The Role of Protein Radicals in Neuroinflammation
As a Model of GWI & Therapeutic Strategies

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Pathogen Environment Disease

Why is the immune perturbation chronic?

Gulf War Illness (GWI)

**Known:**
- Devastating multisymptom illness.
- Symptoms - delayed & persistent.

**Unknown:**
- Specific cause(s) unknown. (But we have some good hypotheses.)
- Pathobiology - poorly understood. (But we have some good hypotheses.)
- Markers & treatments are elusive.

Many Symptoms Linked to Peripheral & Central Inflammation

- Impaired Learning
- GI Dysfunction
- Headaches & Pain
- Memory Deficits
- Behavioral Impairments: Hippocampal Volume
- Chronic Fatigue

**Inflammation**
Microglia are a Source of Chronic Inflammation and Oxidative Stress

Microglia Activation

- LPS
- Air Pollution
- CPF

Inflammatory Trigger

Microglial Activators

- (Laminin, MMP3, α-Synuclein, μ-calpain)

Neurotoxic Factors

(PGE₂, IL-1, TNFα, NO, NOO⁻, O₂⁻, H₂O₂)

Reactive Microgliosis

Self-propelling Neurotoxicity

Neuron Death/Damage

Direct Neurotoxic Insult

Lessons from a Parkinson’s Disease Model:
Inflammation-mediated Neuropathology is Delayed & Progressive

TH-IR neurons, Saline

TH-IR neurons, LPS

(in vivo)

(Qin, Wu, Block, et al., 2007, Glia)

Early time points (0-6 m) – Model of chronic neuroinflammation without overt toxicity.
M1 Polarization & Dysregulated Activation
(The Deleterious Phenotype: Exaggerated & Chronic)

(M1) Pro-inflammatory Response
(M2) Wound Healing/Alternative Response

Impaired Resolution

Amplified & Chronic

Disrupted Balance

M1

M2

Increase

Decrease

TNFα
IL-1β
NO
O₂⁻
H₂O₂
PGE₂
IL-10
IL-4
TGFβ
Arginase 1
IL-4
IL-13

NOX2: Common Mechanism of Microglia-Mediated Neurotoxicity

<table>
<thead>
<tr>
<th>Microglia Activator/Toxin</th>
<th>Extracellular Superoxide</th>
<th>Pro-inflammatory Factor Production</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lipopolysaccharide</td>
<td>Yes</td>
<td>Yes</td>
<td>(Qin et al., JBC, 2004)</td>
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<tr>
<td>Diesel Exhaust Particles</td>
<td>Yes</td>
<td>No</td>
<td>(Block et al., FASEB J, 2004)</td>
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<tr>
<td>Paraquat</td>
<td>Yes</td>
<td>No</td>
<td>(Wu et al., Ant. Red. Sig., 2005)</td>
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<tr>
<td>α Synuclein</td>
<td>Yes</td>
<td>No</td>
<td>(Zhang et al., FASEB J, 2005)</td>
</tr>
<tr>
<td>Substance P</td>
<td>Yes</td>
<td>No</td>
<td>(Block et al., FASEB J, 2006)</td>
</tr>
<tr>
<td>MMP3</td>
<td>Yes</td>
<td>Yes</td>
<td>(Kim et al., FASEB J, 2006)</td>
</tr>
<tr>
<td>μ Calpain</td>
<td>Yes</td>
<td>No</td>
<td>(Levesque et al., Brain, 2010)</td>
</tr>
</tbody>
</table>
Microglial ROS are Detrimental Through Two Mechanisms

Microglial NOX2 Activation → Gulf War-like Exposures?

1. ROS → Neuron Damage or Impaired Function
2. Amplification of Pro-inflammatory Genes

TNFα, PGE₂

Dual Roles of NF-κB p50 in Inflammation

NF-κB Subunits
- p50
- p52
- p65
- c-rel
- relB

Initiator (Pro-inflammatory Gene Expression)
- p50, p65 → Expression → Pro-inflammatory Gene

Repressor (Constitutively Bound & Resolution)
- p50, p50

(Black & Hong 2005, Progress in Neurobiology)
The Multiple Hit Hypothesis of GWI: Many Potential Causes/Exposures/Stressors May Act in Synergy for Persistent Impact

Psychological Stressors

Jet Fuel
CARC Paint
Infectious Diseases (LPS)
Vaccines
Depleted Uranium
Kuwaiti Oil Well Fires
Cholinergic & Related Neurotoxicants (Chlorpyrifos)

Combustion Products From Tent Heaters
Sand & Particulate Exposures

Protein Radicals & the Immune Hypothesis of GWI

Chronic Peripheral Immune Perturbation
Chronic CNS M1 Activation & Priming
Multiple Interacting GW Exposures

Therapeutic Window

Multiple Peripheral & CNS Symptoms
Peripheral Immune Marker
Protein Radical Formation (NF-κB p50-)

Chronic ROS/Intermittent Cytokines
NOX2 Inhibition
Chronic ROS & Cytokines
NOX2 Inhibition

Peripheral Immune Marker
Protein Radical Formation (NF-κB p50-)

NOX2 Inhibition
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