Inflammation as a Therapeutic Target in Alzheimer’s Disease

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“Cocktail” approach to AD therapy
Hallmarks of Alzheimer's Disease Pathology

- ß-amyloid containing plaques
- Neurofibrillar tangles
- Neuronal loss → dementia

AD  Control

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UCI MIND
AD as a Progressive Disease

Preclinical Phase
- Oligomeric Aβ
- Diffuse plaques
- Initiating Factor (?)
- Promoting Factor(s) (?)
- Reparative Activities (?)

Catastrophic Phase
- fAβ, C’ activation, tangles, neuron and synapse loss
- Clinical symptoms appear
- Loses independence
- Death

Adapted from Institute for Memory Impairments and Neurological Disorders, University of California, Irvine.
Overview

• **NeuroInflammation** is a component of Alzheimer’s Disease

• The **Complement** Cascade is inducer of AD Neuroinflammation

• Some components of this response can be reparative or protective.

→ Identifying and screening novel **Candidate Therapeutics**
Immune Response

Goal: Recognition and Elimination of Danger

**Innate**
- Phagocytes/Microglia
- Complement
- Molecular Danger Sensors
- Cytokines

**Adaptive**
- Antibodies
- T cells
- Cytotoxic T cells
- CD4 T Cell-mediated killing
Inflammation

• An immune response to a perceived injury or infection:
  – Heat (fever)
  – Swelling
  – Redness
  – Pain

Neuroinflammation

-no heat, no swelling, no redness, no pain

**Deleterious to Neurological Function**
Neuroinflammation

Proliferation and activation of microglia
- Induction of proinflammatory cytokines →
**Deleterious to Neurological Function**

Microglia Responses

↑ Inflammation  ↑ Phagocytosis  ↑ Repair/remodeling

- Bad  - Good  - Good
Activation States of Microglia

- **Classical Activation** – stimulated by the “cytokine” IFNγ; attack function
- **Alternative Activation** – stimulated by IL-4 and IL-13; anti-inflammatory
- **Acquired Deactivation** – stimulated by TGF-β, IL-10 and dying cells; phagocytic but immunosuppressive

Can coexist – (provides targets for therapeutics)
Complement

- A group of >30 interacting proteins in blood, extracellular fluids and on cells that are activated by injury or infection.
Protection from Infection

Recognition

Activation

Effector Roles:

- Enhances Phagocytosis - C3b, iC3b, C1q
- Recruitment and Activation of immune cells - C5a, C3a
- Lysis of pathogen - MAC (C5b-9)
Pathological Conditions Associated with Complement Activation

Induction of excessive inflammation → cell damage

Infectious diseases, sepsis, anaphylaxis
I/R injury
Paroxysmal nocturnal hemoglobinuria
Psoriasis
Myasthenia gravis
Systemic lupus erythematosus
Multiple sclerosis
Transplant rejection
Cancer

Stroke
Alzheimer’s disease
Age-related macular degeneration

Asthma
Myocardial infarction
Atypical hemolytic uremic syndrome
Crohn’s disease
Rheumatoid arthritis

Ricklin and Lambris, Nature Biotech., 2007
Indicators of Inflammation in Alzheimer Disease

- Neuritic amyloid plaques colocalize with reactive microglia (infiltrating macrophages?) and astrocytes
- Complement proteins
- Increased proinflammatory cytokines

- GWAS SNPs in CR1, Clusterin and Trem2 as contributing to the risk of AD.
Genetic Clues to Late Onset AD:
GWAS - SNPs -2

Genome wide association studies - single nucleotide polymorphisms

- ApoE4 - lipid biology
- TREM2 - phagocytosis*
- CR1 - phagocytosis; regulation of inflammation
- Beclin1 - endosomal trafficking

*ingestion of pathogens and debris
Neurodegenerative Diseases

- Accumulation of protein that is aggregated/misfolded/modified
- Signals from other injured cells
- Imbalance between pro- and anti-inflammatory processes

Inflammation → Neuronal Dysfunction
“Inflammasome” – activated by infection and debris

Including fibrillar amyloid

- accumulation of undigested protein
- Dysfunctional phagocytosis
- improper trafficking within cells for digestion
- regulation of the inflammatory products

GWAS identified SNPs

Clinical Studies

• Epidemiological
  Prospective studies NSAID use ~ decreased risk / delay onset

• Treatment Trials – without success

• Prevention Trials – celecoxib; naproxen ~ no effect
  • The multiple effects of inflammation make specificity of therapeutic targets critical;
  • Differential properties of peripheral inflammation vs. neuroinflammation may be key
Aβ plaques are fibrillar in AD brain while diffuse in nondemented brain.

- Total amyloid
- Fibrillar amyloid
- C1q

Fibrillar Aβ activates the Complement Cascade.
The complement system in Alzheimer’s disease (AD)

Damaged neurons

APP

$\beta$-amyloid (A$\beta$)

A$\beta$ fibrils

$\beta$-amyloid (A$\beta$)

C1q

C1r

C1s

MAC

Complement activation

C5a

C3b/iC3b

Astrocytes

Bad

Neuronal death

$O^-$, NO$_2^-$, proteases

TNFa, IL-6

IL-1$\beta$

Microglia

Late stages of AD
Hypothesis: 
C5a is “Bad” inflammation in AD

Damaged neurons

Pharmacologic and genetic evidence

C5a

Late stages of AD

Microglia

Astrocytes

O\(^{-}\), NO\(_2\), proteases

TNF\(\alpha\), IL-6

IL-1\(\beta\)

Neuronal death

Bad

Complement activation

C5a

C3b/iC3b

TLR2/4
Therapeutic Target: C5a receptor - CD88

C5a Receptor antagonist: PMX205

- Cyclic six amino acid peptide based on the C-terminus of C5a.
- Interacts specifically with the C5a receptor, CD88.
- Therapeutic effects in animal models of inflammatory diseases, including a rat model of neurodegeneration (Woodruff et al., 2005).

PMX205: cyclo-hydrocinnamate-[Orn-Pro-D-cyclohexylalanine-Trp-Arg]

Tg2576 - AD model
12 weeks - oral
12-15 months old
C5aR antagonist decreases pathology in the Tg2576 AD mouse

Fibrillar Ab

- Thioflavine Field Area
- UT vs. PMX205
- n=5, n=6
- p<0.003

Astrocytes

- GFAP Field Area
- UT vs. PMX205
- n=11, n=17
- p<0.001

Microglia

- CD45 Field Area
- UT vs. PMX205
- n=11, n=17
- p<0.002

44-54% ↓

n=11, n=17
p<0.001

n=5, n=6
p<0.003

n=11, n=17
p<0.002
C5aR antagonist treatment decreases Tau hyperphosphorylation in 3xTG 17-20 months

Hyperphosphorylated Tau (AT100)

Fibrillar Aβ Plaques *p<0.05

Reactive microglia (CD45) p<0.12

UT, n=9, PMX, n=9

* p<0.02
C5a - C5a RECEPTOR INTERACTION

C5a

C5a Receptor (CD88)

Peter Ward, 2010
Genetic Deletion of CD88/C5aR Partially Protects Against Loss of Spatial Memory

Object Location Memory

Arctic APP AD mouse model \( \times \) C5aR\(-/-\)

10 Months

Tracy Cole, 2013

*Kruskal-Wallis ANOVA < .01
Overproduction of C5a Accelerates Loss of Spatial Memory in AD Mouse Models

Object Location Memory

Arctic APP x GFAP-C5a

5 Months 7 Months

One way ANOVA is significant  p=0.001

Tracy Cole

*One way ANOVA is significant  p=0.001
GFAP-C5a/3xTg Mice at 16 mo of age

Trend Toward Deficits in Spatial Memory

Object Location Memory

Fibrillar Aβ Plaques

Exhibit Increased Fibrillar Plaques

Exhibit Increased Fibrillar Plaques
Mechanism of PMX205 Protection?

- Blocking a synergistic effect of C5a-C5aR and plaque fAβ - TLRs that induces more robust inflammatory response of microglia and/or infiltrating macrophages?

- Is PMX205 signaling in neurons? Astrocytes? And/or Endothelial cells?

- Is this in the CNS or in periphery?
Summary #1

- Amyloid deposits in AD brain activate the complement pathway.

- Inflammatory markers increase in part as a result of complement activation in mouse models of AD brain.

- Targeted Complement inhibitors may be one approach to prevent and/or slow the progression of cognitive loss in AD.
The complement system in Alzheimer's disease (AD)

Damaged neurons

APP

Aβ

Aβ fibrils

C1q

C1s

C1r

Benoit et al., 2013
**C1q can regulate microglial inflammatory responses**

Damaged neurons

\[
\text{APP} \quad \rightarrow \quad \text{Aβ}
\]

Microglia

Clearance of dead neurons (↓inflammation)

**C1q**

Fan & Tenner, 2004; 2005
Fraser, et al., 2010

C1q suppresses TLR-induced proinflammatory cytokine secretion

Fraser, et al, 2010

**Secreted cytokines - microglia + apoptotic neurons +/- C1q**

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Fold difference from control</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1a</td>
<td>1.0 (±0.5)</td>
</tr>
<tr>
<td>IL-1b</td>
<td>1.0 (±0.5)</td>
</tr>
<tr>
<td>IL-6</td>
<td>1.0 (±0.5)</td>
</tr>
<tr>
<td>TNFα</td>
<td>1.0 (±0.5)</td>
</tr>
<tr>
<td>IL-10</td>
<td>1.0 (±0.5)</td>
</tr>
<tr>
<td>MCP-1</td>
<td>1.5 (±0.5) **</td>
</tr>
</tbody>
</table>

n=5-7

*= p<.05
**= p<.01
In Vitro model of Aβ Injury

Damaged neurons

24h post-stimulation, n = 3
ANOVA, *, p < 0.05 and **, p < 0.01

Mature neurons

Immature neurons

Pisalyaput, et al., 2008; Benoit and Tenner, 2011; Benoit et al., 2013
C1q increases specific protein expression in Aβ-injured neurons

**LRP1B (LDL receptor-related protein 1B)**
- binds Aβ and APP
- retains APP at the cell surface
  → ↓ amyloid-β production

**GPR6 (G protein-coupled receptor 6)**
- increases neurite growth by increasing intracellular levels of cAMP
although I like this slide, it can be removed if you need time
Marie, 10/4/2012
Does the C1q-neuroprotective response to Aβ require LRP1B and GPR6? Yes!

Inhibition of LRP1B and GPR6 expression after siRNA transfection

10 nM scrambled, LRP1B or GPR6 siRNA

2-way ANOVA test

N = 3, 5 fields per condition

***, p < 0.001 and **, p < 0.01
Summary:
*C1q - Good Cop*

- *C1q* promotes neuron survival directly
- Enhancement of clearance of neuronal blebs and apoptotic debris
- Suppression of inflammatory cytokines
Perspective: possible therapeutics

- **Target downstream effectors of C1q**
  - Neuronal death
  - Prevent damage
  - Bad
  - Promote repair
  - Good
  - Enhance neuronal survival
  - Microglia
  - Clearance of dead neurons (↓ inflammation)
  - Early stages of AD
  - Late stages of AD

- Damaged neurons
- APP
- Ab fibrils
- Ab
- O-, NO2-, proteases
- MAC
- C1s
- C1r
- C5aR antagonist
- C5a
- C3b/iC3b
- TNF, IL-6
- IL-1b
- Astrocytes
- Complement activation
- Fonseca, Ager et al., J Immunol, 2009
Conclusion: Cocktail Approach to Therapy

• promotion of complement neuroprotective activity (C1q and C3)

+ 

• Targeted inhibition of detrimental complement events (C5aR)

→ approach to slow the progression of neurodegenerative or developmental disorders
Potential Targets for Therapies in AD

Pathogenic Event

Genetics/Injury

Deposition of $\alpha\beta_{1-42}$

Activation of glia

Neuronal damage

Target Therapy

Secretase Inhibitors
Enhance Clearance
Block oligomer/fibril formation

Block Complement Activation
Block Inflammation

Prevent neurotoxicity
Enhance neuronal function
Basic Science-Discovery

Molecules → Pathways → Pathology

Drug Discovery
Therapeutics
Clinical Trials
Benefit to patients
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