Treating inflammation in epilepsy
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<table>
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<tr>
<th>Company</th>
<th>Role</th>
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<tr>
<td>UCB Pharma SA</td>
<td>Research grant</td>
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<td>Harbor Therapeutics, Inc.</td>
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Learning Objectives

• To understand the role played in the pathogenesis of seizures by the inflammatory pathways activated in epilepsy
• To provide insight into emerging anti-inflammatory treatment options for drug-resistant forms of epilepsy
Challenge for epilepsy treatment: searching new targets for drug development
- Treatment of resistant seizures
- Disease-modifying drugs

Pitkanen and Lukasiuk, Epilepsy & Behavior, 2008
Chronic activation of immune system in epilepsy

Innate immune system
- Microglia, astrocytes
- Macrophages
- Cytokines, Chemokines, PGE2, complement

Adaptive immune system
- Cytotoxic T cells
- Auto-Abs

NE, Lennox-Gastaut, Landau-Kleffner, Dravet, FIRES
- ACTH, Steroids, IVIG, PEX

mTLE, TS, Glioneuronal Tumors, FCD

Inflammation leads to brain damage/dysfunction

Drugs

Autoimmune disorders
- RE
- Viral Enc.
- Limbic Enc.

Inflammation leads to brain damage/dysfunction
Innate immunity: IL-1Receptor/Toll-like Receptor signaling

**PAMPs**
- Microbial-clearance and killing pathways
- Apoptotic and necrotic cell-death pathways

**DAMPs**
- IL-1β
- HMGB1
- IL-1R1
- TLR4

**Glia (±RAGE)**
- IκB
- NF-κB
- AP1
- E-selectin
- ICAM1
- VCAM1
- Chemokines
- COX2

**Cytokines, Chemokines, mTOR, Complement cascade**
- Cell adhesion Molecules
- Metalloproteases
- Cox-2

**Generation of the inflammatory cascade**
- Gorter et al, 2006; Becker et al, 2006; Lukasiuk et al, 2006

**Sterile inflammation**
- Brain injury
- Seizures

**Post-translational modifications**
- Neuronal hyperexcitability
- BBB/astrocyte dysfunction

**EPILEPSY**
Inflammatory mediators are neuromodulators: Cytokines and acquired channelopathies

Adapted from Pitkanen & Lukasiuk, 2009
Activation of innate immunity in human epilepsy: glia & neurons

**IL-1β in mTLE**

**IL-1β in FCD type 2b**

- BBB damage
- Albumin+IgG

**IL-1R1 upregulated in the same cell types**

Ravizza et al, Neurobiol Dis, 2006 and 2008

Activation of this signaling is pro-ictogenic in experimental models

**IL-1/IL-1R1**

Boer et al, J Neuroimmunol, 2006

**Microglia**
Activation of HMGB1 signaling in epilepsy: TLR4 & RAGE

Epileptogenic brain injury
Seizures

HMGB1 (astrocytes & microglia)

Temporal Lobe Epilepsy
TLR4 (neurons & astrocytes)

RAGE (astrocytes & vessels)

Signaling activation occurs in various forms of pharmaco-resistant epilepsies and is pro-ictogenic in experimental models

Adaptive immunity in Rasmussen Encephalitis

Bien et al, Ann Neurol, 2002; Pardo et al, Epilepsia, 2004

Innate immunity in RE

by courtesy of Jan Bauer
Cellular localization of TLRs in RE Cerebral Cortex

- **TLR2** localizes to Astrocytes
- **TLR3** localizes to Neurons
- **TLR4** localizes to microglia & Neurons

**Extracellular matrix proteins**
- Fibrinogen
- dsRNA
- HMGB1, Hsp60/70, Hyaluronan, Fibrinogen

**Gram + Viruses**
- HMGB1

**Gram -**
- HMGB1/TLR4 induces MHCI

*Zong et al, Ann Rheum Dis, 2013*
EVIDENCE FROM EXPERIMENTAL MODELS: triggering factors & functional consequences

✓ Experimental seizures and epileptogenic brain injuries induce brain inflammation:
  • long lasting
  • precedes the development of epilepsy
  • inadequately controlled by endogenous antiinflammatory molecules

✓ Pharmacological experiments:
  • specific anti-inflammatory treatments reduce seizures and delay their onset (see also transgenic models)
  • proinflammatory insults decrease seizure threshold

IL-1β and HMGB1 signaling is induced in glia, neurons, BBB endothelium in human and experimental epileptogenic foci

P2X7 antagonists
(Engel et al, Faseb J, 2012)

Anticonvulsive effects

VX765
ICE inhibitors
Phase 2b
Akin et al, 2011
Maroso et al, 2011

Anticonvulsive effects

**Anakinra/IL-1ra**
- Vezzani et al, PNAS, 2000
- Marchi et al, Neurobiol Dis, 2009
- Auvin et al, Epilepsia, 2010

**Ifenprodil**

**Rahimian & Jyonouchi, J Ped Neurol, in press**

*Therapeutic option in Children intractable seizures*

**Maroso et al, Nature Med, 2010**
Anticonvulsive efficacy of anti-inflammatory treatments

*IL-1R1/TLR signaling*

- Seizures induced by kainic acid (lesional) or bicuculline and FS (non lesional)
  
  (Vezzani et al, 1999; 2000; Dube’ et al, 2005; 2011; Ravizza et al, 2006)

- Status epilepticus in rats is reduced by anakinra
  
  (De Simoni et al, 2000; Marchi et al, 2009)

- Electrical kindling in adult rats: delayed + no seizure generalization
  
  (Ravizza et al, 2008; Auvin et al, 2010; 2011)

- Chronic recurrent seizures in epileptic mice (mTLE model)
  
  (Maroso et al, 2009; 2010)

- SWD in GAERS & WAG/Rij (absence seizures)
  
  (Akin et al, 2011; Kovács et al, 2011)

50-70% decrease in seizure recurrence, delayed seizure onset, reduced generalization

*Resolution of inflammation in areas involved in seizures*

TNF-alpha, IL-6, COX-2 & complement system

(reviewed in Kukarni & Dhir, 2009; Vezzani et al, 2011; Aronica et al, 2012)
COX-2 & PGE2 promote cell loss:
Induction in neurons and astrocytes in mTLE (Desjardins et al, 2003)

Reduced hippocampal cell loss in COX-2 KO mice (Serrano et al, J Neurosci, 2011)

+reduced inflammation and BBB damage

EP2 antagonists are neuroprotective (Jiang et al, PNAS, 2011; 2012)

Parecoxib, Polascheck et al, 2010; Celocoxib, Jung et al, 2005: neuroprotective
SC-58236 worsens SE outcome, Holtman et al, 2010
The yin and yang of the EP2 receptors in the brain

Butaprost/EP2 agonist is neuroprotective

Jiang et al, PNAS, 2011
2012
2013

Jiang & Dingedine, 2013
Neuroprotection after SE: cocktail of antiinflammatory drugs

Blockade of IL-1β system in adult rats *(Noe’ et al, Neurobiol Dis, 2013)*

VX765+Anakinra

Anakinra+COX2 inhibitor

in PN21 rats

*Kwon et al, J Neuroinflamm., 2013*
Inflammation, ictogenesis & epileptogenesis

Inciting event

Disease or Syndrome Modification

Reversal of pathology

Co-morbidity modification

+ attenuation of neuropathology

NSAID: Celecoxib, Parecoxib, Aspirin

Anti-integrins antibodies

Glia activation inhibitors:

Minocycline
Resveratrol
Fingolimod
(and immunosuppressant)

Adapted from A. Pitkanen, Epilepsia, 2010

Finding master regulators
Treatments combination
Prevention & Resolution

Open questions
Activation of innate immunity and inflammation contribute to:

- Seizures generation and recurrence
- Cell loss (cytokines, PGE2)
- BBB damage (losartan; hrAnnexin-1)
- Comorbidities (cytokines)
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Inflammation in epilepsy: what is new?

**Sterile inflammation**

Brain: *cell injury, chronic stress, seizures*

**Innate immune response:** microglia, astrocytes

**Inflammatory mediators are neuromodulators:**

these “immune molecules” have CNS-specific roles independent of their role in the classical immune/inflammatory response

*(Graeber et al, FEBS Letters, 2011)*
Link between brain inflammation and epilepsy

• Various inflammatory mediators are overexpressed in epileptogenic foci in human epilepsy with differing etiologies (e.g. RE, LE, MCD, TLE). Antiinflammatory mechanisms are inefficient in epilepsy (Ravizza et al, Neurobiol Dis, 2006; Pernhost et al, Seizure, 2013)

• Microglia/astrocytes are common sources of inflammatory mediators in brain tissue (also neurons and endothelial cells)

• Leukocytes contribute to different extent depending on etiology of epilepsy

• BBB damage is often detected in areas of perivascular inflammation involving astrocytic endfeet

Extent of glia-derived inflammation positively correlates with frequency of seizures and epilepsy duration