The Role of Amyloid in the Development and Treatment of Alzheimer's Disease

Dr. Charles Glabe
Department of Molecular Biology and Biochemistry
University of California, Irvine
cglabe@uci.edu
Overview

• A short history of AD and senile dementia.
• Familial AD and the amyloid cascade hypothesis.
• Therapeutic strategies based on the amyloid cascade hypothesis and their failure in human clinical trials.
• An alternative amyloid hypothesis based on intraneuronal amyloid accumulation leading to neuronal death and neuritic plaque formation.
• New potential therapeutic strategies.
Amyloid deposits in AD brain.

Auguste Deter, 1905. Alois Alzheimer’s patient

Brown: amyloid Aβ, (senile plaques)
Black: Tau, (neurofibrillary tangles, NFT)
Timeline of Alzheimer’s milestones

Alzheimer describes plaques and tangles in a middle aged woman with dementia. 1907

Peter Davies reports the loss of cholinergic function in AD brain, initiating the development of cholinergic therapeutics like Aricept and Exelon. 1976

Several groups show that NFTs contain the protein, tau. 1986

Goate and Hardy: Mutations in APP associated with familial AD. 1991

Spectacular failure of the Aβ secretion inhibitor, Semagacestat. 2010

Terry and Wisniewski show that AD pathology is identical to senile dementia. 1967

St. George-Hyslop: Mutations in presenilin associated with familial AD. 1995

George Glenner isolates the Aβ peptide, the major component of plaques. 1985

Aβ is derived from a larger protein, APP. 1987

Failure of monoclonal antibody, Bapineuzemab in human clinical trials. 2012
Aβ is derived by proteolytic cutting of APP

Thinakaran and Koo, JBC 2008
Alzheimer’s disease has a familial or inherited early onset form (FAD).

3 Genes are associated with FAD:

Amyloid precursor protein
Presenilin 1 (gamma secretase)
Presenilin 2 (gamma secretase)
FAD genetics supports the amyloid cascade hypothesis

Hardy and Selkoe 2002  
Karran, Mercken and De Strooper, Nature Reviews 2011
Effect of APP mutations on Aβ

“Swedish” mutation increases β-secretase cleavage and Aβ production 5-8 fold.

Amino acid substitution mutations increase Aβ aggregation and oligomerization.

Transmembrane domain substitution mutations increase production of Aβ42 over Aβ40. Aβ42 aggregates much faster.
Presenilin mutations increase the ratio of Aβ42/Aβ40

Increased amyloid-β42(43) in brains of mice expressing mutant presenilin 1

Karen Duff*†, Chris Eckman†, Cindy Zehr*†, Xin Yu*, Cristian-Mihail Prada†, Jordi Perez-tur*†, Mike Hutton*†, Luc Buee‡, Yasuo Harigaya†, Debra Yager†, David Morgan§, Marcia N. Gordon§, Leigh Holcomb§, Lawrence Refolo†, Brenda Zenk†, John Hardy*† & Steven Younkin†

* Suncoast Alzheimer’s Disease Laboratories, University of South Florida, Tampa, Florida 33612, USA
† Birdsall Building, Mayo Clinic Jacksonville, 4500 San Pablo Road, Jacksonville, Florida 32224, USA
‡ Unite 422 INSERM, Place de Verdun, 50945 Lille Cedex, France
§ Alzheimer’s Research Laboratory, Department of Pharmacology, University of South Florida, Tampa, Florida 33613, USA

Mutations in the genes encoding amyloid-β precursor protein (APP), presenilin 1 (PS1) and presenilin 2 (PS2) are known to cause early-onset, autosomal dominant Alzheimer’s disease. Studies of plasma and fibroblasts from subjects with these mutations have established that they all alter amyloid β-protein (βAPP) processing, which normally leads to the secretion of amyloid-β protein (relative molecular mass 4,000; M, 4K; ~90% of the total amyloid-β40 (Aβ40) and amyloid-β42 (Aβ42) production. Aβ42 aggregation much faster.
Investigational new drugs based on the amyloid cascade hypothesis

Golde, T., et al., Exp. Neurol. 2010
Pharma counts just 3 Alzheimer's drug wins in 13 years (101 losses!)
September 14, 2012 | By Ryan McBride

Alzheimer's drug research has riddled biopharma with some of the worst odds of success in the already risky R&D game. … drug developers have scrapped or halted development of 101 meds for the complex disorder and brought to market only three treatments for symptoms of the disease, according to the Pharmaceutical Research and Manufacturers of America (PhRMA). This should come as no surprise to those who have followed the recent late-stage disasters of Johnson & Johnson ($JNJ) and Pfizer's ($PFE) bapineuzumab and Eli Lilly's ($LLY) solanezumab. Do the math on PhRMA's figures and from 1998 to 2011 you end up with a sad win-to-loss ratio of one to 34.
Semagacestat (gamma secretase inhibitor) actually made the treated group cognitively worse.

INDIANAPOLIS, Aug 17, 2010 /PRNewswire via COMTEX News Network/ --

Eli Lilly and Company (NYSE: LLY) will halt development of semagacestat, a gamma secretase inhibitor being studied as a potential treatment for Alzheimer's disease, because preliminary results from two ongoing long-term Phase III studies showed it did not slow disease progression and was associated with worsening of clinical measures of cognition and the ability to perform activities of daily living.
So why have all the new drugs based on the amyloid cascade hypothesis failed miserably?

1. The amyloid cascade is wrong and Aβ is not important.

2. We are not thinking about the amyloid cascade hypothesis correctly.
The Transcellular Spread of Cytosolic Amyloids, Prions, and Prionoids

Adriano Aguzzi¹,* and Lawrence Rajendran²,*

¹Institute of Neuropathology, University Hospital of Zürich, Schmelzbergstrasse 12, CH-8091 Zürich, Switzerland
²Systems and Cell Biology of Neurodegeneration, Psychiatry Research, University of Zürich, CH-8008 Zürich, Switzerland
*Correspondence: adriano.aguzzi@usz.ch (A.A.), rajendran@bli.uzh.ch (L.R.)
DOI: 10.1016/j.neuron.2009.12.016

<table>
<thead>
<tr>
<th>Phenotype/Function</th>
<th>Protein</th>
<th>Molecular Transmissibility</th>
<th>Bona Fide Infectivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prion diseases</td>
<td>PrPSc (luminal)</td>
<td>yes</td>
<td>yes</td>
</tr>
<tr>
<td>Alzheimer’s disease</td>
<td>Aβ (luminal)</td>
<td>yes</td>
<td>in APP-overexpressing mice</td>
</tr>
<tr>
<td>Tauopathies</td>
<td>Tau (cytosolic)</td>
<td>possibly</td>
<td>not shown</td>
</tr>
<tr>
<td>Parkinson’s disease</td>
<td>α-synuclein (cytosolic)</td>
<td>host-to-graft</td>
<td>not shown</td>
</tr>
<tr>
<td>AA amyloidosis</td>
<td>SAA (luminal)</td>
<td>yes</td>
<td>probable</td>
</tr>
<tr>
<td>Huntington’s disease</td>
<td>PolyQ (nuclear)</td>
<td>yes</td>
<td>not shown</td>
</tr>
<tr>
<td>Suppressed translational termination (yeast)</td>
<td>Sup35</td>
<td>yes</td>
<td>limited</td>
</tr>
<tr>
<td>Biofilm production (bacteria)</td>
<td>bacterial curin</td>
<td>yes</td>
<td>questionable</td>
</tr>
<tr>
<td>Heterokaryon incompatibility (fungi)</td>
<td>Het-s</td>
<td>yes</td>
<td>limited</td>
</tr>
<tr>
<td>Pituitary secretory granules</td>
<td>peptide hormones</td>
<td>not shown</td>
<td>not shown</td>
</tr>
<tr>
<td>Mammalian skin pigmentation</td>
<td>Pmel17</td>
<td>not shown</td>
<td>not shown</td>
</tr>
</tbody>
</table>

The fundamental mechanism of prion replication involves misfolding of PrP and its aggregation into a β-sheet fibril where the amyloid domain is resistant to degradation.
Prion replication involves “seed” formation, elongation, fragmentation and transmission of seeds to other cells or individuals.
Uptake of Aβ42 oligomers seeds the prion-like accumulation of insoluble, intracellular APP and amyloidogenic fragments of APP.

<table>
<thead>
<tr>
<th>Control HEK Cells</th>
<th>APP751-expressing HEK cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>no peptide</td>
<td>no peptide</td>
</tr>
<tr>
<td>Aβ1-28</td>
<td>Aβ1-28</td>
</tr>
<tr>
<td>Aβ1-42</td>
<td>Aβ1-42</td>
</tr>
</tbody>
</table>

Yang, et al., 1995

*JBC 270:14786-14792*
Digestion of insoluble intracellular amyloid with Proteinase-K results in the accumulation of degradation-resistant Aß.

\[43K-\quad 29K-\quad 6.2K-\quad 3K-\]

Time of digestion (hours)

\[4 \text{ kDa Aß}\]

\[^{35}\text{S-labeled intracellular amyloid was digested with proteinase K for varying lengths of time, solubilized and immunoprecipitated with 4G8 anti-Aß.}\]

Yang, et al., 1999 JBC 274:20650-20656
Prion-like mechanism for Aβ amyloid accumulation

Initiation:

Aggregation of Aβ42 → Protease Resistance

Replication:

APP fragments normally targeted to lysosomes for degradation

Addition of amyloidogenic fragments onto insoluble Aβ42 aggregates

Growth of aggregates

Proteolytic conversion of amyloidogenic APP fragments to protease resistant Aβ core
So is there any evidence for a prion-like mechanism for amyloid Aβ accumulation in AD brain?
Conformation dependent fibril specific monoclonal antibodies

Antigen: Aβ42 fibrils.
NZW rabbits boosted 6 times at monthly intervals.
Titer for Aβ42 fibrils > 1:20,000
Primary screen: Aβ42 fibrils, prefibrillar oligomers and monomer.
120 primary pools of hybridomas selected
Secondary Screen

- IHC on human AD brain.
- 24 clones selected based on differential reactivity on array and on IHC of AD brain.
M78 recognizes a “generic” aggregation specific fibril epitope.

M78 recognizes a generic fibril epitope because it reacts with amyloid fibrils from Aβ, synuclein and islet amyloid polypeptide. M78 does not recognize the monomeric or natively folded forms of these peptides and proteins.
M78 immunoreactivity first appears intracellularly in 3XTg-AD mice.

3 mo: 6E10 is perinuclear in some neurons and no M78 is observed.

12 mo: M78 colocalizes with perinuclear 6E10 and accumulates in nuclei.

M78 – Red  
6E10 (Aβ) – Green  
DAPI (DNA) - Blue
At 12 mo, M78 is predominantly nuclear while at 14 mo, it is primarily extracellular.

12 mo: M78 accumulates in nuclei with perinuclear 6E10 immunoreactivity.
14 mo: M78 stains only extracellular plaques.
14 mo wild type mice: No 6E10 or M78 staining is observed.

M78 – Red  
6E10 (Aβ) – Green  
DAPI (DNA) - Blue
M78 and DAPI DNA fluorescence are found in the center of neuritic plaques in 12 mo 3XTG-AD mice. This indicates that neuritic plaques are derived from neurons with intranuclear M78 staining.
The core of neuritic plaques also contains NeuN immunoreactivity; a marker of neuronal nuclei. This is further evidence that neuritic plaques are derived from neurons with intracellular amyloid.
Perinuclear and nuclear M78 IR is also observed in human neurons and neuritic plaques.
M78 immunoreactive nuclei are specifically elevated in early AD.
• An alternative amyloid hypothesis based on intraneuronal amyloid accumulation leading to neuronal death and senile plaque formation.

1. Initiation of APP-CTF aggregation in cell by uptake of Aβ oligomers

2. Accumulation in nucleus and/or in perinuclear autophagic vacuoles

3. Lysis and demise of neuron releasing cytosolic contents and ultimately uptake and processing by microglia.
Summary

- The conformation dependent, generic fibril specific monoclonal antibody M78 stains nuclei in aged human brain is correlated with intracellular APP and Aβ immunoreactivity.
- Nuclear M78 co-localizes with APP-CTF immunoreactivity in and around the nucleus.
- The same spatial relationship of the nucleus, M78 staining and APP-CTF immunoreactivity is observed in senile plaques, suggesting that senile plaques are initiated by the demise of these neurons.
Implications: The alternative amyloid cascade hypothesis provides a facile explanation for FAD mutations and the results of the Semagacestat clinical trial.

Alzheimer’s Disease-Linked Mutations in *Presenilin-1* Result in a Drastic Loss of Activity in Purified γ-Secretase Complexes

Matthias Cacquevel, Lorène Aeschbach, Jemila Houacine, Patrick C. Fraering*
École Polytechnique Fédérale de Lausanne, Brain Mind Institute, Laboratory of Molecular and Cellular Biology of Alzheimer’s Disease, Lausanne, Switzerland

*Conclusion/Significance:* Our data support the view that PS1 mutations lead to a strong γ-secretase loss-of-function phenotype and an increased Aβ1–42/Aβ1–40 ratio, two mechanisms that are potentially involved in the pathogenesis of Alzheimer’s disease.

Both PS mutations and Semagacestat partially inhibit PS proteolytic function. They both increase the levels of β-CTF which would be expected to drive its misfolding, aggregation and the alternative pathway leading to cognitive dysfunction.
Therapeutic implications

Intracellular amyloid accumulation begins early, before significant cognitive impairment is evident, so treatment must also begin early if it is going to arrest disease progression.

The same kind of strategies that have been tested in mild to moderate AD may also work if they are started early, like immunotherapy and beta secretase inhibitors and gamma secretase modulators.

Inhibitors of intracellular amyloid aggregation would need to gain access to the inside of the cell.

Extracellular aggregation inhibitors and immunotherapy may work by preventing the spreading of disease by cell to cell seeding.
Glabe Lab

- Dr. Suhail Rasool
- Dr. Anna Pensalfini
- Dr. Leonid Breydo
- Dr. Jessica Wu
- Hiromi Arai
- Asa Hatami
- Ricardo Albay III

- Collaborators:
  - Dr. Carl Cotman
  - Dr. Claudia Kawas
  - Dr. Wayne Poon
  - Dr. Ralf Langen
  - Dr. Hudel Leucke

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