Microglia: Blame or Thank

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### Astrocytes: Interlinked Gatekeepers of Glutamate

**Astrocytes**

- **Synapse formation & Maintenance and plasticity**
- **Regulation of blood flow and extracellular potassium**
- **Transport nutrients and metabolic precursors**
- **Catabolism of several amino acids**
- **Astrocytic glutamate transporters**
- **Networked together by gap junctions - Ca^{++} waves**

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“Microglia: resident macrophages of the CNS”

- **Role in neuronal pruning during development**
- **Remove toxic debris - garbage collectors of CNS**
- **Inflammatory response when there is damage**
- **Both sides of coin:**
  - Neurotoxic effects
  - Neurotrophic effects

Acute and chronic neurodegenerative diseases

Neurotropic viral infections (polio, measles, rabies, HIV)

Stroke, TBI, MS, paraneoplastic disorders

When the immune system attacks the brain when fighting cancer
Glial cells as intrinsic components of non-cell-autonomous neurodegenerative disease

Christian S Lobsiger & Don W Cleveland

A lesson from dominantly inherited forms of diverse neurodegenerative diseases, including amyotrophic lateral sclerosis, spinocerebellar ataxia and Huntington’s disease, is that the selective dysfunction or death of the neuronal population most at risk in each disease is not mediated solely by damage from the mutant protein within the target neurons. The disease-causing toxic process, which in each case is caused by mutation in a gene that is widely or ubiquitously expressed, involves damage done by mutant proteins within the non-neuronal glial cells of the central nervous system, especially astrocytes and microglia. The disease mechanism is non-cell-autonomous, with toxicity derived from glia as a prominent contributor driving disease progression and in some instances even disease initiation.
“Classic view of neurotoxicity – a specific neuronal population is vulnerable to a cumulative toxic burden – which overwhelms the neuron’s defense mechanisms triggering degeneration and neuronal death.”

- Current research suggests that it is not all about the neuron!
  - Neurodegeneration is also influenced by toxic protein expression in glia too.
- How many of the classic neurodegenerative diseases are in fact caused by toxins in glia?

Figure adapted from: Forman, M. S. et al (2004) Nature Medicine 10, 1055 - 1063

Under normal conditions, resting microglia, through their highly mobile filopodia, continuously survey the brain. Microglia became activated in response to various danger signals posed by neurons and/or astrocytes. At this stage, microglia are recruited and have local protective effects via regulated release of cytokines and phagocytosis of cellular debris. At certain point, however, microglia gain reactive phenotype characterized by uncontrolled release of inflammatory mediators. These cells furiously attack neurons, became neurotoxic and extend the damage.

Microglia are part of innate immune system in the brain. Microglia have 3 functionally and morphologically distinct forms.
“Activation of microglia, the resident immune effector cells in the CNS, occurs as a prominent feature early in CNS inflammation—and is a consistent finding in the common late-onset neurodegenerative disorders.”

Monk, P. & Shaw, P. (2006), NATURE MEDICINE VOLUME 12 | NUMBER 8
1. Signals from damaged neuron.

2. Protein aggregates
   Proinflammatory signals
   Autocrine stimulation

3. Dead cells
   Sustained inflammation
   Phagocytosis of cell debris

Figure adapted from: Monk, P. & Shaw, P. (2006), NATURE MEDICINE VOLUME 12 | NUMBER 8
Since microglia are chiefly responsible for phagocytosis and clearance of cellular detritus why don’t they phagocytose Aβ peptides?

Can the innate immune system be hijacked to clear Aβ in AD by correcting the dysfunctional anti-inflammatory signaling?

Guillot-Sestier et al., 2015, Neuron 85, 534–548
Inflammatory responses are kept under control by two key immunoregulatory cytokines: transforming growth factor-β (TGF-β) and interleukin-10 (IL-10).

Note also: there is elevated IL-10 signaling observed in reactive glia neighboring β-amyloid plaques in aged mice.

Blocking TGF-β-Smad 2/3 signaling in innate immune cells mitigates cerebral amyloidosis and behavioral deficits in the Tg2576 mouse model.

Guillot-Sestier et al., 2015, Neuron 85, 534–548
A unified model of the IL-10 signal transduction pathway in human mononuclear phagocytes. IL-10 signal transduction in monocytes is initiated by binding of homodimeric IL-10 to the extracellular domains of two adjoining IL-10R1 molecules. Ligation of IL-10R1 by IL-10 activates the receptor-associated Janus tyrosine kinases, JAK1 and Tyk2, which then trans-phosphorylate the intracellular domains of the IL-10R1 chains on specific tyrosine residues. These phosphorylated tyrosine residues and their flanking peptide sequences serve as temporary docking sites for the latent, cytosolic, transcription factor, STAT3. STAT3 transiently docks on the IL-10R1 chain via its SH2 domain, and is in turn tyrosine phosphorylated by the receptor-associated JAKs. Once activated, it dissociates from the receptor, homodimerizes with other STAT1 molecules, and translocates to the nucleus where it binds with high affinity to SBEs in the promoters of IL-10-inducible genes. One of these genes, SOCS-3, is a member of a newly identified family of JAK/STAT inhibitory genes. The ability of IL-10 to rapidly induce expression of SOCS-3 may explain, at least in part, how IL-10 inhibits induction of many genes in monocyte.
The regulation of IL-10 production by immune cells

Margarida Saraiva* and Anne O’Garra†

Abstract | Interleukin-10 (IL-10), a cytokine with anti-inflammatory properties, has a central role in infection by limiting the immune response to pathogens and thereby preventing damage to the host. Recently, an increasing interest in how IL-10 expression is regulated in different immune cells has revealed some of the molecular mechanisms involved at the levels of signal transduction, epigenetics, transcription factor binding and gene activation. Understanding the specific molecular events that regulate the production of IL-10 will help to answer the remaining questions that are important for the design of new strategies of immune intervention.

Nature Reviews Immunology 10, 170-181 (March 2010)
The immune response has evolved to protect the host from a wide range of potentially pathogenic microorganisms, but parallel mechanisms to control overexuberant immune responses and prevent reactivity to self are required to limit host damage. Interleukin-10 (IL-10) is an anti-inflammatory cytokine with a crucial role in preventing inflammatory and autoimmune pathologies. IL-10-deficient mice develop inflammatory bowel disease following colonization of the gut with particular microorganisms and show other exaggerated inflammatory responses to microbial challenge. Although the absence of IL-10 leads to better clearance of some pathogens with no enhanced immunopathology, during other infections the absence of IL-10 can be accompanied by an immunopathology that is detrimental to the host but does not necessarily affect the pathogen load. This suggests that an absence of IL-10 is not always compensated by other regulatory mechanisms and thus that there is a non-redundant role for IL-10 in limiting inflammatory responses in vivo.

“So, IL-10 is a cytokine with important effects on the development of an immune response. An understanding of how IL10 expression is regulated in different innate and adaptive immune cells is therefore of importance for the development of immune intervention strategies in various pathologies.”
II10 Deficiency Rebalances Innate Immunity to Mitigate Alzheimer-Like Pathology

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APP/PS1 cerebral amyloidosis mouse

mouse deficient in interleukin-10 (Il10) (anti-inflammatory cytokine)

\[ \text{APP/PS1}^{+} \text{Il10}^{-/-} \]

Guillot-Sestier et al., 2015, Neuron 85, 534-548
At 12 to 13 months of age, \textit{APP/PS1\textsuperscript{+/i10\textminus/\textminus}} mice manifested significantly reduced amyloid deposition in cingulate cortex (CC), entorhinal cortex (EC), and hippocampus (HC) as measured by thioflavin S histochemistry.

Surprisingly, \textit{APP/PS1\textsuperscript{+/i10\textplus/\textplus}} mice had modest but statistically significant increases in abundance of small (<25 μm) plaques in the CC and EC versus \textit{APP/PS1\textsuperscript{+/i10\textplus/\textminus}} animals.
IL-10 Signaling Is Elevated in AD Patient Brains

Evaluating IL-10 signaling in postmortem samples from AD patient brains versus age-matched, non-demented controls.

Hippocampal sections were stained for IL-10 receptor alpha chain (IL-10Rα) and microtubule-associated protein 2 (MAP2, a neuronal marker). Interestingly, IL10Rα expression was elevated in AD compared to control brains, and some of these signals could be found colocalized with MAP2⁺ neurons (white arrowheads.)

Phospho-Jak1, a key downstream effector kinase of the IL-10 pathway, was elevated in AD brains in close proximity to thioflavin S⁺ amyloid plaques.
1. Stimulation of microglia by recombinant IL-10 induces nuclear translocation of the downstream signal transducer STAT3 and reduces Aβ phagocytosis.

2. *Il10* deficiency or *Stat3* knockdown increases Aβ uptake by cultured microglia.

3. *Il10* deficiency increases microglial activation and promotes Aβ uptake into Lamp1+ and CD68+ phagolysosomes in vivo.

*Il10* deficiency in *APP/PS1* mice seems to restore physiologic ability to phagocytose Aβ.

Guillot-Sestier et al., 2015, Neuron 85, 534–548
A 3-D reconstruction illustrates immune cells (shown in blue) clearing the brain of plaque deposits (in red).

Guillot-Sestier et al., 2015, Neuron 85, 534-548
This is a 3-D reconstruction of an immune cell (red) containing β-amyloid (blue) within an intracellular degradation compartment (yellow).

Deletion of the anti-inflammatory cytokine, Il10, activates innate immune cells to clear the brain of toxic β-amyloid plaques.

Credit: Marie-Victoire Guillot-Sestier, Ph.D.
**In vitro:**

knockdown of microglial *Il10-Stat3* signaling endorsed Aβ phagocytosis, while exogenous IL-10 had the converse effect.

**IL-10 signaling pathway is abnormally elevated in AD patient brains**

If the IL-10 anti-inflammatory response is blocked then it could be potential therapy for AD.

**AD pathology is driven by an imbalance between Aβ production and clearance**

Guillot-Sestier et al., 2015, Neuron 85, 534–548