Neuroinflammation

Chair: David J. Loane, Ph.D., University of Maryland School of Medicine
Co-Chair: Alan I. Faden, M.D., University of Maryland School of Medicine
Members:
  Monica J. Carson, Ph.D., University of California, Riverside
  Cristina Morganti-Kossmann, Ph.D, Monash University, Melbourne
  Rachel J. Schindler, M.D. Pfizer, Inc
Working Group on Neuroinflammation

Monica J. Carson, Ph.D.
Professor and Chair of Biomedical Sciences,
University of California Riverside, School of Medicine, Riverside, CA

Cristina Morganti-Kossmann, Ph.D.
Adjunct Associate Professor, Department of Epidemiology and Preventive Medicine, Monash University, and Australian New Zealand Intensive Care Research Centre, Melbourne, Australia

Research Associate Professor, Barrow Neurological Institute, Phoenix Children’s Hospital, Department of Child Health, University of Arizona, Phoenix, AZ

Monica J. Carson, Ph.D.
Professor and Chair of Biomedical Sciences,
University of California Riverside, School of Medicine, Riverside, CA

Cristina Morganti-Kossmann, Ph.D.
Adjunct Associate Professor, Department of Epidemiology and Preventive Medicine, Monash University, and Australian New Zealand Intensive Care Research Centre, Melbourne, Australia

Research Associate Professor, Barrow Neurological Institute, Phoenix Children’s Hospital, Department of Child Health, University of Arizona, Phoenix, AZ

Rachel J. Schindler, M.D.
Clinical Disease Area Expert, Neurosciences, Vice President Pfizer, Inc.

Alan I Faden, M.D.
David S. Brown Professor in Trauma, Director, Center for Shock, Trauma & Anesthesiology Research (STAR), University of Maryland School of Medicine, Baltimore, MD

David J. Loane, Ph.D.
Assistant Professor, Center for Shock, Trauma & Anesthesiology Research (STAR), University of Maryland School of Medicine, Baltimore, MD
Why is neuroinflammation important in TBI?

**Actions**
- **Homeostatic**
  - Release of gliotransmitters, neurotrophic factors, cytokines
  - Vasodilation
  - Phagocytosis
- **Maladaptive**
  - Release of pro-inflammatory factors
  - Plasma extravasation
- **Neurotoxic**
  - Release of pro-inflammatory factors
  - Excitotoxicity, apoptosis
  - Blood-CNS-barrier breakdown
- **Anti-inflammatory**
  - Release of anti-inflammatory cytokines, neuroprotectins, resolvins, neurotrophic factors, neurotransmitters, neuropeptides, cell adhesion molecules
  - Vasodilation

**Outcomes**
- **Adaptation**
  - Microbe elimination
  - Synaptic plasticity
  - Enhanced perfusion
  - Neuroprotection, repair, regeneration
- **Dysfunction**
  - Hyperexcitability and/or impaired inhibition
  - Reduced computational power
- **Degeneration**
  - Progressive CNS loss of function
  - Chronic disease
- **Resolution**
  - Termination of inflammatory response

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Xanthos and Sandkühler, Nat Rev Neurosci. 15, 43-53, 2014
Long term effects
Neuroinflammation

Mechanisms

Activation of microglia and astrocytes, and infiltration of blood leukocytes

Immune mediators:
- Pro-/anti-inflammatory cytokines
- Chemokines
- Neurotrophic factors

Blood brain barrier (BBB) disruption

Secondary factors:
- Oxidative stress, Excitotoxicity
- Angiogenesis, Scarring

Outcomes

Pathological:
- Chronic neurodegeneration
  - Brain/hippocampal atrophy

Clinical:
- Cognitive decline/loss of executive function
- Neuropsychiatric changes
  - Depression, anxiety, aggression

Post-traumatic epilepsy

Post-traumatic pain

Associated disorders:
- Alzheimer’s disease
- Chronic traumatic encephalopathy (CTE)
Clinical evidence of an early and robust inflammatory response after TBI

<table>
<thead>
<tr>
<th>Mediator</th>
<th>Tissue/fluid</th>
<th>Major Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1β</td>
<td>Post-mortem tissue CSF, Serum</td>
<td>• IL-1 mRNA expressed within minutes of TBI</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Low expression in CSF and serum that is associated with poor outcomes and elevated ICP</td>
</tr>
<tr>
<td>IL-1β/IL-1ra</td>
<td>Brain parenchyma</td>
<td>• High IL-1ra/IL-1β ratio is associated with better outcome</td>
</tr>
<tr>
<td>TNF</td>
<td>Post-mortem tissue CSF, Serum</td>
<td>• TNF mRNA expressed within minutes of TBI</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• TNF detected at minutes-hours-weeks post TBI; no correlation with GCS, ICP, or outcomes</td>
</tr>
<tr>
<td>IL-6</td>
<td>CSF, serum, plasma</td>
<td>• IL-6 increases to a greater extent in CSF than serum, and is associated with poorer outcomes</td>
</tr>
<tr>
<td>IL-10</td>
<td>CSF, serum, plasma</td>
<td>• IL-10 rapidly increased in CSF and serum following TBI; no association with outcomes</td>
</tr>
<tr>
<td>IL-8 (CXCL8)</td>
<td>Post-mortem tissue CSF, serum</td>
<td>• IL-8 mRNA expressed after TBI</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• IL-8 can be measured in CSF and to a lesser extent in serum after TBI</td>
</tr>
<tr>
<td>MCP-1 (CCL2)</td>
<td>Post-mortem tissue CSF</td>
<td>• MCP-1 mRNA expressed after TBI</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• MCP-1 can be measured in CSF for several days after TBI</td>
</tr>
<tr>
<td>Inflammasome</td>
<td>CSF</td>
<td>• Increased ASC, caspase 1, an NALP-1 expression after TBI that is correlated with neurological outcomes</td>
</tr>
</tbody>
</table>

Adapted from Woodcock and Morganti-Kossmann, Frontiers in Neurology, 4, 18, 2013
Recent clinical research demonstrates a **chronic** pro-inflammatory response after TBI

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<tr>
<th>Mediator</th>
<th>Tissue/fluid</th>
<th>Major Findings</th>
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</table>
| IL-1β, TNF, IL-6, IL-10, IL-8 | Serum        | • Increased cytokine load score was associated with increase in odds of unfavorable GOS score at 6 and 12 months  
• High IL-6/IL-10 ratio is associated with poorer outcome | Kumar et al., J Head Trauma Rehabil. [in press Jun 4, 2014] |
| TNF            | Serum, CSF   | • Acute and chronic serum TNFα was associated with disinhibition and suicidal endorsement at both 6 and 12 months  | Juengst et al., Brain Behav Immun, 41, 134-143, 2014 |
TBI triggers a chronic inflammatory response associated with microglial activation in humans

Imaging biomarker of neuroinflammation

- Translocator protein 18 kDa (TSPO) is dramatically up-regulated in microglia during neuroinflammation.
- TSPO PET ligand = [11C]PK11195

Longitudinal TSPO imaging in TBI subjects: [11C](R)PK11195
Prolonged neuroinflammation in former NFL players is associated with hippocampal atrophy and linked with deficits in verbal learning and memory

\[
\text{[11C]/[18F] DPA-713 (2nd generation TSPO ligand)}
\]


- Longer half-life of [18F] facilitates distribution of the tracer across PET centers

*Coughlin et al., Neurobiology of Disease 74, 58–65, 2015

*Analysis corrected for rs6971 genotype because Ala147Thr polymorphism is associated with reduced affinity for TSPO target (Owen et al., J Cereb Blood Flow Metab 32, 1-5, 2012)
Neuroinflammation and white matter degeneration persist for years after a single TBI

37-year-old male 4 years following a single severe TBI

Johnson et al., Brain 136, 28-42, 2013

See also: Gentleman et al., Forensic Sci Int 146, 97-104, 2004
The immune response to TBI is highly complex

- Injury type?
  - mild, moderate/severe diffuse, focal, hemorrhagic blast, penetrating repeated

- Age?
  - pediatric, geriatric

- Genotype?

- Gender?

- Pre-existing medical conditions?

- Regulation of inflammation
  - Neural-microglia interactions (e.g. Fractalkine-CX3CR1, CD200-CD220R)
  - Cell signaling pathways (e.g. TLR4; IRF5/STAT1; IRF4/STAT6; PPARγ/δ)
  - miRNA and epigenetic (e.g. miR-146a, miR-155/miR-124, Jmjd3)
  - metabolic and redox (e.g. HIF1α, Nrf2, NOX2)

- Immune cell infiltration
  - B lymphocyte
  - T lymphocyte
  - Macrophage
  - Neutrophil

- BBB disruption

- Neuronal injury

- Mitochondrial dysfunction

- Microglial activation

- Pro-inflammatory cytokines (IL-1β, TNFα, IL-6)

- Chemokines (CCL2, CXCL8, CCL20)

- Anti-inflammatory cytokines (IL-10, TGFβ)

- Neurotrophic factors (IGF-1)

- Oxidative stress

- Oxidative metabolites (NO, ROS, RNS)

- Edema

- Scar formation
**Functional roles of microglia**

- Activated Microglia
  - Injured Neuron
  - DAMPs
  - PRR
  - TLR

**Spectrum of activation**

**M2-like**
- CD163
- CD206
- FcγR
- Arg-1
- Ym1
- TGFβ

**M1-like**
- IL-4/IL13
- IL-10
- IFNγ

**IL-1β**, **LPS, GM-CSF**

- IL-1β
- TNFα
- IL-6
- iNOS
- IL-12p40

**Neurotrophic factors**
- 

**Anti-inflammatory cytokines**
- 

**Protease secretion**
- 

**Phagocytosis**
- 

**Resolution of inflammation**
- 

**Clearance of debris**
- 

**Axonal remodeling and neural repair**
- 

**Angiogenesis and neurovascularization**
- 

**Neurogenesis**
- 

**Oligodendrogenesis and remyelination**
- 

**Chronic neuroinflammation**
- 

**Oxidative stress**
- 

**Neuronal dysfunction**
- 

**Pro-inflammatory cytokines**
- 

**Chemokines**
- 

**iNOS**
- 

**ROS/RNS**
- 

**Functional roles of microglia**

- IL-4/IL13
- IL-10
- IFNγ
- LPS, GM-CSF
- CD206
- CD163
- FcγR
- Arg-1
- Ym1
- TGFβ
Coordinated inflammatory responses play a critical role in wound healing after injury.

- **Pro-inflammatory (M1) phase**
  - Function: clear debris
  - (Injury)

- **Anti-inflammatory (M2) phase**
  - Function: wound healing and resolution of M1
  - (Resolution & Repair)

- **TBI**

- **Chronic inflammation**

Ideal therapy will not abolish inflammatory response, but will alter/modulate it.
Critical questions to be addressed:

What mechanisms prime reactivity of glia acutely after TBI and sustain their immune activation for weeks, months and years?

   Cytokines?  Reactive microgliosis?

How does peripheral immune activation impact TBI outcomes, and vice versa?

How does post-traumatic neuroinflammation precipitate cognitive and psychiatric dysfunction?

Is there a role for autoantibodies as pathological parameter of an autoimmune degenerative condition that is triggered after TBI?

Will delayed interventions that modulate chronic neuroinflammation be effective for treatment?
Treatment Development Opportunities
Near Term (in the clinic)

Anti-inflammatory therapies:

**Rovastatin**
Title: Effect of Rosuvastatin on Immunological Markers After Traumatic Brain Injury: Clinical Randomized Double Blind Study Phase 2
Phase: 2
Location: Mexico

**Atorvastatin**
Title: Multi-Center, Randomized, Placebo-Controlled, Double Blind Study of the Atorvastatin: Effect on Patients With Chronic Subdural Hematoma
Phase: 4
Location: China

**Interleukin-1 receptor antagonist**
Title: A single center, phase II, open label, randomized-control study of recombinant human IL1ra (rhIL1ra, Anakinra) in severe TBI.
Phase: 2
Location: UK
Publication: Helmy et al., J Cereb Blood Flow Metab. 34, 845-51, 2014

Multi-potential therapies:

**Erythropoietin**
Title: A Randomised, Placebo-controlled Trial of Erythropoietin in ICU Patients With Traumatic Brain Injury
Phase: 3
Location: Australia
Treatment Development Opportunities
Longer Term (experimental)

CCR2 antagonist\(^1\) (CCX872)
Mesenchymal stem cells\(^2,3\) (e.g. mGluR5 PAM\(^4\), minocycline\(^5,6\), FAAH inhibitor\(^7\), miR-155/miR-124\(^8\))

Alter M1/M2 balance

Delayed anti-inflammatory therapies (e.g. voluntary exercise\(^9\), ibudilast\(^10\), mGluR5 agonist\(^11\))

mGluR5: therapeutic target for post-traumatic neuroinflammation

mGluR5 PAM are being developed for psychiatric disorders

VU0360172
Orally bioavailable
Good pharmacokinetic profile
Biased receptor signaling – reduced side effects

TBI model (CCI; VU0360127, 50mg/kg i.p. at 3h post-injury)
Neuroprotective
Improves motor function
Attenuates NOX2/CD68/iNOS expression
Promotes M2 expression

Alters M1/M2 balance in microglia

miR-155 expression

miR-124 expression

Con LPS LPS+VU LPS+CHPG

Con LPS LPS+VU LPS+CHPG

Arg1 Ym1 β-actin

TBI (Vehicle)

TBI (VU0360172)

A

B

C

miR-124 (Mander's overlap coefficient)

iNOS+CD11b+ (Mander's overlap coefficient)

Arg1+CD11b+ (Mander's overlap coefficient)
**mGluR5: therapeutic target for post-traumatic neuroinflammation**

Chronic microglial activation

- **TBI**
- **Neuron Injury Signals**
  - α-Synuclein, µ-Calpain, MMP3 Neuronmelanin
- **Reactive Microgliosis**
- **NOX2 Activation**
- **ROS & Amplified Cytokines**
- **Neuron Death/Damage**
- **ongoing neurodegeneration & tissue loss**

**NOX2 inhibition and TBI:**

- **NOX2 inhibition**
  - Dohi et al., J Neuroinflammation. 7:41, 2010

- **mGluR5 activation**
  - Byrnes et al., Glia. 57:550-60, 2009
  - Byrnes et al., J Neuroinflammation. 9:43, 2012
  - Loane et al., J Neurotrauma. 30:403-12, 2013
mGluR5: therapeutic target for post-traumatic neuroinflammation

Delayed treatment with the mGluR5 agonist, CHPG, arrests lesion progression and attenuates chronic microglial activation after TBI

Byrnes et al., J Neuroinflammation 9:43, 2012

Experimental protocol:

Byrnes et al., J Neuroinflammation 9:43, 2012
Biomarkers of neuroinflammation

Need improved predictive serum biomarkers, particularly for mild TBI

Immune fingerprint/signature: cytokines, microRNAs

Urgent need for biomarker/s for chronic phase

Need new stable PET ligands or MRI based methods to image neuroinflammation

Combine PET imaging of neuroinflammation with tau/amyloid imaging to study associated chronic pathologies

Future collection of inflammation biomarkers in CDE
Future progress will require:

A greater understanding of complex immunological responses to TBI

Development of new inflammatory biomarkers to evaluate mechanisms and therapeutic responses

More clearly define the target – what pathway/s is drugable?

Timing: When to intervene?

Match therapeutic intervention with type of injury