Role of astrocytes in MS and their modulation by disease modifying therapies

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courtesy of S. Ludwin

Physiologic role of astrocytes in adult CNS

Comprise 20-40% of all glia; 4:1 ratio with neurons

- Blood brain barrier (BBB) - end processes contribute to BBB
  - effect on endothelial cells - sonic hedgehog - (Alavarez - Science 2011)
- Structural support - contribute to extra-cellular matrix (ECM)
- Extra-cellular environment homeostasis
  - fluid and ion balance - aquaporins
  - “glymphatics (Iliff - Sci Transl Med 2012) - protein clearance
  - scavengers of reactive oxygen species
- Neuron - metabolic support - lactate shuttle
  - synaptic maintenance and plasticity
  - neurotransmitter regulation – glutamate
- Oligodendrocytes - trophic support; pro-myelinating factors
Primary human neuron cultures are a model of astrocyte-neuron interactions

Neuron displacement is consequent to astrocyte injury
Role of astrocytes in MS

“Negative“ role
- promote inflammation
  - interact with infiltrating immune cells via chemokines/cytokines
  - production of effector molecules
  - reduced protective functions
  - inhibit repair- “glial scar”

“Positive“ role
- maintain physiologic functions
- promote recruitment and differentiation of oligodendrocyte progenitor cells (OPC)

Contribution to symptoms
- Cognition – maintenance of neural networks
- Pain - Chen G. - Connexin-43 induces chemokine release from spinal cord astrocytes to maintain late-phase neuropathic pain in mice. Brain. 2014
GLIOSIS (from S Ludwin)
Acute MS Reactive Astrocytes

Chronic MS Plaque

Lessons from Celiac Disease - Coordinated induction by IL15 of a TCR-independent NKG2D signaling pathway converts CTL into lymphokine-activated killer cells in celiac disease Meresse B
Mechanisms of Injury

NKG2D is implicated in killing of oligodendrocytes by NK cells, γδ T cells and CD8 T cells


Expression of NKG2D ligands on oligodendrocytes in MS lesions

Saikali et al J Neurosci 2007
IL15 expression by astrocytes in MS lesions.


T cell Subsets

- Th1
  - anti-CD3
  - anti-IL-4
  - IL-12
  - anti-IFNγ
  - anti-IL-10

- Th2
  - anti-CD3
  - anti-IL-4
  - IL-4
  - anti-IFNγ
  - anti-IL-12

- Th17
  - anti-CD3
  - anti-CD28
  - anti-IFNγ
  - anti-IL-23

Myeloid cell subsets

- M1
  - LPS
  - IFNγ
  - IL-12
  - IL-23

- M2
  - IL-4
  - IL-13

PBMCs

Direct and indirect injury of myelin lineage cells

Astrocytes

Human A2B5

Ruffini et al., AJNP, 2004

O4+ Cells

(Oligodendrocyte differentiation marker)
Figure 4. Human M1 supernatants decrease % of O4+ cells in vitro (Moore CS J Immunology 2015)

Figure 6. Effects of astrocyte released mediators on OPCs

A

Control | T1,1 ACM | CXCL10 Inhibitor

B

C

Unpolar ACM | M1 ACM | M2 ACM
- Astrocyte injury in MS: GFAP Cleavage in MS plaques, not normal controls

- Failure to buffer micro-environment – glutamate, free radicals
- Failure to produce supportive molecules – neurotrophins
- Production of inhibitory molecules

Darlington et al.; J. of Neuropathology and Experimental Neurology 2008

Neuromyelitis optica (NMO): anti-aquaporin 4 antibody

Effects of NMO serum in astrocyte HBEC co-cultures (Vincent et al J Immunology 2008)

- Decreased permeability of BSA in Boyden chamber assay

Internalization of AQP4 from astrocyte end feet

Increased permeability of BSA in Boyden chamber assay

HFAs alone HFAs + BBB-ECs + NMO-IgG + HC serum
Effects of therapeutic agents on astrocytes

**Indirect effects**
- via altered systemic inflammatory activity - applies both to agents that doA* or do notB* cross the BBB
  A* - fingolimod, dimethylfumarate
  B* - interferon β, glatiramer acetate, teriflunomide
  - ?monoclonal antibodies

**Direct effects** - applies to agents that cross the BBB
- effects via compartmentalized immune activity or biologic properties of primary neural cells**

** shown in animal models and/or in vitro studies

1- Tallantyre 2008

Indirect effects of therapeutic agents on astrocytes

Reduce trafficking of immune cells into CNS
- overall trafficking
- select populations - T cells, B cells, monocytes, “stem” cells

Polarization of T cell populations
- Th1, Th2, Th17/Treg lymphocytes
Can Interferon β antibodies alter astrocyte function?

Potential for interferon beta-induced serum antibodies in multiple sclerosis to inhibit endogenous interferon-regulated chemokine/cytokine responses within the central nervous system.
– Shapiro - Arch Neurol – 2006

We used an in vitro assay involving toll-like receptor 3 ligand (polyinosinic-polycytidylic acid) signaling to assess the effect of serum samples containing high titers of NAbs (1800-20 000 U) on production of the chemokine CXCL10 and the cytokine interleukin 6 by human astrocytes

Serum samples positive for NAbs significantly inhibited polyinosinic-polycytidylic acid-induced CXCL10 and IL-6 production by astrocytes.

Signaling pathways in astrocytes: relevance for emerging therapies

**Inflammation related**
- NFκB - can be inhibited by antioxidant molecules
- JNK and p38MAPK
- JAK/Stat pathway - transducer of inflammatory signals mediated by growth factors and cytokines (IL-6, CNTF, EGF and TGF-α)

**Neurotrophins** – secreted by as pro-neurotrophins that are then proteolytically cleaved to mature neurotrophins in the synaptic cleft and rapidly degraded
FTY720 is present in the CNS following oral administration

Light microscopy autoradiography in rats following multiple oral doses of [14C]FTY720. [14C]-FTY720 7.5 mg/kg was given p.o. once a day for 7 days.

- Dr. Alain Schweitzer ED DMPK/ADME EU

Modes of action of oral fingolimod

- Lymph node
- Peripheral blood circulation
- Central nervous system

Immunomodulatory effects of fingolimod:
- Blockade of lymphocyte egress from lymph node (e.g., Th17 cell)
- Promotion of adherent junction assembly
- Decreased permeability of the blood-brain barrier
- Increased remyelination

Activation and recruitment of other immune cells

Potential effects of fingolimod on the blood-brain barrier

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Sphingosine 1-phosphate (S1P)–S1P receptor-mediated cellular functions in the CNS

S1P receptor subtypes 1, 2, 3, and 5.

**Astrocyte**
S1P<sub>1</sub>, and S1P<sub>3</sub>
- Proliferation, migration, gap junction communication, growth factor production

**Oligodendrocyte**
S1P<sub>1</sub>, S1P<sub>3</sub>, and S1P<sub>5</sub>
- Survival, migration, differentiation, morphology (process extension and retraction)

**Neuron**
S1P<sub>1</sub>, S1P<sub>2</sub>, and S1P<sub>3</sub>
- Neurogenesis, neural progenitor migration, survival, neurotransmission

**Microglia**
S1P<sub>1</sub>, S1P<sub>2</sub>, and S1P<sub>3</sub>
- Pro-inflammatory cytokine production

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Dual actions of FTY720 in the CNS

**S1P**
- Internalizes and recycles S1P<sub>1</sub>
- Internalizes and degrades S1P<sub>1</sub>

**FTY720 (fingolimod)** efficacy in an animal model of multiple sclerosis requires astrocyte sphingosine 1-phosphate receptor 1 (S1P<sub>1</sub>) modulation

**Persistent signaling induced by FTY720-phosphate is mediated by internalized S1P<sub>1</sub> receptors**


FTY720 (fingolimod) efficacy in an animal model of multiple sclerosis requires astrocyte sphingosine 1-phosphate receptor 1 (S1P1) modulation.

Abstract
To identify CNS cells functionally contributing to FTY720 activity, genetic approaches were combined with cellular and molecular analyses. These studies relied on the functional assessment, based on clinical score, of conditional null mouse mutants lacking S1P(1) in CNS cell lineages and challenged by experimental autoimmune encephalomyelitis (EAE), an animal model of multiple sclerosis. All conditional null mutants displayed WT lymphocyte trafficking that responded normally to FTY720. In marked contrast, EAE was attenuated and FTY720 efficacy was lost in CNS mutants lacking S1P(1) on GFAP-expressing astrocytes but not on neurons. In situ hybridization studies confirmed that astrocyte loss of S1P(1) was the key alteration in functionally affected mutants. Reductions in EAE clinical scores were paralleled by reductions in demyelination, axonal loss, and astrogliosis. Receptor rescue and pharmacological experiments supported the loss of S1P(1) on astrocytes through functional antagonism by FTY720-P as a primary FTY720 mechanism. These data identify nonimmunological CNS mechanisms of FTY720 efficacy and implicate S1P signaling pathways within the CNS as targets for multiple sclerosis therapies.

Antagonist function of FTY720 - inhibits S1P induced astrocyte proliferation in vitro

IL1b induction of Ca²⁺ mobilization in astrocytes (Wu et al J Neuroinflammation 2013)

![Graph showing Ca²⁺ increase evoked by IL-1b injection]

**Implications of IL-1b induction of [Ca²⁺]:**

- Ca²⁺ serves as the primary substrate for glial excitability
- iNOS expression induced by IL-1b could be blocked by reducing Ca²⁺ concentrations (Dal Pra, Chiarini et al. 2005)

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**Agonist function of FTY720 - daily FTY720 treatment inhibits IL1b-induced Ca²⁺ mobilization**

Wu et al. 2013

![Graphs showing IL1b and Ca²⁺ mobilization with and without FTY720 treatment]

FTY720 induces expression of glutamate transporter in astrocytes
Gris et al. ECTRIMS 2013

Figure 11. FTY-720 induces the expression of glutamate transporter in the inflamed astrocytes.

FTY720 enhances remyelination in demyelinated mouse cerebellar slice cultures (Miron et al)
Demyelinated with lysolecithin (0.5mg/ml) overnight at 21 days in vitro
Treated with FTY720 (100 pM and 1 µM) for 14 days in vitro after demyelination
FTY720 enhances microglia cell number and GFAP immunoreactivity in demyelinated mouse cerebellar slice cultures (Miron et al)

FTY720 and S1P daily or q3days promote ensheathment of rat DRGN axons by human OPCs

Cui et al – GLIA 2014
**Differential activation of ERK1/2, p38MAPK, and CREB by FTY720 in OPCs and astrocytes**

**Early cell cultures**

**Later cell Cultures**

**Conclusion** - FTY720p can act directly on OPCs to impact differentiation via p38MAPK, and also potentially indirectly via neurons and astrocytes by activating ERK1/2, p38MAPK, and/or CREB.
Dimethyl fumarate

- **Inflammation** - impaired expression of nuclear factor kappa B (NFκB) dependent genes by interfering with the nuclear translocation of this transcription factor. This may involve a reduced expression of chemokines or adhesion molecules and shift in cytokine production from a "Th1" to a "Th2" pattern of lymphocytes and myeloid cell polarization to from M1 to M2.

- **Neuroprotection** - Effects on endogenous cellular antioxidative pathways involving the transcription factor nuclear (erythroid-derived 2) related factor (Nrf2). Nrf2 is a redox-sensitive leucine zipper transcription factor,

  *from Linker and Gold*

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Teriflunomide

- **Inhibits** INOS production in rat astrocytes in vitro (Miljkovic 2001)
From clinic to bench to clinic - IGF-1


- insulin-like growth factor-1 (IGF-1) is a critical signal for oligodendrocytes, which promotes their development and ultimately myelin formation.