Targeting Soluble Aβ Oligomers by Passive Immunization for AD Therapy

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Development of Amyloid Pathology

Cellular Metabolism:
APP, BACE, \( \gamma \)-secretase

Soluble A\( \beta \) Oligomers

A\( \beta \) Fibrillization vs. A\( \beta \) Degradation

Fibril

TOXICITY???
Oligomer-Selective Monoclonal Antibody

NAB228

NAB61

NAB228

NAB61

St - 38.1 - 28.4 - 18.2 - 9.2 - 4.3 -

Aβ NO2 UV HNE

Aβ NO2 UV HNE

Aβ NO2 UV HNE

Aβ NO2 UV HNE
NAB61 Does Not Recognize APP or C-terminal APP Fragments
NAB61 Immunoreactivity Does Not Co-localize with APP
NAB61 Recognizes Amyloid Deposits
Regional Specificity of NAB61

Ban50             NAB61             Ban50             NAB61

Hipp

Entorhinal Cortex

Mid-Frontal Cortex

Down’s Syndrome          Alzheimer’s Disease
NAB61 Recognizes Fibrillar Aβ, Angiopathy and Mature Senile Plaques

Ban50  NAB61  anti-Aβ42  NAB61  Merge

Diffuse Plaque

Amyloid Angiopathy

Mature Plaque
Mechanisms of Aβ Immunotherapy

- Active or passive immunization improves cognitive function and inhibits amyloid pathology \textit{in vivo}

- Acute passive immunization improves cognitive function without affecting pathology
  - Synaptotoxic soluble Aβ oligomer???

- Peripheral sink hypothesis
  - Antibodies bind Aβ in plasma and sequester Aβ from the central nervous system

- Central action
  - Antibodies enter the CNS and neutralize Aβ
Targeting Soluble Oligomers by Passive Immunization of Aged tg2576 Mice with NAB61

Probe Trials

visible

hidden

NAB61 or IgG
Passive Immunization Does Not Affect Performance on the Visible Water Maze

Repeated measures ANOVA $p=0.0952$
Passive Immunization Reverses Learning and Memory Deficits in Aged tg2576 Mice

Repeated measures ANOVA p<0.0001
IgG vs. wt or NAB61 p<0.001
wt vs NAB61 p>0.05
Probe Trial Outcomes

- Three probe trials (early, middle, late)
- Platform removed from pool for trial of 60 seconds
Spatially Oriented Swimming Behavior: Early Probe

One-way ANOVA
Fisher’s post-hoc, T vs. L, T vs R
Spatially Oriented Swimming Behavior: Middle Probe

![Graph showing % Time in Quadrant for IgG, NAB61, and Wild Type with statistical significance symbols for comparisons between groups.]

- **IgG**
- **NAB61**
- **Wild Type**

**Legend:**
- **Left**
- **Target**
- **Right**
- **Opposite**
Spatially Oriented Swimming Behavior: Late Probe

![](image)

**Probe 3**

- **% Time in Quadrant**
- **Left**
- **Target**
- **Right**
- **Opposite**

![Chart showing % Time in Quadrant for IgG, NAB61, and Wild Type](chart)

- **IgG**:
  - Left
  - Target
  - Right
  - Opposite

- **NAB61**:
  - Left
  - Target
  - Right
  - Opposite

- **Wild Type**:
  - Left
  - Target
  - Right
  - Opposite

*** indicates statistical significance.
Spatially Oriented Searching Behavior in NAB61 Immunized tg2576

Platform Crossing Index:
# Crosses over target platform/
Average # crosses over other platforms

Repeated measures ANOVA p=0.0301
(Bonferroni) IgG vs. NAB61 p<0.05
(Neuman-Keul, Fisher) IgG vs. NAB61/wt p<0.05
Improved Behavior is Not Due to Differences in Swimming Speed

Two-way ANOVA (Group x Probe)
Treatment p=0.3540
Probe p=0.8284
NAB61 Immunization Does Not Affect APP Processing

Full Length APP

sAPPβswe NL

β-cleavage C99

β'-cleavage C89

α-cleavage C83

APPfl

sAPPβswe

CTF's
Quantification of Brain Aβ After Short Term NAB61 Immunization

![Graph showing quantification of Aβ levels after short term NAB61 immunization. The graph compares IgG, NAB61, and wt conditions in both RIPA and Formic Acid preparations.](image)
Peripheral Accumulation of Oligomeric Aβ Species???

- tg2576 mice passively immunized with 500 μg of NAB61 i.p. or i.v.
- 24 hours post-injection, mice were exsanguinated
- Accumulation of peripheral Aβ was analyzed by electrophoresis of formic acid denatured serum on step gradient acetic acid/urea gels
- Immunoblotting with two different monoclonal antibodies that recognize different epitopes within Aβ demonstrates the accumulation of a higher molecular weight Aβ species
Production Paradox and Intermediate Impairment

Cellular Metabolism:
APP, BACE, γ-secretase

Soluble Aβ Oligomers

Aβ Fibrillization vs. Aβ Degradation

Protofibril

Fibril
Acknowledgements

- Eddie Lee
- CNDR members
- John Trojanowski
- Ted Abel
- Harry Ischiropoulos
- David Teplow
- Tom Montine

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Some Points For Discussion On Protein Misfolding And Neurodegenerative Diseases

- Why do only selected brain proteins misfold, fibrillize and deposit with advancing age in neurodegenerative disease brains?
- What is it about the aging that drives this process?
- If misfolding is a core neurodegenerative disease mechanisms, are there interventions to counter misfolding in these disorders?
- Can this be done regardless of the disease protein?
- When should such therapies be given (birth, prodrome, onset, etc.)?
- Why do aggregates of misfolded proteins cause neurodegeneration?
- Do they kill by occupying space, by disrupting cellular communications and transport, by loss of function due to sequestration in the aggregates), by toxic gains of functions, by sequestering other key proteins thereby taking them out of action, etc.
Society for Neuroscience, Neurobiology of Disease Workshop
“Protein Misfolding in Neurodegenerative Diseases”
Friday, 22 Oct., 2004; 8:30 AM – 5:00 PM

Speakers/Agenda
Dennis Selkoe: Protein Misfolding in Alzheimer's, Parkinson's and Other Neurodegenerative Diseases (with a live or video presentation of an AD or PD patient)
Virginia M.-Y. Lee: Convergence of Tau and Alpha-synuclein Amyloids in Neurodegenerative Diseases
Rick I. Morimoto: Genome-wide Screen for Genes that Regulate Protein Quality Control
Stuart Lipton: Nitrosative/oxidative Stress E3 Ligases and the Ubiquitin Proteasome System in Neurodegeneration

12:00-1:30     Lunch
Breakout Groups: Translational Research in Diseases of Protein Misfolding Each breakout lasts 1-1/2 hour. Students attend 2.
NAB61 Induced Meningoencephalitis
Disruption of the BBB

Non-encephalitic NAB61 immunized tg2576

Encephalitic NAB61 immunized tg2576
Vasocentric Mononuclear Infiltrates
Lymphocytic Infiltrates

- Macrophages (Mac-3)
- T Lymphocytes (CD3)
- B Lymphocytes (CD45R)
**Limited Deposition of Truncated Aβ**

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<tr>
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<th>NAB228</th>
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<td>AD Mid-frontal Cortex</td>
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Limited Deposition of Truncated Aβ

- **APP x BACE-Lo**
- **APP x BACE-M**
- **APP x BACE-Hi**
Limited Deposition of Truncated Aβ
Anti-amyloid Serum Titers

![Graph showing O.D. for different samples: wt IgG, tg2576 IgG, wt NAB61, tg2576 NAB61]
NAB61 Titer by ELISA

![Graph showing NAB61 Titer by ELISA](image-url)
Serum Aβ After Passive Immunization