchers analyzed serum and plasma samples from a Brazilian LHON population to assess various markers, including total antioxidant levels, lipid peroxidation products, inflammatory cytokines (IL-3 and TNF-α), and neuron-specific enolase (NSE). Our group, as well as that in Miami, observed that there were no significant differences in antioxidant levels, lipid peroxidation, or inflammatory cytokines between affected LHON patients, asymptomatic carriers, and healthy controls. Nevertheless, LHON carriers showed significantly elevated levels of NSE compared to healthy controls, even in the absence of overt vision loss. The LHON carriers with elevated NSE also exhibited subtle subclinical visual deficits, such as Tritan color vision defects and prolonged visual evoked potential latencies (9).

Moreover, LHON typically affects both eyes, but the involvement is often asynchronous. Although vision loss is usually painless, mild discomfort may occasionally occur due to inflammation of the optic nerves (19, 20). Bargiela et al. discussed the role of mitochondrial dysfunction in neuro-inflammation, and explored the overlap between the pathogenic mechanisms of LHON and MS. Mitochondrial dysfunction can be both inherited (as in LHON) and acquired (as in some cases of MS). There is significant clinical and molecular overlap between LHON and a condition called LHON-MS, where patients have features of both LHON and MS. This suggested shared underlying mechanisms involving mitochondrial pathways (21).

The precise role and mechanisms by which pro-inflammatory responses contribute to the development and progression of LHON remain unclear. This pro-inflammatory process appears to occur at the optic nerve head, leading to some pro- inflammatory changes, distinct from the patterns observed in other optic nerve disorders. Further research is needed to fully elucidate the detailed involvement of pro-inflammatory processes in this mitochondrial disease.

Our specific aim is to analyze the expression patterns of key cytokines, such as IL-1, IL-6, and TNF-α, the study aims to elucidate the underlying pro-inflammatory mechanisms that contribute to optic nerve degeneration in LHON. Understanding these pathways could pave the way for the development of targeted cytokine-based interventions, potentially offering new hope for patients with this devastating optic neuropathy.

Study Objectives

- To characterize the cytokine profiles in LHON optic nerve head samples and identify the key pro-inflammatory mediators involved in the pathogenesis of optic nerve degeneration.
- To explore the potential of existing cytokine inhibitors, such as TNF-α and IL-6 blockers, as repurposed therapeutic strategies to mitigate the pro-inflammatory response and protect the optic nerve in LHON.

Research Design

- Obtain paraffin-embedded optic nerve head samples from at leasat 18 eyes from 9 confirmed LHON patients and 12 eyes from 6 healthy controls.
- Perform immunohistochemical analysis on the optic nerve head sections to assess the expression and localization of key pro-inflammatory cytokines, including IL-1, IL-6, TNF-α, and other relevant markers.
- Quantify the levels of these cytokines and inflammatory mediators in the optic nerve head samples using ELISA or multiplex assays.
- Compare the cytokine profiles in LHON optic nerve heads to age-matched healthy control samples.
- Assess the presence and extent of RGC loss, optic nerve degeneration, and glial activation (astrocytes and microglia).
- Evaluate the correlation between cytokine profiles and the severity of optic nerve degeneration.

Study Duration and Timeline

The study is expected to be completed within 12 months, including data analysis and manuscript preparation.

Month 1-10

- Collect retinal and optic nerve samples
- Conduct initial analysis of cytokine and inflammatory marker levels in the samples
- Conduct immunohistochemistry for microglia, astrocytes, activated astrocytes, and macrophages in the samples.
- Correlate cytokine levels with structural and functional measures of disease severity (like retinal ganglion cell loss, visual acuity)

Month 10-12

- Submit the completed article
- Conduct
- Initiate the planning and preparation for preclinical studies evaluating the efficacy of anti-inflammatory agents or cytokine inhibitors in animal models of LHON

References

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PROPOSED BUDGET

Salary support for Dr. Maryam Golmohammadi - $30,000 as a direct stipend from IFOND to her (to avoid the indirect costs of going through Doheny Eye Institute).

Reagents for testing cytokines and inflammatory markers:	$500 X 8 =	$4,000
Reagents for immunohistochemistry of cells:	750 X 5 =	3,750

Total = $37,750